



**EMBO WORKSHOP**



# **EPITHELIAL-MESENCHYMAL TRANSITIONS**

**10-12 September 2007  
Krakow Poland**

## **Co-Chairs:**

<b>Pierre Savagner</b>	<b>Aristidis Moustakas</b>
<b>Antonio Garcia de Herreros</b>	<b>Amparo Cano</b>

## **International committee:**

<b>Shoukat Dedhar, Canada</b>	<b>Raghu Kalluri, USA</b>
<b>Suresh Mohla, USA</b>	<b>Donald Newgreen, Australia</b>
<b>Angela Nieto, Spain</b>	<b>Raymond Runyan, USA</b>
<b>Jean-Paul Thiery, Singapore</b>	<b>Kristin Verschueren, Belgium</b>

**Co-organized by TEMTIA and  
Marie-Curie Epiplasticarcinoma EU-RTN network**

<http://www.mtci.com.au/temtia.html>

<http://www.epiplasticarcinoma.org>

<http://www.cwp.embo.org/w07-41/>

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***WE WISH TO EXPRESS OUR THANKS  
TO OUR SPONSORS FOR THEIR GENEROUS FUNDING OF THE 3rd EMT MEETING***



Website: <http://mtci.com.au/temtia.html>

eMail address: [temtia@mtci.com.au](mailto:temtia@mtci.com.au)

## ... from the Convenors

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We are delighted to welcome you to the 3rd Epithelial-Mesenchymal Transition (EMT) Meeting, held in Larischa Palace, in Poland's historic city of Krakow, which in 2007 celebrates its 750th Anniversary. The 3rd Meeting in EMT series is the first to be held in Europe, and is convened as an EMBO Workshop, co-sponsored by TEMTIA and the Marie-Curie Epiplasticarcinoma EU-RTN network. At this point we wish to thank our sponsors: The Ludwig Institute for Cancer Research, and Merck Serono, EMBO, TEMTIA, and the EU-RTN Network. We are grateful for your support without which this meeting would not be possible.

Unfortunately, we must begin this introduction with some sad news. EMT pioneer, Elisabeth Hay passed away in mid-August. Her historical impact on the EMT field will be commemorated early in this meeting.

EMT has been recognized as an essential mechanism in embryological development. The process integrates several regulatory pathways converging towards a classic pattern of cell dissociation and migration. EMT-like events are also involved during carcinoma progression and metastasis, as well as fibrotic processes. The EMT Meetings draw together the top researchers in these fields from all over the world.

As convenors, we would like to thank the International Program Committee, for their invaluable contribution to the success of the meeting, and all our presenters in the program - the invited and contributed papers will bring a novel and stimulating academic perspective to our shared ideas. In the 3rd EMT Meeting, we are introducing the Elizabeth D Hay Lecture, so named to honour a founding scientist in the field of EMT. The Inaugural Lecture will be presented by leading researcher and academic, Jean-Paul Thiery. We have continued the tradition of incorporating two debates into the program - these prove to be not only an opportunity to confront interactively the real problems and seeming conundrums of the field, but also a great source of quick-witted humour and opportunity for theatrics from erstwhile seemingly dour scientists!! Light relief – interactivity – academic rigour ~ not to be missed.

The program is an exciting mix of presentations: Abstract topics range from new insights into developmental control of EMT to the participation of EMT and related events in diverse pathological processes from cancer to organ fibrosis.

Poster Presentations are always a highlight of the EMT Meetings, with vigorous discussions and new collaborations developed. They are an integral part of the program and will feature each extended lunchtime and in the morning and afternoon breaks, and each day at the end of the conference sessions. We ask poster presenters to provide short oral presentations at specific times to be noted on their poster board.

The inaugural EMT Meeting organised by Don Newgreen and Rik Thompson was held in Port Douglas, Far North Queensland, Australia. After this meeting, an international association which dedicates its activities to EMT was formed ([www.mtci.com.au/TEMTIA.html](http://www.mtci.com.au/TEMTIA.html)). The 2nd EMT Meeting, chaired by Shoukat Dedhhar was held in Vancouver - and now it remains to say:

Thank you all for coming to Krakow - and Welcome to the 3rd International EMT Meeting and a feast of scientific ideas and debate!

The Convenors

*Pierre Savagner*

*Antonio Garcia de Herreros*

*Aristidis Moustakas*

*Amparo Cano*

*Krakow, 2007*

## ... General information

### THE CITY

In 2007 Krakow, pulsating with cultural and scientific life in their various forms, and with exceptional collections of museums, galleries and churches, celebrates its 750th Anniversary. Known for centuries as the Athens of the north, or the second Rome, the city's status is further enhanced by its wonderful buildings, among them The Royal Castle and Cathedral at Wawel, St Mary's Basilica, the Jagiellonian University's Collegium Maius and the church of SS Peter and Paul. Krakow is included on the UNESCO world heritage list.

### CONFERENCE PROGRAM

The conference begins at 8am on Monday the 10<sup>th</sup> of September with registration, and concludes at 4pm on Wednesday the 12<sup>th</sup>. There is the inaugural Elizabeth Hay named Lecture, invited and contributed oral presentations, debates and Poster presentation sessions. The language of the conference is English and all talks and papers must be presented in English. There is no translation. Abstracts are noted as INV (Invited), SYM (Symposium) and POS (Poster) in the abstract section of the program.

### VENUE

Larischea Palace, (Jagiellonian University building), 12 Bracka Street, Krakow, Poland. The various rooms used for the sessions are listed in the program.

### NAME BADGES

Delegates must wear name badges at all times for entry to scientific sessions and social events.

### POSTERS

Posters are numbered in the program; please display your poster on the numbered boards, upon arrival. The means to attach your poster is available at your numbered board. **Poster presenters are also requested to display clearly on their poster board/s, two specific days/times during the meeting when they will make a short, 5-minute oral presentation in front of their poster/s.**

Posters are viewed in all the tea breaks, at lunchtimes and at the end of the day. This latter Poster Session runs concurrently with the AGMs on both days.

### REFRESHMENTS

The registration fee includes morning tea and lunch on all days of the conference and afternoon tea on Monday and Tuesday; also, the Welcome Reception on Monday and the Conference Dinner on Tuesday night.

### SLIDE PREVIEW/DATA PROJECTION

Slide and data-projection preview are available at the conference rooms. Please make sure that your talk is loaded before your session and preferably on the day before.

### ACCOMMODATION and PAYMENT OF ACCOUNT

Accommodation is booked and paid for prior to arrival. On check in, delegates will be asked to provide a credit card imprint for full payment on check-out of incidentals such as telephone/data access, use of mini-bar etc. If sharing a room, please check out together to facilitate payment of incidentals.

### BANKING

Recognised international credit cards are usually accepted in hotels, shops and restaurants. Bank opening hours are usually from 8.00 until 18.00. The official Polish currency is Polish Zloty. The Exchange rate (Aug. 2007):  
1 Euro ~ 3.8 zlotys (PLN); 1 USD ~ 2.8 zlotys (PLN)

### CLIMATE

Krakow's weather in September is generally mild, being warm and usually sunny. A jacket for night time events - and an umbrella, just in case!!

**Average high temperatures:** 19.0° C / 65.8° F

**Average low temperatures:** 9.0° C / 47.5° F

### ELECTRICITY

The electricity supply is 220 volts, 50 Hz. Foreign appliances may require an adapter.

### KRAKÓW AIRPORT INFORMATION

Phone no: (0) 801 055 000; 295-58-00

<http://www.krakow-info.com/airport.htm>

### MEDICAL AND EMERGENCY SERVICES

Ambulance: 999; Police: 997; Fire Brigade: 998

From a mobile telephone: 112

### NOTICE BOARD

A small notice board outside the conference room will display any messages left for delegates. Please check as messages will not be delivered individually.

### SMOKING

The university buildings, restaurants and some accommodation rooms are non-smoking areas. Smoking is permitted outside.

### SOCIAL PROGRAM

Registration includes: Lunches and teas, Welcome Reception and Conference Dinner as outlined above.

For dinners with friends at the end of Monday, after the Posters and Reception, there is a good selection of restaurants, cafés, and coffee-bars in the centre of the city. Please see tourist leaflets and locals! for suggested restaurants and cafés. Also consult this site for a comprehensive City-wide list:  
<http://www.krakow.pl/en/turystyka/restauracje/>

### TOURS AND PLACES TO SEE

Enquire from ORBIS at the Registration desk for details of post conference touring options.

### TRANSPORT - PUBLIC

Tram tickets may be purchased in kiosks, some shops, and post offices. Single-trip tickets can be also purchased from the driver, with a small surcharge. If you are thinking of hiring a car or a bike etc., please consult this site first!  
<http://www.krakow.pl/en/turystyka/?id=transport.html>

### TRANSPORT - TAXIS

**Taxi corporation fleet cars** are clearly marked, and are much cheaper than other unmarked taxis. Also, pre-booking some taxis entitles you to a price reduction. Please note that there are supplements for Sundays, holidays, and at night. **Payment by Credit card is not always available.**

### 3rd EMT MEETING ~ PROGRAM AT-A-GLANCE

<b>Monday, September 10</b>		
0800	<i>Registration</i>	Larischka Palace, Conference Room Foyer
	<b>EMT and Development:</b> Chairperson: Raghu Kalluri	Larischka Palace, Conference Room
0830	Opening Remarks	
0900	<b>Session I</b> (Invited)	Inaugural Elizabeth D Hay Lecture: <i>Jean-Paul Thiery: Mechanochemistry of cell adhesion in epithelial cell plasticity</i>
0935	(Invited)	Angela Nieto: <i>EMT-related and EMT-independent regulation of cell adhesion and movement by Snail</i>
1010		Patrick Humbert: <i>The polarity regulators Scribble and DLG control epithelial migration during development, wound healing and tumorigenesis</i>
1030	<i>Coffee &amp; Posters</i>	Conference Room Foyer
1100	<b>Session II</b> (Invited)	David McClay: <i>Gene regulatory network states that control EMT.</i>
1135	(Invited)	Michael Klymkowsky: <i>Conserved and novel features of the NF<math>\kappa</math>B-Slug-Twist network involved in Xenopus mesoderm formation and EMT</i>
1210		Michael Murray: <i>Photoactivatable GFP resolves Drosophila mesoderm migration behaviour</i>
1230	<i>Lunch and Poster viewing</i>	Wierzynek Restaurant / Conference Room Foyer
	<b>Cell signalling and EMT (I).</b> Chairperson: Angela Nieto	Larischka Palace, Conference Room
1400	<b>Session III</b> (Invited)	Aristidis Moustakas: <i>Smad proteins of the TGF<math>\beta</math> pathway instruct a network of transcription factors for the establishment of EMT</i>
1435	(Invited)	Caroline Hill: <i>Searching for novel regulators of EMT</i>
1510		Wolfgang Mikulits: <i>PDGF Links TGF-<math>\beta</math> Signaling to Nuclear <math>\beta</math>-Catenin Accumulation in Hepatocellular Carcinoma Progression</i>
1530		Eileen Whiteman: <i>The Transcription Factor Snail Represses Crumbs3 Expression and Disrupts Apico-Basal Polarity Complexes</i>
1550	<i>Afternoon Tea and Posters</i>	Conference Room Foyer
1630	<b>Session IV</b> (Invited)	John Collard: <i>The Par/Tiam1 polarity complex controls epithelial and mesenchymal properties of epithelial cells.</i>
1705	(Invited)	Shoukat Dedhar: <i>Regulation of EMT and Wnt signaling by Integrin-Linked Kinase</i>
1740		William Schiemann: <i>EMT and Oncogenic Signaling by TGF-<math>\beta</math> in Mammary Epithelial Cells</i>
1800		<b>POSTER SESSION</b> / EpiPlast Carcinoma Committee Meeting
<b>Tuesday, September 11</b>		
	<b>EMT and Cancer (I)</b> Chairperson: Antonio Garcia de Herrerros	Larischka Palace, Conference Room
830	<b>Session V</b> (Invited)	Paolo Comoglio: <i>Invasive growth: a MET-driven genetic programme for cancer and stem cells</i>
905	(Invited)	Don Newgreen: <i>EMT in the neural crest model: Does functional manipulation of adhesion and cytoskeletal systems reset the sensitivity to pro-EMT growth factors?</i>
940		Greg Goodall: <i>The microRNA-200 family regulates the E-cadherin repressors, ZEB1/deltaEF-1 and SIP1/ZEB2 and EMT</i>
1000	<i>Coffee &amp; Posters</i>	Conference Room Foyer
1030	<b>Debate</b>	EMT-MET cycles in tumors , Amparo Cano, Gerhard Christofori, Pierre Savagner and Erik (Rik) Thompson
		Larischka Palace, Conference Room

### 3rd EMT MEETING ~ PROGRAM AT-A-GLANCE

<i>Tuesday's program continued ...</i>		
1130	<b>Session VI</b> (Invited)	Gerhard Christofori: <i>Distinct mechanisms of tumor invasion and metastasis</i> Larisha Palace, Conference Room
1205	(Invited)	Erik (Rik) Thompson: <i>Epithelial Mesenchymal Transition Traits in Human Breast Cancer Cell Lines Parallel the Human Breast Cancer Stem Cell Phenotype and Sub-classify Human Breast Cancers</i>
1240	<i>Lunch and Poster viewing</i>	Wierzynek Restaurant / Conference Room Foyer
	<b>EMT and Cancer (II)</b> Chairperson John Collard.	Larisha Palace, Conference Room
1415	<b>Session VII</b> (Invited)	Amparo Cano: <i>Understanding the role of Snail factors and other EMT regulators in tumour progression</i>
1450	(Invited)	Thomas Brabletz: <i>Malignant progression in colorectal cancer: EMT, <math>\beta</math>-Catenin &amp; cancer stem cells</i>
1525		Andreas Eger: <i>The transcription factor ZEB1 promotes tumor progression by repressing master regulators of epithelial differentiation</i>
1545	<i>Afternoon Tea and Posters</i>	Conference Room Foyer
1615	<b>Session VIII</b> (Invited)	Avri Ben-Ze'ev: <i>Cell motility at the invasive front of tumors: The role of novel Wnt/<math>\beta</math>-catenin target genes</i>
1650	(Invited)	Arne Östman: <i>PDGF-dependent cancer fibroblasts and pericytes as novel cancer drug targets</i>
1725		Laura Rosano: <i><math>\beta</math>-Arrestin-1 as messenger of endothelin A receptor-driven <math>\beta</math>-catenin signaling pathway and epithelial to mesenchymal transition: implication for an effective combined therapy in ovarian cancer</i>
1745		Hector Peinado: <i>LOXL2 as a player in epidermal homeostasis and early marker of squamous cell carcinomas</i>
1805		<b>POSTER SESSION / TEMTIA Annual General Meeting</b>
1900		<i>Conference Dinner</i> Folwark Zalesie
<b>Wednesday, September 12</b>		
	<b>Physiological aspects of EMT.</b> Chairperson Aristidis Moustakas	Larisha Palace, Conference Room
0830	<b>Session IX</b> (Invited)	Pierre Savagner: <i>Slug regulates early epithelial differentiation by maintaining a metastable phenotype</i>
0905	(Invited)	Raghu Kalluri: <i>The Effects of BMP-7 on EndMT and Cardiac Fibrosis</i>
0940		Jiri Zavadii: <i>Gene Regulation Network Analysis Suggests Epigenetic Mechanism for Silencing of TIMP3 in EMT</i>
1000	<i>Coffee &amp; Posters</i>	Conference Room Foyer
1030	<b>Debate</b>	EMT in development and physiology: Michael Klymkowsky, Raghu Kalluri and Angela Nieto
1130	<b>Session X</b> (Invited)	Raymond Runyan: <i>Regulation of EMT in the atrioventricular canal of the developing heart</i>
1205	(Invited)	Claire Sharpe: <i>Delineation of GTPase pathways in EMT: A role for every Rho?</i>
1225		Andrew Garrod: <i>New concepts in desmosomal adhesion and their implications for EMT</i>
1245	<i>Lunch/Poster Viewing</i>	Conference Room Foyer
	<b>Cell signalling and EMT (II).</b> Chairperson Paolo Comoglio	
1415	<b>Session XI</b> (Invited)	Lionel Larue: <i>The role of IGF/AKT pathways in the induction of epithelium mesenchyme transition</i>
1450	(Invited)	Antonio García de Herreros: <i>The adhesion junction protein E-cadherin plays a central role in the process of epithelial morphogenesis</i>
1525	(Invited)	Geert Berx: <i>Functional analysis of EMT-inducing transcription factors in invasion and metastasis</i>
1600	Concluding remarks, and Invitation to the 4th EMT Meeting in 2009	

10 September 2007

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8:00 AM

Registration

Conference Room Foyer

8:30 AM - 10:30 AM

**EMT and Development: Session I** Larischa Palace, Conference Room  
Chair: Raghu Kalluri

- 8:30 AM 1 **THE INAUGURAL ELIZABETH HAY LECTURE:  
Mechanochemistry of cell adhesion in epithelial cell plasticity**  
**Thiery, JP\***, Dufour, S#, Chu, HS\*, Pincet, F§, Eder, O#, Thomas, W#, Martinez-Rico, C# and Nassoy, P+  
*\*Systems Biology, Institute of Molecular and Cell Biology, 61 Biopolis Drive (Proteos), Singapore, Singapore 138673, Singapore; #CNRS Institut Curie Paris; § Ecole Normale Supérieure Paris*
- 9:35 AM 2 **EMT-related and EMT-independent regulation of cell adhesion and movement by Snail**  
**Nieto, MA**  
*Instituto de Neurociencias de Alicante, CSIC-UMH Apartado 18. San Juan de Alicante. Spain.  
E-mail: anieto@umh.es*
- 10:10 AM 3 **The polarity regulators Scribble and DLG control epithelial migration during development, wound healing and tumourigenesis**  
**Lukas E. Dow\***, Jeffrey S. Kauffman\*, Ryan Galea\*, Jacinta Caddy<sup>^</sup>, Stephen M. Jane<sup>^</sup>, Sarah M. Russell# and Patrick O. Humbert\*  
*\*Cell Cycle & Cancer Genetics Laboratory, #Immune Signalling Laboratory, Peter MacCallum Cancer Centre, Melbourne, Australia. <sup>^</sup>Rotary Bone Marrow Research Laboratories, Parkville, Australia.*

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10:30 AM

Coffee

Conference Room Foyer, Larischa Palace

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11:00 AM - 12:30 PM

**EMT and Development: Session II** Larischa Palace, Conference Room  
Chair: Raghu Kalluri

- 11:00 AM 4 **Gene regulatory network states that control EMT.**  
**McClay, D**  
*Developmental Biology, Duke University, Box 90338, Durham, NC 27708, USA;*
- 11:35 AM 5 **Conserved and novel features of the NFkB-Slug-Twist network involved in Xenopus mesoderm formation and EMT**  
**Klymkowsky, M**  
*Molecular, Cellular & Developmental Biology, University of Colorado, Boulder, 347 UCB, Boulder, CO 80309-0347, USA*
- 12:10 PM 6 **Photoactivatable GFP resolves drosophila mesoderm migration behaviour**  
**Murray, MJ and Saint, R**  
*Centre for the Molecular Genetics of Development, Research School of Biological Sciences, The Australian National University, Building 46, Acton, Canberra, ACT 0200, Australia*

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12:30 PM - 2:00 PM

**Lunch and Poster viewing** Wierzynek Restaurant and Conference Foyer

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2:00 PM - 3:50 PM

**Cell signalling and EMT (I): Session III**

*Larisha Palace, Conference Room*

*Chair: Angela Nieto*

- 2:00 PM 68 **Smad proteins of the TGF  $\beta$  pathway instruct a network of transcription factors for the establishment of EMT**  
Moustakas, A, Thuault, S, Vanlandewijck, M, Tan, E-J, Raja, E and Heldin, C-H  
*Ludwig Institute for Cancer Research, Box 595, BioMedical Center, Uppsala University, Uppsala SE-751 24, Sweden.*
- 2:35 PM 69 **Searching for novel regulators of EMT**  
Wu, MY, Daly, A and Hill, CS  
*Developmental Signalling Laboratory, London Research Institute, Lincoln's Inn Fields Laboratories, 44 Lincoln's Inn Fields, London, WC2A 3PX, UK*
- 3:10 PM 70 **PDGF Links TGF- $\beta$  Signaling to Nuclear  $\beta$ -Catenin Accumulation in Hepatocellular Carcinoma Progression**  
Fischer, ANM, Fuchs, E, Mikula, M, Huber, W, \*Beug, H and Mikulits, W  
*Department of Medicine I, Division: Institute of Cancer Research, Medical University of Vienna, Borschke-Gasse 8A, Vienna, 1090, Austria; Research Institute of Molecular Pathology, Dr. Bohr-Gasse 7, A-1030 Vienna, Austria*
- 3:30 PM 71 **The Transcription Factor Snail Represses Crumbs3 Expression and Disrupts Apico-Basal Polarity Complexes**  
Whiteman, EL, Liu, Chia-Jen, Fearon, ER and Margolis, B  
*Internal Medicine, University of Michigan, 109 Zina Pitcher Place, 1698 Biomedical Science Research Building, Ann Arbor, MI 48105, USA*

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3:50 PM

**Afternoon Tea**

*Conference Room Foyer, Larisha Palace*

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4:30 PM - 6:00 PM

**Cell signalling and EMT (I): Session IV**

*Larisha Palace, Conference Room*

*Chair: Angela Nieto*

- 4:30 PM 72 **The Par/Tiam1 polarity complex controls epithelial and mesenchymal properties of epithelial cells.**  
Collard, J  
*Division of Cell Biology, The Netherlands Cancer Institute, Plesmanlaan 121, Amsterdam, 1066 CX, The Netherlands*
- 5:05 PM 73 **Regulation of EMT and Wnt signaling by Integrin-Linked Kinase**  
Dedhar, S  
*Biochemistry and Molecular Biology, University of British Columbia/BC Cancer Research Centre, 675 West 10th Avenue, Vancouver, BC V5Z1L3, Canada;*
- 5:40 PM 74 **EMT and Oncogenic Signaling by TGF- $\beta$  in Mammary Epithelial Cells**  
Schiemann, WP  
*Department of Pharmacology, MS8303, University of Colorado Health Sciences Center, RCI South Tower, Room L18-6110, 12801 East 17th Avenue, PO Box 6511, Aurora, CO 80045, USA*

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6:00 PM

**POSTERS / EpiPlast Carcinoma AGM**

*Larisha Palace, Conference Room*

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7:00 PM

**Welcome Reception**

*Main Hall, Larisha Palace*

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## 11 September 2007

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8:30 AM - 10:00 AM

**EMT and Cancer (I): Session V** Larischa Palace, Conference Room  
Chair: Antonio Garcia de Herreros

- 8:30 AM 75 **Invasive growth: a MET-driven genetic programme for cancer and stem cells**  
**Comoglio, P**  
*IRCC, Institute for Cancer Research and Treatment, University of Torino School of Medicine, Candiolo, Torino, 10060, Italy*
- 9:05 AM 76 **EMT in the neural crest model: Does functional manipulation of adhesion and cytoskeletal systems reset the sensitivity to pro-EMT growth factors?**  
**Don Newgreen<sup>1</sup>, Dong Zhang<sup>1</sup>, Honor Hugo<sup>1</sup>, Peter Farlie<sup>2</sup>, Sonja McKeown<sup>1</sup>**  
*<sup>1</sup>Embryology Laboratory, and <sup>2</sup>Craniofacial Sciences Laboratory, Murdoch Childrens Research Institute, The Royal Children's Hospital, Parkville, 3052, Australia.*
- 9:40 AM 77 **The microRNA-200 family regulates the E-cadherin repressors, ZEB1/deltaEF-1 and SIP1/ZEB2 and EMT**  
**Philip A Gregory, Andrew G Bert, Emily L Paterson, Anna Tsykin, Mathew A Vadas, Yeessim Khew-Goodall and Gregory J Goodall**  
*Hanson Institute, Institute of Medical and Veterinary Science, Adelaide, South Australia, Australia, 5000*

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10:00 AM

**Coffee**

*Conference Room Foyer, Larischa Palace*

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10:30 AM - 11:30 AM

**Debate: EMT-MET Cycles in tumours***Larischa Palace, Conference Room*  
**Amparo Cano, Gerhard Christofori, Pierre Savagner and Erik (Rik) Thompson.**

11:30 AM - 12:40 PM

**EMT and Cancer (I): Session VI** Larischa Palace, Conference Room  
Chair: Antonio Garcia de Herreros

- 11:30 AM 78 **Distinct mechanisms of tumor invasion and metastasis**  
**Lehembre, F, Wicki, A, Yilmaz, M, Grotgut, S, Kren, A, Fantozzi, A, Achermann, C and Christofori, G**  
*Institute of Biochemistry and Genetics, University of Basel, Mattenstrasse 28, Basel, CH - 4058, Switzerland*
- 12:05 PM 79 **Gene signatures defining Epithelial Mesenchymal Transition and stem cell lineage are a defining characteristic of human breast cancer subtypes**  
**Blick, T<sup>#</sup>, Widodo, E. <sup>^</sup>,<sup>§</sup>, Waltham, M<sup>#</sup>,<sup>^</sup>, Hugo, H<sup>\*</sup>, Newgreen, DF<sup>\*</sup>, Lenburg, ME<sup>##</sup> and Thompson, EW<sup>#</sup>,<sup>^</sup>**  
*<sup>#</sup>St. Vincent's Institute and <sup>^</sup>University of Melbourne Dept. of Surgery, St. Vincent's Hospital, Fitzroy, 3065, Australia; <sup>§</sup> Faculty of Medicine, Brawijaya University, East Java 65141, Indonesia; <sup>\*</sup>Embryology Laboratory, Murdoch Children's Research Institute, The Royal Children's Hospital, Parkville, 3052, Australia; <sup>##</sup>Department of Genetics and Genomics, Boston University School of Medicine, Boston, MA 02118, USA; <sup>\*\*</sup>Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94270, USA*

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12:40 PM - 2:15 PM

**Lunch/ and Poster viewing** *Wierzynek Restaurant and Conference Foyer*

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2:15 PM - 3:45 PM

**EMT and Cancer (II): Session VII** *Larisch Palace, Conference Room*  
Chair: John Collard

- 2:15 PM 80 **Understanding the role of Snail factors and other EMT regulators in tumour progression**  
Cano, A  
*Biochemistry, Universidad Autonoma de Madrid, Instituto de Investigaciones Biomedicas "Alberto Sols" CSIC-UAM, Arzobispo Morcillo, 4, Madrid, 28029, Spain*
- 2:50 PM 81 **Malignant progression in colorectal cancer: EMT,  $\beta$ -Catenin & cancer stem cells**  
Brabletz, T  
*Molecular Oncology, Surgery, Univ. of Freiburg, Hugstetter Str. 55, Freiburg, 79095, Germany*
- 3:25 PM 82 **The transcription factor ZEB1 promotes tumor progression by repressing master regulators of epithelial differentiation**  
Aigner, A, Descovich, L, Sultan, A, Brabletz, T\* and Foisner, R and Eger, A  
*Max F. Perutz Laboratories, Department of Medical Biochemistry, Medical University Vienna, Dr. Bohrgasse 9, Vienna, Vienna A-1030, Austria; \*Department of Visceral and General Surgery, Albert-Ludwigs-University, Freiburg, Germany*

3:45 PM

**Afternoon Tea**

*Conference Room Foyer, Larisch Palace*

4:15 PM - 6:05 PM

**EMT and Cancer (II): Session VIII** *Larisch Palace, Conference Room*  
Chair: John Collard

- 4:15 PM 83 **Cell motility at the invasive front of tumors: The role of novel Wnt/ $\beta$ -catenin target genes**  
Ben-Ze'ev, A  
*Molecular Cell Biology, Weizmann Institute of Science, Hertzl Street, Rehovot, 76100, ISRAEL*
- 4:50 PM 84 **PDGF-dependent cancer fibroblasts and pericytes as novel cancer drug targets**  
Östman, A  
*Molecular Oncology and Cancer Therapies, Karolinska Institute, Department of Oncology - Pathology Building, Karolinska University Hospital 171, Stokholm, 76, Sweden*
- 5:25 PM 85  **$\beta$ -Arrestin-1 as messenger of endothelin A receptor-driven  $\beta$ -catenin signaling pathway and epithelial to mesenchymal transition: implication for an effective combined therapy in ovarian cancer**  
\* Rosano', Laura, \* Cianfrocca, Roberta, \* Masi, Stefano, \* Spinella, Francesca, \* Di Castro, Valeriana, \*\*\* Nicotra, Maria Rita, \*\* Natali, Pier Giorgio and \* Bagnato, Anna  
*\*Laboratories of Molecular Pathology and Ultrastructure, and \*\*Immunology, Regina Elena Cancer Institute, via delle Messi D'Oro 156, Rome, 00158, Italy; \*\*\*Molecular Biology and Pathology Institute, National Research Council, 00137 Rome, Italy.*
- 5:45 PM 86 **LOXL2 as a player in epidermal homeostasis and early marker of squamous cell carcinomas**  
Peinado, H., Moreno-Bueno, G, Santos, V., Perez-Gomez, E., Hardisson, D.\*; De Diego J.I.\*, Quintanilla, M., Portillo, F. and Cano, A.  
*Departamento de Bioquímica. Instituto de Investigaciones Biomedicas Alberto Sols Consejo Superior de Investigaciones Científicas-Universidad Autonoma de Madrid. Arturo Duperier, 4, 28029 Madrid. Spain. \*Hospital Universitario de la Paz. 28029 Madrid. Spain*

6:05 PM

**POSTERS / TEMTIA AGM**

*Larisch Palace, Conference Room*

7:00 PM

**Conference Dinner**

*Folwark Zalesie*



2:15 PM

**Cell signalling and EMT (II): Session XI** *Larischa Palace, Conference Room*  
*Chair: Paolo Comoglio*

- 2:15 PM 93 **The role of IGF/AKT pathways in the induction of epithelium mesenchyme transition**  
**Lionel LARUE**  
*Institut Curie, Bat 110, Orsay, Paris, 91405, France.*
- 2:50 PM 94 **The adhesion junction protein E-cadherin plays a central role in the process of epithelial morphogenesis**  
**Garcia de Herreros, A**  
*Unitat de Biologia Cel·lular i Molecular, Institut Municipal d'Investigacio Medica, Universitat Pompeu Fabra, Dr Aiguader 80, 08003, Barcelona, Catalunya 08003, Spain*
- 3:25 PM 95 **Functional analysis of EMT-inducing transcription factors in invasion and metastasis**  
**De Craene, B1,2, Vandewalle, C1,2, Raspé, E 1,2, Seflek Unay, Z1,2, Van Roy, F1,2, Berx, G1,2**  
*Department of Molecular Biomedical Research, VIB and Ghent University, Technologiepark 927, Ghent (Zwijnaarde), 9052, Belgium*

4:00 PM

**Close and Announcement of 2009 Meeting**

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**POSTER PRESENTATIONS**

- 7 **The role of deltaEF1 family proteins, in the regulation of TGF-beta-induced epithelial-mesenchymal transdifferentiation**  
**Saitoh, M, Horiguchi, K, Shirakihara, T and Miyazono, K**  
*Molecular Pathology, Tokyo University, 7-3-1 Hongo, Bunkyo-ku, Tokyo, Tokyo 113-0033, Japan*
- 8 **Activation of NF-kB by Akt upregulates Snail expression and induces epithelium mesenchyme transition.**  
**Julien, S\*, Puig, I\*, Caretti, E\*\*, Bonaventure, J\*, Nelles, L\*\*\*, van Roy, F\*\*\*\*, Dargemont, C\*\*\*\*\*, Garcia de Herreros, A\*\*\*\*\*, Bellacosa, A\*\* and Larue, L\***  
*Developmental genetics of melanocytes, Institut Curie, Batiment 110-Centre Universitaire, Orsay, 91405, France*
- 9 **Snail1 and Snail2 dependent epithelial-mesenchymal transition in breast carcinoma cells**  
**Hugo, HJ\*, #Kokkinos, MI, \$Ackland, ML, ^Thompson, EW and Newgreen, DF**  
*\* Embryology, Murdoch Childrens Research Institute, Flemington Rd, Parkville, Melbourne, VIC 3052, Australia; #Department of Surgery, Royal Melbourne Hospital, Melbourne, Australia; \$Centre for Cellular and Molecular Biology, School of Biological and Chemical Sciences, Deakin University, Burwood Campus, Burwood, Australia; ^Department of Surgery St. Vincent's Hospital, Melbourne, Victoria, Bernard O'Brien Institute for Microsurgery, Fitzroy, Melbourne, Australia and St. Vincent's Institute of Medical Research, Fitzroy, Melbourne, Victoria*
- 10 **Periphelial blood monocytes induce a phenotypic transition and an increase of a motile activity of human pancreatic carcinoma (HPC-4) cells via secretion of tumour necrosis factor (TNF)-alpha**  
**Bechne, I, Baran, B, Sroka, S, Madeja, Z, Siedlar, M\* and Czyz, J**  
*Department of Cell Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, ul. Gronostajowa 7, Cracow, 30-387, Poland; \*Department of Clinical Immunology, Polish-American Children Hospital, Jagiellonian University Medical College, Cracow, Poland*
- 11 **The role of the Snail family of transcription factors in human carcinomas**  
**Bram De Craene \*, Petra Vermassen \* and Geert Berx \***  
*\* Department for Molecular Biomedical Research, VIB & Department of Molecular Biology, Ghent University, B-9052 Ghent, Belgium.*
- 12 **Genetic alterations in epithelial and stromal cells of ovarian carcinomas**  
**Hanna Tuhkanen 1,2,3, Maarit Anttila 1,3, Veli-Matti Kosma 1,4, Seppo Heinonen 3, Matti Juhola 4, Seppo Helisalmi 5, Vesa Kataja 2 and Arto Mannermaa 1**  
*1 Institute of Clinical Medicine, Pathology and Forensic Medicine 2 Oncology 3 Obstetrics and Gynecology, University of Kuopio and Kuopio University Hospital, 70210 Kuopio, Finland. 4 Department of Pathology, Jyväskylä Central Hospital, 40620 Jyväskylä, Finland. 5 Institute of Clinical Medicine, Neurology, Brain Research Unit, Clinical Research Center / Mediteknia, University of Kuopio, 70210 Kuopio, Finland.*

- 13 **Role of p120ctn in cell migration and invasion**  
**Kuemper, S and Ridley, AJ**  
*Cell Signalling in Invasion and Migration Laboratory, Randall Division of Cell & Molecular Biophysics, King's College London, Guy's Campus, 2nd floor New Hunt's House, London, SE1 1UL, UK*
- 14 **Role of epithelium- mesenchymal transition in tissue fibrosis in asthma**  
**Letuve, S, Maret, M, Grandsaigne, M, Taille, C, Dombret, MC, Aubier, M and Pretolani, M**  
*INSERM U700, Faculte de Medecine Xavier Bichat, and Service de Pneumologie, Hopital Bichat, Paris, France*
- 15 **ERK MAPK and NF-kB activation control peritoneal mesothelial cell EMT**  
**Strippoli R, Benedicto I\*, Perez Lozano ML\*, Lopez Cabrera M\* and Del Pozo MA**  
*Vascular Biology and Inflammation, CNIC Fundacion Centro Nacional de Investigaciones Cardiovasculares Carlos III, Madrid, Spain; \*Unidad de Biologia Molecular, Hospital Universitario de la Princesa, Madrid, Spain*
- 16 **The Protein Tyrosine Phosphatase Pez Regulates TGF-beta, Epithelial-Mesenchymal Transition and Organ Development**  
**Wyatt, L\*^, Wadham, C\*, Crocker, LA\*, Lardelli, M# and Khew-Goodall, Y\*^**  
*\*Department of Human Immunology, Hanson Institute, Institute of Medical and Veterinary Science, Frome Road, Adelaide, SA, 5000, Australia; ^Discipline of Biochemistry and #Discipline of Genetics, School of Molecular and Biomedical Science, The University of Adelaide, Adelaide, SA, 5005, Australia*
- 17 **EGF-induced Epithelial-mesenchymal Transition of Human Ovarian Surface Epithelial Cells: Molecular and Signaling Profiles and Possible Role of Amphiregulin**  
**Huang, RYJ, Ozbun, L, Choi, JH, Salamanca, CM, Pelech, S, Birrer, MJ and Auersperg, N**  
*Dept. Anatomy and Cell Biology, College of Medicine, National Taiwan University, Taipei, Taiwan, Dept. Obstetrics and Gynecology, University of British Columbia, Vancouver, B. C., Canada; Cell and Cancer Biology Branch, NCI, Bethesda, Maryland, USA, Dept. Medicine, University of British Columbia, Vancouver, B.C., Canada, Kinexus Bioinformatics Corp., Vancouver, B.C., Canada.*
- 18 **Role of HMGA2 in TGF-beta signaling and cancer progression**  
**Thuault, S, Tan E-J, Peinado, H\*, Cano, A\*, Heldin, C-H and Moustakas, A**  
*TGF-beta Signaling Group, Ludwig Institute for Cancer Research, Box 595, Biomedical Center, 75124 Uppsala, Sweden. \* Departamento Biologia Molecular del Cancer, Instituto de Investigaciones Biomedicas "Alberto Sols", C/ Arturo Duperier 4, 28029 Madrid, Spain.*
- 19 **Mechanisms of palatal seam EMT by Transforming Growth Factor (TGF) beta 3**  
**Shaheen Ahmed, ChangChih Liu and Ali Nawshad**  
*Department of Oral Biology, University of Nebraska Medical Center, 40th and Holdrege, Lincoln, NE 68583, USA*
- 20 **Downregulation of polarity factor LGL2 at the invasion front of colorectal carcinomas - a region exhibiting high amounts of nuclear b-catenin**  
**Simone Spaderna\*, Otto Schmalhofer\*, Mandy Wahlbuhl#, Dennis Strand∞, Andreas Eger##, Jorgen Behrens§ and Thomas Brabletz\***  
*\*Department of Surgery, University of Freiburg, Hugstetter Str. 55, Freiburg, 79095, Germany; #Dept. of Pathology and §Nikolaus-Fiebiger-Center, University of Erlangen; ∞First Dept. of Internal Medicine, Univ. of Mainz; ##Max F. Perutz Laboratories, Medical University Vienna*
- 21 **Genes associated with metastasis and epithelial-mesenchymal transition (EMT)-like phenotype in human colon cancer cells**  
**Tay, Puei Nam, Tan, Tze Chin, Laban, Mirtha, Leung, Carol Ho-Wing and Hooi, Shing Chuan**  
*Physiology, National University of Singapore, Yong Loo Lin School of Medicine, Blk MD9, 2 Medical Drive, Singapore, Singapore 117597, Singapore*
- 22 **Inhibition of TGFbeta-mediated effects by BMPs across the spectrum of breast cancer progression**  
**Van Overveld, PGM (1), Henriquez, NV (2) and Van der Pluijm, G (1, 2)**  
*(1)Department of Urology, Leiden University Medical Center, Albinusdreef 2, PO Box 9600, 2300 RC, Leiden, The Netherlands; (2) Department of Endocrinology and Metabolic Diseases, Leiden University Medical Center, Albinusdreef 2, PO box 9600, 2300 RC, Leiden, The Netherlands*
- 23 **Functional characterization of Snail2 repression complex**  
**Molina-Ortiz P, McPherson M, Cano A and Portillo F**  
*Universidad Autonoma de Madrid. Dept. Bioquimica, Instituto de Investigaciones Biomedicas "Alberto Sols" (CSIC-UAM), Arturo Duperier 4, Madrid, 28029, Spain*
- 24 **Regulation of transcription factor Snail1 through Phosphorylation.**  
**Matthew MacPherson\*, Ihor Yakomovych#, Serhiy Souchelnytskyi#, Paco Portillo\* and Amparo Cano\***  
*\*Departamento de Bioquimica, Alberto Sols, Universidad Autonoma de Madrid, Calle Arturo Duperier 4, Madrid 28029, Spain; #Institutionen for Onkologi-Patologi, Karolinska institute, Stockholm, Sweden.*

- 25 **New insights in the regulation of E-cadherin and EMT: the role of bHLH factors E2-2 and their relationship with other EMT inducers**  
**Sobrado, VR, Holt H, Moreno-Bueno G, Portillo F and Cano A**  
*Departamento de Bioquímica, Instituto de Investigaciones Biomedicas Alberto Sols (CSIC-UAM), Arturo Duperier 4, (28029)Madrid, Spain*
- 26 **Chronic Allograft Nephropathy: Can TGFbeta-expressing intraepithelial T cells induce EMT?**  
**Pekalski, M, Robertson, H, Rygiel, K, Al-Hamidi, AH, Burt, AD and Kirby, JA**  
*Applied Immunobiology and Transplantation Research Group, Institute of Cellular Medicine, Newcastle University, Framlington Pl., Newcastle upon Tyne, Tyne and Wear NE2 4HH, UK*
- 27 **The Transition of Intrahepatic Biliary Epithelium to Mesenchymal Cells during Chronic Inflammatory Liver Disease.**  
**Rygiel, KA, Robertson, H, Pekalski, M, Burt, AD\*, Jones, DEJ\*\* and Kirby, JA**  
*Institute of Cellular Medicine, Applied Immunobiology and Transplantation Research Group, Newcastle University, Framlington pl, Newcastle upon Tyne, NE2 4HH, England; \*Institute of Cellular Medicine, Applied Immunobiology and Transplantation Research Group, Royal Victoria Infirmary, Department of Pathology, Newcastle University; \*\*Institute of Cellular Medicine, Liver Research Group, Newcastle University*
- 28 **Regulation of EMT during Avian Gastrulation by FGF Signaling and the Receptor Tyrosine Kinase EPHA1.**  
**Hardy, KM\*, Yatskievych, TA\*, Konieczka, JH<sup>^</sup> and Antin, PB\*<sup>^</sup>**  
*Depts of Cell Biology and Anatomy\* and Molecular & Cellular Biology<sup>^</sup>, University of Arizona, Tucson, AZ, USA*
- 29 **MUC1 and MUC4 epithelial mucins: actors of epithelial-mesenchymal transition?**  
**Perrais, M, Aubert, S, Hemon, B, Porchet, N, Leroy, X and Van Seuningen, I**  
*INSERM U837 - Jean-Pierre Aubert Research Center, Batiment Biserte, Place de Verdun, Lille cedex, 59045, France*
- 30 **Oxidative stress drives Epithelial To Mesenchymal Transition (EMT) in human lung epithelial cells via the TGF-beta pathway**  
**Nazarowicz, MR, Parker, S, Borthwick, L, Saretzki, GC, Kirby, JA, Corris, PA and Fisher, AJ**  
*Applied Immunobiology and Transplantation Research Group, Institute of Cellular Medicine, Newcastle University, UK*
- 31 **Snail and Smad act as co-repressors of coxsackie- and adenovirus receptor in TGF-beta-induced EMT**  
**Neve, EPA\*, Vincent, T\*, Kukalev, A#, Moustakas, A, †, Pettersson, RF\* and Fuxe, JB**  
*\*Ludwig Institute for Cancer Research, Stockholm Branch, Nobels vag 3, Stockholm, SE-17177, Sweden; #Department of Cell and Molecular Biology, Karolinska Institute, SE-17177 Stockholm, Sweden; †Ludwig Institute for Cancer Research Uppsala Branch, SE-75124 Uppsala, Sweden; βDepartment of Anatomy and Cardiovascular Research Institute (CVRI), University of California San Francisco, San Francisco, CA 94143, United States*
- 32 **MUC1 and MUC4 epithelial mucins: actors of epithelial-mesenchymal transition?**  
**Perrais, M, Aubert, S, Hémon, B, Porchet, N, Leroy, X and Van Seuningen, I**  
*Inserm U837, Place de Verdun, Lille cedex, 59045, FRANCE*
- 33 **HLH transcription factors E47 and Id1 in E-cadherin promoter repression and EMT**  
**Cubillo, E (1), Peinado, H (1), Moreno-Bueno, G (1), Palacios, J (2) and Cano, A (1)**  
*Instituto de Investigaciones Biomedicas (UAM-CSIC), C/ Arturo Duperier, 4, Madrid, 28029, Spain*
- 34 **Involvement of NF-kB in embryonic vascular remodelling and endothelial-mesenchymal transition process**  
**Arciniegas, E, Carrillo, LM and DeSanctis, JB**  
*Lab Estructura y Biología Celular, Servicio Autonomo Instituto de Biomedicina. Universidad Central de Venezuela. , Esq San Nicolas a esq Providencia, San Jose, Caracas, Dto Capital 1010-A, Venezuela*
- 35 **EMT of hepatocyte through increase of TGF-b1 and 2 contributes to hepatocyte dysfunction and liver fibrosis**  
**Wang, JH, Han, JH, Kim, JH, Kim, MD, Lee, JH and Kim, WH**  
*Department of surgery, Ajou University School of Medicine, San5 Wonchun-Dong , Yeongtong-Gu, Suwon, 443-749, Korea*
- 36 **Dissecting regulatory networks controlling EMT and mesoderm formation by in vivo RNAi**  
**Morkel M, Brouwer-Lehmitz A, Liu F, Leushacke M, Werber M and Herrmann BG**  
*Developmental Biology, Max-Planck-Institute for Molecular Genetics, Ihnestr. 73, Berlin 14195, Germany*

- 37 **The mutual repression between SNAIL2 and SOX3 regulates the EMT at gastrulation**  
Hervé Aclouque, Oscar Ocaña and M. Angela Nieto  
*Instituto de Neurociencias de Alicante CSIC-UMH, 03550 San Juan de Alicante, Spain*
- 38 **Identification of novel genes and signaling networks involved in tumor associated EMT**  
Marc Leushacke\*, Ralf Spoerle\*, Lorenz Neidhardt\*, Anne-Kristin Heninger#, Mirko Theis#,  
Frank Buchholz#, Bernhard G. Herrmann\* and Markus Morkel\*  
*\*Max Planck Institute for Molecular Genetics, Ihnestr. 73, Berlin 14195, Germany; #Max-Planck-Institute of Molecular Cell Biology and Genetics, Pfotenhauerstr. 108, Dresden 01307, Germany*
- 39 **The small GTPase RhoV is an essential regulator of neural crest induction in Xenopus**  
Vignal, E., Guemar, L., de Santa Barbara, P., Donnay, JM., Fort, P. and Faure, S.  
*CRBM, CNRS UMR 5237, 1919 Route de Mende, Montpellier cedex 5, 34293, France*
- 40 **Phosphoproteomic identification of effectors of the breast tumour suppressor Syk**  
Larive, RM, Mascre, G, Poncet, J\*, Urbach, S\*, Jouin, P\*, Mangeat, PH, Coopman, PJ and Bettache, N  
*CRBM, CNRS-UMR5237, IFR 122, Univ. Montpellier II, Place Eugene Bataillon, CC107, Montpellier Cedex05, 34095, France; \*IGF, CNRS-UMR5203, INSERM-U661, IFR 3, Univ. Montpellier I, Univ. Montpellier II, 141 rue de la Cardonille, 34094 Montpellier Cedex 5 FRANCE*
- 41 **Distinct bone morphogenetic protein expression profiles and Smad pathway activation in different phenotypes of experimental canine mammary tumors**  
Helena Wensman\*, Nils-Erik Heldin\*\*, Gunnar Pejler\* and Eva Hellmen\*  
*\*Department of Anatomy, Physiology and Biochemistry, Swedish University of Agricultural Sciences, Uppsala, Sweden; \*\*Department of Genetics and Pathology, Uppsala University, Uppsala, Sweden*
- 43 **Overexpression of thioredoxin reductase 1 inhibits Protein Kinase C-dependent induction of phenotypic transition and motility of HEK-293 cells**  
Sroka, J\*, Antosik, A\*, Czyz, J\*, Nalvarte, I\*\*, Ollson, JM#, Spyrou, G§ and Madeja, Z\*  
*\*Department of Cell Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Gronostajowa 7, Cracow, 30-387, Poland; \*\*Department of Biosciences at Novum, Center for Biotechnology, Karolinska Institute, Huddinge, Sweden; #Department of Molecular Medicine and Surgery, Karolinska Institutet, Karolinska University Hospital, Solna, Stockholm, Sweden; §Foundation for Biomedical Research, Academy of Athens, Athens, Greece*
- 44 **Smad3 is a key mediator of TGFβ-induced transcriptional responses and EMT in mouse mammary epithelial cells**  
Joanna Dzwonek, Lena Preobrazhenska, Ann Schellens, Silvia Cazzola, Maarten van Dinther, Anke Klippel, Peter ten Dijke and Kristin Verschuere  
*Department of Molecular and Developmental Genetics, VIB, K.U.Leuven, Herestraat 49, Box 812, Leuven, 3000, Belgium*
- 45 **Snai1 and Snai2 silencing effectively suppresses tumor growth and invasiveness.**  
Olmeda, D, Peinado, H, Montes, A, Santos, V, Fabra, A and Cano, A  
*Instituto de Investigaciones Biomedicas "Alberto Sols", C/Arturo Duperier 4., Madrid, Madrid 28029, Spain*
- 46 **The E-cadherin-repressed hNanos1 gene induces tumor cell invasion by upregulating MT1-MMP expression**  
Arnaud Bonnomet\*,β, Béatrice Nawrocki-Raby\*, Kristin Strumane§, #, Christine Gillesβ, Geert Berx§, Frans van Roy§, Myriam Polette\* and Philippe Birembaut\*  
*\*INSERM UMRS 514, Laboratory of Histology, IFR 53, CHU Maison Blanche, Reims, France; βLaboratory of Developmental and Tumor Biology, University of Liège, CHU Sart-Tilman, B23, Liège, Belgium; §Department for Molecular Biomedical Research, VIB-Ghent University, Ghent, Belgium; #The Netherlands Cancer Institute, Amsterdam, the Netherlands*
- 47 **Regulation of CXCL8/IL-8 by Zonula Occludens-1 during breast tumor-associated epithelial-to-mesenchymal transitions**  
Mestdagt M\*, Polette M\*\*, Bindels S\*, Hunziker W§, Buendia M#, Birembaut P\*\*, Foidart JM\* and Gilles C\*  
*\*University of Liege, Laboratory of Tumor and Developmental Biology, Liege, 4000, Belgium; \*\*Unité I.N.S.E.R.M. U514, Laboratoire Pol Bouin, I. F. R. 53, C.H.U. Maison Blanche, 51100 Reims, France; §Institute of Molecular and Cell Biology, Epithelial Cell Biology Laboratory, Singapore, Singapore; #Unité I.N.S.E.R.M. U163, Department of Molecular Medicine, Institut Pasteur, 75015 Paris, France.*
- 48 **Positive and Negative Cooperation of p38 MAPK with TGF beta in Epithelial to Mesenchymal Transition (EMT)**  
Wang,B and Zhu, HJ  
*Department of Surgery (RMH), The University of Melbourne, Level 5, Clinical Sciences Building, The Royal Melbourne Hospital, Parkville, Melbourne, VIC 3050, Australia*

- 49 **Epithelial-Mesenchymal Transition Induces Migratory/Invasive Phenotype, While Inhibiting Tumor Cell Growth, To Promote Metastasis in Lung Cancer.**  
**Jun Chen, Rork Kuick, Shalini Anthwal, Gilbert S. Omenn, Theodore J. Standiford and Venkateshwar G. Keshamouni**  
*Internal Medicine/ Pulmonary Division, University of Michigan, 109 Zina Pitcher Place, Ann Arbor, Michigan 48109, USA*
- 50 **Mesenchymal to epithelial transition in the establishment of secondary tumours: insights from a bladder carcinoma progression series**  
**Williams, ED, Chaffer, CL, Blick, T\*, Thompson, EW#**  
*Monash Institute of Medical Research, Monash University, Clayton, Australia; \*Department of Surgery (St. Vincent's Hospital), University of Melbourne, Parkville, Australia; #St. Vincent's Institute, Fitzroy, Australia*
- 51 **PTEN repression: a novel mechanism involved in Snail1 induced resistance to apoptosis**  
**Escriva, MJ, Peiro, S and Garcia de Herreros, A**  
*Unidad de Biología Molecular y Celular; Instituto Municipal de Investigación Médica (IMIM) Barcelona, SPAIN*
- 52 **A natural antisense transcript regulates Zeb2/Sip1 gene expression during Snail1-induced epithelial-mesenchymal transition**  
**Beltran, M\*, Puig, I, #, Alvarez, B\* and Gracia de Herreros, A\***  
*\* Unitat de Recerca Biologia Celular i Molecular, Institut Municipal de Investigacions Mèdiques (IMIM), Dr Aiguader 83, Barcelona, E-08003, SPAIN; #Developmental Genetics of Melanocytes, UMR 146, CNRS Institut Curie, Orsay Cedex, France*
- 53 **Snail1 and 1alpha,25-dihydroxyvitamin D3 have opposite effects on Wnt/beta-catenin signaling and gene expression profile in human colon cancer cells**  
**Larriba, MJ\*, Valle, N\*, Palmer, HG\*, Ordonez-Moran, P\*, Alvarez-Diaz, S\*, Garcia de Herreros, A^, Gonzalez-Sancho, JM\* and Munoz, A\***  
*\*Instituto de Investigaciones Biomédicas, Consejo Superior de Investigaciones Científicas - Universidad Autónoma de Madrid, Madrid, Spain; ^Institut Municipal d'Investigació Mèdica - Universitat Pompeu-Fabra, Barcelona, Spain*
- 54 **The Role of Interleukin-like EMT Inducer (ILEI) in Liver Carcinoma Progression**  
**Lahsnig, C, Mikula, M, Huber, H, Beug, H and Mikulits, W**  
*Department of Medicine I, Division: Institute of Cancer Research, Medical University of Vienna, Borschkegasse 8a, Vienna, Vienna 1090, Austria*
- 55 **Modulation of EMT and TGFbeta signal by extracellular matrix components in vivo and in vitro**  
**Shizuya Saika (1), Toshimitu Uede (2), Shigeyuki Kon (2), Susan R. Rittling (3), David T. Denhardt (3) and Winston Kao (4)**  
*1: Ophthalmology, Wakayama Medical University School of Medicine, 811-1 Kimiidera, Wakayama, 641-0012, Japan; 2: Hokkaido Univ; 3: Rutgers Univ., Piscataway, NJ; 4: Univ. of Cincinnati Med Ctr, Cincinnati, OH.*
- 56 **Study of gene activation mediated by Snail1: requirements of the Fibronectin promoter**  
**Porta de la Riva, M and Agusti, C and Baulida, J**  
*Unitat de Biologia Cel·lular i Molecular, Institut Municipal d'Investigació Mèdica, Doctor Aiguader 88, Barcelona, 08003, Spain*
- 57 **Oral administration of GW788388, a kinase inhibitor of the TGF beta type I and type II receptors, reduces renal fibrosis in db/db mice**  
**Maj Petersen**  
*Molecular Cell Biology, LUMC, Einthovenweg 20, Leiden, 2333 RC, The Netherlands;*
- 58 **Genipin suppresses fibrogenic behaviors of C<sub>2</sub>-TN4 lens epithelial cell line.**  
**Ai Kitano, Osamu Yamanaka, Yuka Okada, Kumi Shirai and Shizuya Saika**  
*Ophthalmology, Wakayama Medical University School of Medicine, 811-1 Kimiidera, Wakayama, 641-0012, Japan*
- 59 **NF-kB mediates matrix metalloproteinase-induced EMT in mammary epithelial cells**  
**Przybylo, JA, Obr, AE and Radisky, DC**  
*Mayo Clinic Jacksonville, 4500 San Pablo Rd. Griffin Bldg., Rm. 351b Jacksonville, FL 32224*
- 60 **Epithelial to Mesenchymal Transition of Mesothelial cells: Pathologic Significance in Peritoneal Dialysis Patients and Potential Therapeutic Interventions**  
**Lopez-Cabrera, M**  
*Unidad de Biología Molecular, Hospital de la Princesa, Diego de Leon, 62, Madrid-28006, Spain*

- 61 **Analysis of the BRCA1 breast tumors identifies a novel oncogene and EMT inducer ~ YAP.**  
**Smolen, GA, Overholtzer, M, Zhang, J, Muir, B, Sgroi, DC, Deng, CX, Brugge, JS, and Haber, DA**  
*Cancer Center, Massachusetts General Hospital, Harvard Medical School, Bld. 149, 13th Street, Charlestown, MA 02139, USA*
- 62 **TRIP6, a novel molecular partner of the MAGI-1 scaffolding molecule, promotes invasiveness**  
**Larissa Kotelevets<sup>1</sup>, Alexey Kruglov<sup>1</sup>, Erik Bruyneel<sup>2</sup>, Marc Bracke<sup>2</sup>, Yolande Di Gioia<sup>1</sup>, Mary C Beckerle<sup>3</sup>, Frans van Roy<sup>4,5</sup> and Eric Chastre<sup>1</sup>**  
<sup>1</sup> INSERM, U773, Centre de Recherche Biomedicale Bichat Beaujon CRB3, BP 416, F-75018, Paris, the Université Paris 7 Denis Diderot, site Bichat, BP 416, F-75018, Paris, France; <sup>2</sup>Laboratory of Experimental Cancerology, Ghent University Hospital, B-9000 Ghent; <sup>3</sup>Huntsman Cancer Institute, Departments of Biology and Oncological Sciences, University of Utah, Salt Lake City, Utah 84102, USA; <sup>4</sup>Department for Molecular Biomedical Research, VIB, B-9052 Ghent, Belgium; <sup>5</sup>Department of Molecular Biology, Ghent University; B-9052 Ghent, Belgium
- 63 **Following Snail Trails: A screen for EMT regulators in vivo**  
**Mary Y.W. Wu and Caroline S. Hill**  
*Developmental Signalling Laboratory, Cancer Research UK, 44 Lincoln's Inn Fields, London, WC2A 3PX, UK*
- 64 **Control of TGFbeta signalling by Smad4 ubiquitination**  
**Mamidi, A, Dupont, S, Morsut, L, Cordenonsi, M and Piccolo, S**  
*Department of Medical Biotechnologies - University of Padua - Italy*
- 65 **ILEI, a novel cytokine essential for tumor progression: does proteolytic processing matter?**  
**Csiszar, A, Goepfert, A, Waerner, T, Alacakaptan, M, Gal, A and Beug, H**  
*Research Institute of Molecular Pathology, Dr. Bohr-Gasse 7, Vienna, 1030, Austria*
- 66 **An inducible system to study gene function in EMT and tumor progression - on the example of ILEI overexpression**  
**Soelch, S, Beug, H and Csiszar, A**  
*Research Institute of Molekular Pathologie, Dr. Bohr Gasse 7, Vienna, Austria 1030, Austria*
- 67 **Chemokines: Key players in function of the Interleukine-like EMT Inducer (ILEI)?**  
**Gal, A, Alacakaptan, A, Csiszar, A and Beug, H**  
*Radiation Oncology and Biology, Radiobiology Research Institute, Churchill Hospital, Headington, Oxford, OX3 7LJ, United Kingdom*
- 96 **Characterization of signal transduction pathways regulating Snail in primary human endometrial carcinoma**  
**Hipp, S, Becker, KF**  
*Insitut fuer Pathologie, Technische Universitaet Muenchen, Trogerstrasse 18, Muenchen, Germany 81675*
- 97 **Ha-ras induced EMT in human colon cells: microarray analysis and transcriptional regulation of vimentin and S100A4 genes**  
**Andreolas, V, Kalogeropoulou, M, Voulgari, A, Madziar, B, Makrodouli, E, Roberts, M, Joyce, T, Pintzas, A**  
*Lab Signal Mediated Gene Expression, Inst Biol Res Biotech, National Hellenic Research Foundation, 48, Vas Constantinou Avenue, Athens, 116 35, Greece*
- 98 **A Snail-Smad transcriptional repressor complex promotes TGF-beta-mediated epithelial-mesenchymal transition**  
**Vincent, T, Neve, EPA, Kukalev, A, Pietras, K, Moustakas, A, Virtanen, I, Pettersson, RF**  
*Ludwig Institute for Cancer Research, Stockholm Branch, Karolinska Institute, Nobels vag 3, Stockholm, 171 77*
- 99 **p53 is a novel regulator of EMT**  
**Laureline Roger, Veronique Gire and Pierre Roux**  
*CRBM, CNRS, Montpellier, France*
- 100 **Control of Rho/ROCK signalling by p53: Consequences on cell migration and invasion.**  
**Gadea Gilles, Roger Laureline, Anguille Christelle, Vinot Stephanie and Roux Pierre**  
*CRBM, CNRS, 1919, route de Mende, Montpellier, France 34293, France*

END OF POSTER PRESENTATIONS

## Abstracts

### 1-INV

#### THE INAUGURAL ELIZABETH D HAY LECTURE: MECHANOCHEMISTRY OF CELL ADHESION IN EPITHELIAL CELL PLASTICITY

**Thiery, JP\***, Dufour, S#, Chu, HS\*, Pincet, F§, Eder, O#, Thomas, W#, Martinez-Rico, C# and Nassoy, P+

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Cell adhesion is a primary process in development which plays a major role in morphogenesis and tissue remodeling. The repertoire of putative cell adhesion molecules is now fairly well established representing almost 3% of the human genome. Distinct adhesion mechanisms have been described to operate during early development. A remarkable increase in intercellular adhesion is observed at the morula stage. The compaction mechanism of blastomeres at the 8 cell stage has major consequences on cell lineage determination of the trophectoderm and the inner cell mass. Compaction of blastomeres critically depends on the strengthening of E-cadherin mediated adhesion, the prototypic type cadherin of epithelial cells. Subsequent developmental events implicate epithelial cell plasticity including epithelial-mesenchymal transition leading to the formation of parietal endoderm and later to mesoderm at the gastrula stage. Modulation of intercellular adhesion is controlled by distinct mechanisms at the transcriptional, translational and post translational levels. For example a shift from type 1 N-cadherin to the type 2 cadherin-7 mediated adhesion is observed during epithelial-mesenchymal transition of avian neural crest cells. To unravel mechanisms controlling the strength of adhesion we used Sarcoma S180 cell line which lacks cadherin-mediated intercellular adhesive properties. The stable transfectants of S180 cells, expressing either N-cadherin or cadherin-7, were analyzed in vivo and in vitro cell motility assays. A dual pipette assay was modified to measure forces required to separate S180 cell doublets expressing different levels of type 1 or type 2-cadherins. The force required to separate E-cadherin expressing doublets was critically depending on the duration of contact and levels of expression. An initial adhesion not exceeding several nanonewtons is reinforced only following cortical actin polymerization. This mechanism is dependent on CDC42 and Rac activation. We shall also discuss the role of the cadherin binding protein P120 in the strengthening of cell adhesion. Type II cadherin-7 or -11 is significantly less adhesive than E or N-cadherin. These results suggest that in vivo, cadherin-7 or -11 mediates transient adhesion, which favors motility, and invasion, while E or N-cadherin mediates stable contacts. We are currently designing approaches to analyze integrin-mediated adhesion on fibronectin-coated substrates and reciprocal cross-talk mechanisms between cadherin and integrin mediated adhesion.

Thiery, J.P. (2002) Epithelial-mesenchymal transitions in tumor progression. *Nature Cancer Review*, 2 : 442-454.

Thiery JP., Sleeman J., (2006) Complex networks orchestrate epithelial-mesenchymal transitions. *Nature Rev. Mol. Cell Biol.* 7: 131-142

### 2-INV

#### EMT-RELATED AND EMT-INDEPENDENT REGULATION OF CELL ADHESION AND MOVEMENT BY SNAIL

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The Snail genes have been implicated in processes that involve profound cell movements. Their most prominent described role is the induction of the epithelial to mesenchymal transition (EMT) during embryonic development and tumour progression. Recent evidences extend the implication of aberrant Snail expression to other pathologies. Interestingly, whereas Snail-induced EMT leads to the acquisition of invasive and migratory properties in embryonic and cancer cells, it leads to the development of fibrosis in non-transformed adult epithelia.

Snail proteins can act not only as EMT inducers, but also as more general regulators of cell adhesion and movement even when they fulfil similar functions. I will discuss two situations that occur during mesoderm formation in different species to illustrate that cell movements triggered by Snail may involve the induction of a full EMT or the downregulation of cell-cell adhesion in an EMT-independent manner. When the latter occurs within a group of cells, the changes in adhesion experienced by Snail-expressing cells can affect the behaviour of Snail-negative neighbouring cells. This complex behaviour may have important implications both during embryonic development and cancer progression.

### 3-SYMP

#### THE POLARITY REGULATORS SCRIBBLE AND DLG CONTROL EPITHELIAL MIGRATION DURING DEVELOPMENT, WOUND HEALING AND TUMOURIGENESIS

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Loss of apico-basal polarity is an integral step in the process of epithelial-to-mesenchymal transition (EMT) and is one of the earliest hallmarks of epithelial neoplasia. Loss of apico-basal polarity leads to the disruption of junctional complexes and aberrant communication between the epithelial cell and its microenvironment. Genetic screens in *Drosophila* have identified scribble, discs large (dlg) and lethal giant larvae (lgl) as key epithelial polarity regulators with mutation in any of these genes resulting in loss of polarity, overproliferation and multilayering of epithelial cells leading to 3D-tumorous overgrowth, and in the presence of activated Ras, invasion and metastasis. Evidence from cancer patients suggests that Scribble and Dlg could act as a tumour suppressor in some epithelial cancers with low levels of Scribble or Dlg1 (one of four human Dlg homologues) correlating with increased tumour invasiveness and malignancy. We have undertaken a detailed functional analysis of Scribble and Dlg1 in mammalian epithelial cells and mice mutant for Scribble and Dlg1. We have uncovered a critical role for mammalian Scribble and Dlg in the regulation of epithelial polarity required for directed migration during development and wound healing in vivo. In addition, we show that loss of human Scribble together with activated Ras leads to increased invasion and tumourigenesis similar to what is seen in *Drosophila*. Therefore, mammalian Scribble and Dlg can act to promote or inhibit migration dependent on the cellular context. In addition, we provide the first piece of functional evidence that mammalian Scribble and associated proteins have tumour suppressive activity in vivo. We propose that Scribble, Dlg and other polarity regulators are key signalling molecules involved in a new pathway regulating epithelial tumour progression in mammals.

## 4-INV

## GENE REGULATORY NETWORK STATES THAT CONTROL EMT.

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## 5-INV

## CONSERVED AND NOVEL FEATURES OF THE NFkB-SLUG-TWIST NETWORK INVOLVED IN XENOPUS MESODERM FORMATION AND EMT

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First identified as part of the Dorsal (NFkB)-Snail-Twist network required for mesoderm formation in *Drosophila*, the Snail/Slug and Twist proteins play a key role in the regulation of epithelial-mesenchymal transition in mammalian systems. We will discuss evidence that an NFkB-Slug/Snail-Twist network is active in the vertebrate *Xenopus laevis* embryo. Morpholino-based loss of function studies indicate that Twist, like Slug, is required for mesoderm and neural crest formation and that Slug can rescue at least some of the effects of Twist loss of function. Using hormone-regulated forms of Slug, Twist, and RelA proteins, as well as wild type and dominant negative form of MyD88 (supplied by Ralph Rupp), which acts downstream of Toll-like receptors to activate NFkB, we find i) that Slug, Snail, and Twist are direct targets of NFkB regulation, ii) that Slug regulate NFkB expression and activity through a MyD88 dependent pathway, iii) that both canonical and non-canonical Wnts can negatively regulate NFkB activity, iv) that Slug directly regulates levels of mRNAs encoding specific Wnt inhibitors, and v) that a number of Wnt inhibitors negatively regulate NFkB activity. The vertebrate NFkB-Slug/Snail-Twist loop yet another example of an evolutionary conserve circuit that plays analogous roles in the early development of two quite distinct organisms. *Project supported in part by the American Heart Association.*

## 6-SYMP

## PHOTOACTIVATABLE GFP RESOLVES DROSOPHILA MESODERM MIGRATION BEHAVIOUR

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Mesoderm migration is a pivotal event in the early embryonic development of animals. One of the best studied examples occurs during *Drosophila* gastrulation. Here, mesodermal cells invaginate, undergo an epithelial to mesenchymal transition (EMT), and spread out dorsally over the inner surface of the ectoderm. Although several genes required for spreading have been identified, our inability to visualise mesodermal cells in living embryos has left us to speculate about the cell rearrangements involved. Several mechanisms such as chemotaxis towards a dorsally expressed attractant, differential affinity between mesodermal cells and the ectoderm and convergent extension have been proposed. Here we resolve the behaviour of *Drosophila* mesodermal cells in live embryos using photoactivatable-GFP fused to alpha-tubulin (PAGFP-Tub). By expressing PAGFP-Tub ubiquitously in early embryos, we can photoactivate presumptive mesodermal cells prior to gastrulation, and then observe fluorescent mesodermal cells migrating over non-fluorescent ectodermal cells. We show that the outer-most (outer) cells, which are in contact with the ectoderm, migrate dorsolaterally as a group, but can be "leap-frogged" by more internal (inner) cells. Using laser-photoactivation of individual cells, we then show that inner cells adjacent to the centre of the furrow migrate dorsolaterally away from the midline to reach dorsal positions, while cells at the centre of the furrow disperse randomly across the mesoderm, before intercalating with outer cells. Photoactivated cells can survive fixation, allowing us to immunostain embryos for Twist and thereby determine the final position of labelled cells in the context of the entire mesoderm. Our results suggest that chemotactic movement and differential affinity are the primary drivers of mesodermal cell spreading. With these characterisations in hand we can now begin to analyse the behaviour of cells in embryos mutant for genes involved in spreading.

## Abstracts

7-POS

### THE ROLE OF DELTAEF1 FAMILY PROTEINS, IN THE REGULATION OF TGF-BETA-INDUCED EPITHELIAL-MESENCHYMAL TRANSDIFFERENTIATION

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Epithelial-mesenchymal transdifferentiation (EMT) is a critical morphogenic event which occurs during embryonic development and during the progression of various epithelial tumors. EMT can be induced by transforming growth factor (TGF)- $\beta$  in mouse NMuMG mammary epithelial cells. Here, we demonstrate central roles of transcription factors including E47, Snail, SIP1 and ZEB1 in TGF- $\beta$ -induced EMT. Epithelial cells ectopically expressing SIP1 or ZEB1 suppressed E-cadherin expression but not affected several mesenchymal markers such as fibronectin and N-cadherin, whereas Snail did not appear to enable the cells to induce EMT. In addition, overexpression of E47 also suppressed E-cadherin expression through induction of SIP1 and ZEB1 genes. In contrast, cells transfected with siRNAs against both SIP1 and ZEB1 inhibited TGF- $\beta$ - and E47-induced E-cadherin suppression without any effects on mesenchymal markers, and partially inhibited motility induced by TGF- $\beta$ . Taken together these results suggest that  $\delta$ EF1 family transcription factors, SIP1 and ZEB1, are essential for TGF- $\beta$ -induced EMT, especially regulation of E-cadherin.

8-POS

### ACTIVATION OF NF-KB BY AKT UPREGULATES SNAIL EXPRESSION AND INDUCES EPITHELIUM MESENCHYME TRANSITION.

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Carcinoma progression is associated with the loss of epithelial features, and the acquisition of mesenchymal characteristics and invasive properties by tumour cells. The loss of cell-cell contacts may be the first step of the epithelium mesenchyme transition (EMT) and involves the functional inactivation of the cell-cell adhesion molecule E-cadherin. Repression of E-cadherin expression by the transcription factor Snail is a central event during the loss of epithelial phenotype. Akt kinase activation is frequent in human carcinomas, and Akt regulates various cellular mechanisms including EMT. Here, we show that Snail activation and consequent repression of E-cadherin may depend on AKT-mediated nuclear factor-kB (NF-kB) activation, and that NF-kB induces Snail expression. Expression of NF-kB subunit p65 is sufficient for EMT induction, validating this signalling module during EMT. NF-kB pathway activation is associated with tumour progression and metastasis of several human tumours types; E-cadherin acts as a metastasis suppressor protein. Thus, this signalling and transcriptional network linking AKT, NF-kB, Snail and E-cadherin during EMT is a potential target for antimetastatic therapeutics.

9-POS

### SNAIL1 AND SNAIL2 DEPENDENT EPITHELIAL-MESENCHYMAL TRANSITION IN BREAST CARCINOMA CELLS

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E-cadherin downregulation is commonly associated with the metastasis of breast carcinoma cells. It is essential for epithelial-mesenchymal transition (EMT) and is restored in mesenchymal-epithelial transition (MET) as cancer cells differentiate at secondary tumor sites. We have studied the mechanism of EMT in the mesenchymal breast carcinoma cell line PMC42ET and its epithelial counterpart (PMC42LA). Each line displayed typical morphology, gene expression and immunohistochemistry with regard to epithelial (E-cadherin, occludin, claudin-1, mucin-1, with membranous beta and gamma-catenin) and mesenchymal genes (vimentin, fibronectin, MMP-2, N-cadherin, Snail1, Snail2, Zeb1/delta EF1) hence these were used as EMT markers in our investigation. Of all the known E-cadherin repressor genes, PMC42ET cells expressed Snail1 and Snail2 and the Snail1 downstream gene Zeb1/deltaEF1 higher than PMC42LA, therefore Snail1 and 2 appear to be drivers of endogenous EMT in these cells. Snail1 is predominantly upregulated in invasive breast carcinoma, where it plays a role in orchestrating EMT (Blanco et al., 2002, Come et al., 2004, Elloul et al., 2005) however Snail2 may also play a role (Hajra et al., 2002). In this work we investigate the effectiveness of Snail1 and Snail2 in inducing EMT in PMC42LA through exogenous expression, and by siRNA knockdown, we assess their role in maintaining EMT in PMC42ET. Both induced EMT in PMC42LA to varying degrees. Snail1 and Snail2 knockdown pushed PMC42ET cells towards MET, where Snail1 and Snail2 mediated E-cadherin repression, the former through the Snail1 target Zeb1/deltaEF1, and both Snail1 and 2 were necessary for vimentin and MMP-2 expression. When PMC42LA were treated for 3d with EGF and/or the actin-disrupting chemical staurosporine (ST), endogenous Snail2 signaling (EGF) shifted early to Snail1 (EGF+ST), producing a greater EMT including latent Zeb1/deltaEF1 E-cadherin repressor expression. ST alone had only a mild effect on EMT, however at 3h, ST dramatically reduced actin stress fibres and focal contact size and distribution with a shift of phosphotyrosine immunostaining from at the cell membrane to focal contacts. These changes did not require new protein synthesis and are consistent with the activation of integrin signalling (Levenberg et al., 1998). These findings suggest ST accelerated EGF-mediated EMT in PMC42LA by inducing Snail1 and Zeb1/delta EF1, possibly via a mechanism involving its effects on cell-ECM adherence and integrin signaling. These results combined with exogenous expression and siRNA knockdown comparison studies of Snail1 and Snail2 support a dominant role for these genes in breast carcinoma EMT.

10-POS

**PERIPHERIAL BLOOD MONOCYTES INDUCE A PHENOTYPIC TRANSITION AND AN INCREASE OF A MOTILE ACTIVITY OF HUMAN PANCREATIC CARCINOMA (HPC-4) CELLS VIA SECRETION OF TUMOUR NECROSIS FACTOR (TNF)-ALPHA**

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Monocytes/macrophages are cells of the mononuclear phagocyte system involved in the host response against cancer. They act as tumour-associated and/or antigen presenting cells but also reveal some cytostatic/cytotoxic anticancer activities, thus their role during the tumorigenesis seems to have a complex nature. It includes the limitation of the cancer cell proliferation at early stages of tumour growth, but also a promotion of the neoangiogenesis and the formation of metastases. The latter may correlate with epithelial-to-mesenchymal transition (EMT) of tumour cells. A secretion of pro-inflammatory cytokines, for example TNF-alpha, by activated monocytes may play a role in this process. In the present study, we focused on the effect of human peripheral blood monocytes on the morphology and motility of human pancreatic carcinoma (HPC-4) cells. Using an experimental model based on the co-cultures of both cell types, we demonstrate that tumour cells react to the prolonged presence of monocytes with an increased cell motility correlated with a transition from epithelial to mesenchymal morphology. A corresponding effect was observed in the presence of exogenous TNF-alpha. This result indicates that tumour cell-induced TNF-alpha production by monocytes is involved in the phenotypic transition of pancreatic carcinoma cells. Thus, the interaction between tumour and immune cells, possibly dependent on the exchange of humoral factors, enhances the invasiveness of neoplastic cells. Therefore, TNF-alpha-induced EMT in cancer cell populations may be crucial for the formation of metastases by some tumours.

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11-POS

**THE ROLE OF THE SNAIL FAMILY OF TRANSCRIPTION FACTORS IN HUMAN CARCINOMAS**

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Malignancy of carcinoma cells is characterized by loss of both cell-cell adhesion and cellular differentiation. The epithelial cell-cell adhesion protein E-cadherin is a genuine tumor suppressor as well as an established invasion suppressor. Transcriptional downregulation of E-cadherin in various epithelial tumors appears to be an important event during tumorigenesis. A variety of transcriptional regulators, like Snail, ZEB and some basic helix-loop-helix (bHLH) factors have been identified as potent repressors of E-cadherin. Furthermore these factors are able to drive epithelial mesenchymal transitions (EMT), through which epithelial cells lose their polarity and are converted to a mesenchymal phenotype.

Snail proteins are expressed during embryonic development in mesoderm-derived tissues and their expression correlates with loss of adhesion and induction of cell migration (De Craene et al., 2005a). Likewise, induction of hSnail and hSlug in colorectal cancer cells in vitro resulted in an EMT-like process, coinciding with a dramatic change in morphology. Induced cells showed loss of intercellular adhesion coinciding with induction of invasiveness. Comparative differential gene expression analysis using cDNA microarrays was performed for the different model cell systems (De Craene et al. 2005b). It seems that both hSnail and hSlug use a similar strategy to tackle the epithelial differentiation program. Immediately after induction, the repression of a cohort of genes is initiated (all of which implicated in adhesion, differentiation, cytoskeleton, actin binding, motility/migration) resulting in the loss of the epithelial phenotype. Although Snail family members have been described as transcriptional repressors, there are also upregulated genes like SPARC, CYR61 and TNC coding for secreted macromolecules that interact with cell-surface receptors, ECM, and/or growth factors and proteases. Interestingly, besides commonly regulated genes, the gene expression profiles also indicate distinct groups of genes specifically modulated by only one of the Snail family members. Current identification and further characterization of possible target genes both in vitro (inducible overexpression, invasion, migration & 3D-culture) and in vivo (transgenic mouse models, chemical carcinogenesis) may explain in more detail the role of the different transcriptional repressors in EMT and invasion by cancer cells.

References:

De Craene B, van Roy F, Berx G: Unraveling signalling cascades for the Snail family of transcription factors. *Cell Signal* 2005, 17:535-547.

De Craene B, Gilbert B, Stove C, Bruyneel E, van Roy F, Berx G: The transcription factor snail induces tumor cell invasion through modulation of the epithelial cell differentiation program. *Cancer Res* 2005, 65:6237-6244.

12-POS

**GENETIC ALTERATIONS IN EPITHELIAL AND STROMAL CELLS OF OVARIAN CARCINOMAS**

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Recent studies have highlighted the importance of epithelial-stromal interactions during tumorigenesis. We have previously detected allelic imbalance on chromosome 3p21 in epithelial and stromal cells of ovarian tumours. The objective of this study was to investigate the extent of gene dosage alterations in stromal cells of epithelial ovarian carcinomas.

Thirteen stromal and 24 epithelial samples were microdissected from ovarian carcinomas. Genetic alterations from these samples were studied by analysing the gene dosage of 110 genes across human chromosomes using Multiplex Ligation-dependent Probe Amplification technique.

DNA copy number alterations were detected throughout the chromosomes of both epithelial and stromal cells in ovarian carcinomas [1]. The mean number of quantitatively altered genes per tumour was 23.6 in epithelium and 10.8 in the stroma. More precisely, in the epithelium, the mean number of gains was 13.7 and losses 9.9 and in the stroma 6.6 and 4.2, respectively. Loss of DCC (Deleted in Colorectal Carcinoma) was the most frequent alteration seen in 62 % of all samples. The tumours with high number of changes in the epithelium were associated with advanced tumour stage ( $p = 0.035$ ) and with death due to ovarian cancer ( $p = 0.032$ ). Most importantly, similar genetic alterations (gain - gain or loss -loss) in 38 genes were observed in the epithelia - stroma pair of tumours. This is the first study to reveal the broad level of alterations in the coding areas of cancer associated genes in the stromal tissue surrounding a tumour. The very similar genetic alterations in malignant epithelium and stroma suggest that epithelial-mesenchymal transition (EMT) occurs in ovarian cancer. This may have a major impact on ovarian tumorigenesis.

[1] Tuhkanen et al. *Int J Cancer* 2006, 119:1345-53.

## Abstracts

13-POS

### ROLE OF P120CTN IN CELL MIGRATION AND INVASION

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Cell migration is a key process in a variety of biological processes, for example during development, in response to infection and in the transition to metastasis in tumors. p120catenin (p120ctn) is a multifunctional protein that regulates cell adhesion, motility and invasion through its interaction with classical cadherins and Rho GTPases. P120ctn is widely expressed, but can exist in different isoforms and shows a complex pattern of Tyr as well as Ser/Thr phosphorylation. In order to investigate the function of p120ctn in cell migration and invasion we have used an RNA interference (RNAi) approach in two prostate cancer cell lines (DU145 and PC3) with different capacity of invasion. The results show that downregulation of p120ctn disrupts the formation of adherens junctions in DU145 in an E-cadherin-dependent manner. In PC3 cells, which do not usually form junctions due to a lack of E-cadherin, the depletion of p120ctn leads to the downregulation of N-cadherin as well as some morphological changes. In wound healing and random migration assays, p120ctn-depleted DU145 cells migrate faster, whereas p120ctn-depleted PC3 cells migrate slower in comparison to control cells. The proliferation or adhesion of these cells does not seem to be altered when p120ctn is downregulated as determined using adhesion and proliferation assays as well as FACS analysis.

These results suggest that p120ctn is most likely to regulate cell migration via its effect on cadherins.

14-POS

### ROLE OF EPITHELIUM- MESENCHYMAL TRANSITION IN TISSUE FIBROSIS IN ASTHMA

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**Introduction :** Asthma is a chronic inflammatory disease associated with structural remodelling of the bronchial wall that include sub-epithelial basement membrane thickening and tissue accumulation of fibroblasts/myofibroblasts. These features are critically linked to increased airway stiffness and airflow obstruction and are poorly sensitive to current asthma therapies. Epithelium-mesenchymal transition (EMT) has been implicated in tissue fibrogenesis through the production of TGF-beta1, a cytokine which expression is elevated in the airways of asthmatic individuals and correlates with the extent of bronchial sub-epithelial fibrosis. We hypothesized that EMT is involved in tissue fibrosis in asthma. **Methods :** Human primary bronchial epithelial cells were cultured for 30 minutes to 10 days in medium alone or containing 5 ng/ml TGF-beta1, in the absence or in the presence of 1 microM of the corticosteroid dexamethasone, or 200 nM of the beta2-agonist salmeterol. Expression of epithelial and mesenchymal proteins, E-cadherin and vimentin respectively, was studied by real-time PCR, western-blot and immunofluorescent staining coupled to confocal microscopy. We evaluated Smad-3 activation by phosphorylation using western-blot. Bronchial biopsies obtained from subjects with asthma and normal donors were assessed for cytokeratin and vimentin localisation by immunofluorescence. **Results :** Stimulation of bronchial epithelial cells with TGF-beta1 was associated with smad-3 phosphorylation and resulted in down-regulation of E-cadherin and induction of vimentin. This was concomitant with a loss in cell-cell contacts and the appearance of an organised network of vimentin. Co-treatment with dexamethasone or salmeterol did not prevent TGF-beta1-mediated activation of Smad-3 and alterations in epithelial cell phenotype. Cells co-expressing cytokeratin and vimentin were identified in the bronchial mucosa of asthmatics, adjacent to the sub-epithelial basement membrane area. **Conclusion :** Our observations suggest that induction of EMT by TGF-beta1 may be involved in bronchial sub-epithelial fibrogenesis in asthma. The lack of efficiency of available treatments in blocking EMT may be related to progressive fibrosis and the long-term installation of airflow obstruction observed in severe asthma.

15-POS

### ERK MAPK AND NF-KB ACTIVATION CONTROL PERITONEAL MESOTHELIAL CELL EMT

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EMT has been recently demonstrated to take place during fibrotic processes in many organs and tissues, such as kidney, liver, lung and in peritoneum of patients undergoing continuous ambulatory peritoneal dialysis (CAPD). In the latter case, EMT is particularly frequent upon episodes of recurrent peritonitis and hemoperitoneum. EMT of mesothelial cells has been linked to peritoneal fibrosis and ultrafiltration failure. Ultimately, the establishment of EMT is a limiting step in the peritoneal dialysis and significantly reduces the life quality of these patients. The study of the regulation of EMT of mesothelial cells is thus relevant from both basic and clinical perspectives.

In this study we have used both MET-5A cells, a mesothelial cell line, and omentum primary cells, extracted from samples of surgery patients, as cellular models to induce EMT 'in vitro'. In order to mimic the microenvironment of injured peritoneum, we treated both MET-5A and omentum cells with profibrotic and proinflammatory cytokines such as TGF1beta and IL-1alpha. By using biochemical and confocal microscopy approaches, we found that these cells undergo EMT under our experimental conditions. Similarly to epithelial cells from other tissues, mesothelial cells trans-differentiation is characterized by the progressive loss of the epithelial characteristics and by the acquisition of a fibroblastic phenotype with an elongated shape and increased migratory an fibrotic activities. These complex biochemical and morphological alterations suggest an involvement of several intracellular signalling pathways in the genesis and/or maintenance of EMT.

NF-kB is a transcription factor involved in numerous cellular processes, including differentiation, inflammation, proliferation, and apoptosis. In addition, NF-kB has recently been demonstrated to play a major role in the establishment and maintenance of EMT in a model of breast cancer progression. By using pharmacologic inhibitors and retroviral vectors, we studied the role of NF-kB activation pathway in the establishment of EMT in omentum derived mesothelial cells stimulated with IL-1alpha and TGF1beta. We found that NF-kB activation controls both E-Cadherin and Cytokeratin downregulation during EMT. Moreover, we studied upstream activation pathways regulating NF-kB transcriptional activity. By using different approaches we found that ERK MAPK activation controls NF-kB nuclear internalization and transcriptional activity. We then considered the role of ERK MAPK and of NF-kB in the expression of Snail, the best studied component of a family of zinc finger proteins which is directly implicated in E-Cadherin downregulation during EMT. The results prompted us to hypothesize the existence of an ERK-NF-kB-Snail activation pathway that controls EMT in this experimental model. Finally, by studying 'ex vivo' mesothelial cells from peritoneal effluent of patients undergoing continuous ambulatory peritoneal dialysis (CAPD), we analyzed the role of ERK and NF-kB in the persistence of EMT. Blocking ERK-NF-kB activation pathway induced a marked increase of E-Cadherin. Therefore, the inhibition of this signalling pathway could counteract the progressive deterioration of peritoneal dialysis functional parameters which is observed in these patients.

16-POS

**THE PROTEIN TYROSINE PHOSPHATASE PEZ REGULATES TGF-BETA, EPITHELIAL-MESENCHYMAL TRANSITION AND ORGAN DEVELOPMENT**

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TGF-beta is a pleiotropic cytokine with crucial roles in both organogenesis and tissue homeostasis. It can also play opposing roles in cancer progression, acting as a potent tumour suppressor in the early stages of tumour progression via its cytostatic and apoptotic effects, but serving as an inducer of invasion and metastasis at the later stages of carcinogenesis through promotion of TGF-beta mediated epithelial-mesenchymal transition (EMT). Whilst the signalling pathways initiated down-stream of TGF-beta have been well studied, the molecular mechanisms of TGF-beta induction remain poorly defined. Using zebrafish embryogenesis as a model of development, we have found that the protein tyrosine phosphatase Pez is required for the cell-type specific induction of TGF-beta mRNA in the brain and heart of the developing embryo. In wild-type zebrafish embryos, Pez mRNA expression correlates spatially and temporally with TGF-beta3 mRNA induction in the brain and heart. In embryos made hypomorphic for Pez via micro-injection of specific antisense morpholino oligonucleotides, we see specific defects in the cellular architecture of organs in which Pez is normally expressed during embryogenesis, including defective cardiac valve formation and over-proliferation of neural precursors in the ventricular zone of the brain, suggesting a crucial role for Pez in the development of these organs. Furthermore, TGF-beta3 mRNA expression is absent from the developing brain and heart in embryos hypomorphic for Pez, suggesting that Pez expression is required for TGF-beta3 induction in these organs, and that the defects observed may be due to a loss of TGF-beta expression. Exogenous expression of Pez in the epithelial Madin-Darby Canine Kidney (MDCK) cell line resulted in a dramatic cellular transformation from epithelial to mesenchyme-like morphology, reminiscent of the EMT induced by TGF-beta treatment of these cells. This morphological transformation was accompanied by an increase in migratory capacity, transcriptional down-regulation of the epithelial marker E-cadherin and induction of the mesenchymal marker fibronectin. Induction of the three TGF-beta isoforms (TGF-beta1, -beta2, -beta3), as well as the EMT-promoting transcription factors Snail, Slug, Zeb1 and Zeb2 were also induced by Pez expression. Taken together, our results identify Pez as a novel regulator of TGF-beta signalling during embryogenesis, and suggest that some of the developmental defects observed following knock-down of endogenous Pez expression may be due to an inability to induce developmental EMT. Pez mutations have been identified in colorectal (Wang et al, 2004) and breast cancers (Sjoblom et al 2006), however their role in oncogenesis or cancer progression remains to be elucidated. Our understanding of the role of Pez in regulating TGF-beta signalling in embryogenesis provides valuable insight into its potential role in cancer progression.

References:

Sjoblom et al (2006) Science 314(5797):268-74

Wang et al (2004) Science 304(5674):1164-6

17-POS

**EGF-INDUCED EPITHELIAL-MESENCHYMAL TRANSITION OF HUMAN OVARIAN SURFACE EPITHELIAL CELLS: MOLECULAR AND SIGNALING PROFILES AND POSSIBLE ROLE OF AMPHIREGULIN**

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The ovarian surface epithelium (OSE) is the precursor of the ovarian carcinomas. Epidermal growth factor (EGF) initiates epithelial-mesenchymal transition (EMT), as shown by shape changes, ECM secretion and loss of keratin, in over 95% of cultures of normal human OSE, but not in ovarian carcinoma lines. Here we show that, in contrast to the majority of EGF-responsive OSE cases, named (EMT+)OSE, rare OSE cultures, named EMT(-)OSE, mimic neoplastic cells in their non-responsiveness to EGF, and remain epithelial and keratin positive upon EGF stimulation. Affymetrix U133 Plus 2.0 microarrays were used for gene expression profiling of EMT(-) vs EMT(+) OSE cells before and after EGF treatments. We compared the list of differentially expressed genes in response to EGF-treatment, in EMT(+) OSE and in EMT(-) cultures. Univariate analysis at p less than 0.001 using paired t-test showed differential activation of genes regulating extracellular matrix structure, wound healing, coagulation, cell morphogenesis and cell adhesion. Most importantly, the EGF-like ligand, amphiregulin (AR), stood out by an up to 133-fold increase of expression over basal levels in EMT(+) cells, suggesting that autocrine-loop amplification of EGFR signaling by AR overexpression might play a major role in EGF-induced EMT in OSE cells. AR protein production, as determined by ELISA, significantly increased after EGF (10 ng/ml) incubation in EMT(+) OSE cultures, both in conditioned medium and in cell lysates. We investigated possible roles of AR in wound healing and cell migration by using scratch assays and demonstrated that exogenously added AR (100 ng/ml) significantly increased migration of EMT(+) OSE cells. Interestingly, both EMT(-) and BRCA1-mutated OSE cells had significantly lower AR protein production after EGF incubation and were relatively non-responsive to exogenous AR in wound healing assays. Immortalized OSE cell lines, IOSE385 and IOSE386, were derived by transfecting primary EMT(-) OSE385 and EMT(+) OSE386 cells with SV40-T/t antigen. AR (100 ng/ml) significantly increased migration of IOSE386 cells by 6h compared to IOSE385 cells (p=0.005), and induced the migrating IOSE386 cells to assume a more mesenchymal morphology, indicating a role of AR in EMT. Finally, Kinex™ Antibody Microarray (Kinexus), was used to screen hundreds of proteins for expression and phosphorylation in EMT(-) vs. EMT(+) OSE cells before and after EGF (10 ng/ml) treatment for 30 min. Differential phosphorylation profiles of various signaling molecules such as EGFR (Tyr-1045), Raf1 (Ser-259), and the transcription factor STAT1 (Ser-727) were found. We hypothesize that failure to induce EMT by EGF in OSE might be related to upstream signal attenuation and/or modulation via EGFR and Raf1, while activation of STAT1 might contribute to changes in downstream gene expression in EGF-induced EMT in OSE. Whether the nonresponsiveness to EGF of ovarian cancer cells and of OSE cells reflects common molecular mechanisms requires further investigation. In conclusion, our data suggest an autocrine role of AR in the EMT process of human OSE, and support the hypothesis that lack of the EGF response by (EMT-) OSE may indicate a predisposition to neoplastic progression. Supported by NCIC Canada, and by NIH/NCI USA. \*Ruby YJ. Huang was supported by FIGO/ESRF postdoctoral fellowship program.

## Abstracts

18-POS

### ROLE OF HMGA2 IN TGF-BETA SIGNALING AND CANCER PROGRESSION

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Epithelial-mesenchymal transition (EMT) converts polarized epithelial cells to motile mesenchymal cells. EMT operates during embryonic cell layer movements and tumor cell invasiveness. Transforming growth factor (TGF) beta family members trigger EMT. TGF-beta via intracellular Smad transducers and other signaling pathways that regulate gene expression inhibits epithelial cell proliferation, acting as a tumor suppressor but also promotes carcinoma progression and metastasis. The tumor-promoting actions of TGF-beta are based on its ability to induce EMT, matrix invasiveness and blood vessel intravasation by carcinoma cells, cytostatic effects on surveilling immune cells and proangiogenic effects.

We previously reported that the High Mobility Group A2 (HMGA2) gene is required for TGF-beta to elicit EMT (Thuault S. et al, 2006) by regulating key players of E-cadherin expression regulation and EMT process such as Snail and Twist. HMGA2, together with the highly related HMGA1, constitute the HMGA family of non-histone architectural nuclear factors that bind to AT-rich DNA sequences. Their expression is high during embryonic development and becomes silent in normal adult tissues. However, HMGA factors are abundantly expressed by oncogenically transformed cells or tumors of mesenchymal and epithelial origin. We here investigated the role of HMGA2 in cell migration of breast carcinoma cells that are highly metastatic.

We have additionally investigated the mechanism by which HMGA2 regulates Snail gene expression. Here, we report that HMGA2 is required for full induction of Snail by TGF-beta. In fact, HMGA2 synergizes with the heteromeric Smad3/Smad4 to activate the Snail promoter. The mechanism behind this cooperation involves interaction between these factors leading to an increased binding of Smads to the Snail promoter. The present study demonstrates how HMGA2, downstream of TGF-beta, regulates Snail expression. Whether HMGA2 is required for the transcriptome regulated by TGF-beta is still an open question.

Thuault S., Valcourt U., Petersen M., Manioletti G., Heldin C-H., Moustakas A., Transforming growth factor-beta employs HMGA2 to elicit epithelial to mesenchymal transition, JCB, 2006 Jul 17;174(2):175-83.

19-POS

### MECHANISMS OF PALATAL SEAM EMT BY TRANSFORMING GROWTH FACTOR (TGF) BETA 3

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TGFbeta3 signaling initiates and completes sequential phases of cellular differentiation that is required for complete disintegration of the palatal medial edge seam (MES), that progresses between 14 to 17 embryonic days in the murine system, which is necessary in establishing confluence of the palatal stroma. Understanding the cellular mechanism of palatal MES disintegration in response to TGFbeta3 signaling will result in new approaches to defining the causes of cleft palate and other facial clefts that may result from failure of seam disintegration. We have isolated MES primary cells to study the details of MES disintegration mechanism by TGFbeta3 during palate development using several biochemical and genetic approaches. Our results demonstrate a novel mechanism of MES disintegration where MES, independently yet sequentially, undergoes cell cycle arrest, EMT and apoptosis to generate immaculate palatal confluency during palatogenesis in response to robust TGFbeta3 signaling. The results contribute to a missing fundamental element to our base knowledge of the diverse roles of TGFbeta3 in functional and morphological changes that MES undergo during palatal seam disintegration. We believe that our findings will lead to more effective treatment of facial clefting.

20-POS

### DOWNREGULATION OF POLARITY FACTOR LGL2 AT THE INVASION FRONT OF COLORECTAL CARCINOMAS ~A REGION EXHIBITING HIGH AMOUNTS OF NUCLEAR B-CATENIN

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Overexpression of the transcriptional activator b-catenin, mostly owing to loss-of-function mutations of the adenomatous polyposis coli (APC) tumour suppressor gene, is crucial for the initiation and progression of human colorectal carcinogenesis. Tumor cells at the invasive front exhibit particularly high amounts of nuclear b-catenin. Additionally, this invasive region is characterized by a loss of epithelial differentiation. In many cancer types this is due to activation of an epithelial-to-mesenchymal transition (EMT) program. One hallmark of epithelial differentiation is basal-apical cell polarity and its loss is associated with the unstructured growth of carcinomas. We here show that (1) malignisation and loss of polarity at the invasive front are associated with reduced expression of the cell polarity factor Lgl2, a human homologue of drosophila Lgl, in colorectal and breast cancer. (2) Loss of Lgl2 is mechanistically coupled to EMT, whereby EMT-associated transcriptional repressors bind to and suppress the Lgl2-promoter. (3) ZEB1 is a crucial repressor of Lgl2 expression in colorectal and breast cancer cells. (4) Loss of LGL2 in colorectal cancer cells leads to a loss of cellular polarity and is associated with nuclear accumulation of b-catenin. Our data indicate that loss of cellular polarity in tumors is directly coupled to activation of EMT and further suggest a possible link between EMT and Wnt signaling at the invasive front.

21-POS

**GENES ASSOCIATED WITH METASTASIS AND EPITHELIAL-MESENCHYMAL  
TRANSITION (EMT)-LIKE PHENOTYPE IN HUMAN COLON CANCER CELLS**

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Metastasis is the leading cause of death among colorectal cancer patients. Although the physiological steps in the metastatic cascade are well defined, the underlying molecular mechanisms are poorly understood. One of the mechanisms of metastasis in colorectal tumors appear to involve primarily cells at the invasive front, where the cells exhibit alterations in epithelial structure/function, leading to enhanced motility and invasion: a process known as epithelial-mesenchymal transition (EMT). Therefore, a global transcriptome analysis of whole tumor samples may obscure important genetic changes that occur in the minority of cells within a tumor. We attempt to identify genes associated with colon cancer metastasis by comparing the gene expression profiles of cell lines with different metastatic abilities. To derive metastatic variants, poorly metastatic HCT116 colon cancer cell line was injected into the spleen of nude mice. Metastatic tumors that formed in the liver were harvested to obtain cell lines whose metastatic ability was confirmed by *in vivo* experiments in nude mice and *in vitro* invasion assays. Gene expression profiles of the derived lines were compared against parental HCT116 cell line by microarray analysis. Amongst the derived metastatic lines, the E1 clonal line was highly metastatic and most invasive. This line also exhibited characteristics reminiscent of EMT i.e. a transition from epithelial to fibroblast-like morphology with actin cytoskeleton remodeling and decreased cell-cell adhesion. However, the E1 cells did not show any loss of E-cadherin expression nor gain in vimentin expression, molecular markers associated with the epithelial and mesenchymal phenotype, respectively. E-cadherin and proteins in the E-cadherin-catenin complex (such as beta-catenin, alpha-catenin, p120catenin, alpha-actinin) were localized to the cytoplasm in the E1 cells compared to their presence in the cell-cell junctions of HCT116 cells. Novel candidate genes that have not been previously associated with metastasis were selected from the above microarray analysis to characterize this EMT-like phenotype and its role in metastasis. One of these genes is palladin, an actin-associated phosphoprotein that binds to several actin-binding proteins such as alpha-actinin, VASP, ezrin and profilin. Palladin was most down-regulated in the E1 cell line, both at mRNA and protein levels. As palladin links to the E-cadherin-catenin complex and actin cytoskeleton via interactors such as alpha-actinin and VASP, its down-regulation may be responsible for loss of cell-cell contact as well as actin remodeling observed in E1 cells. Inhibition of the Erk pathway (using chemical inhibitor U0126), one of the pathways known to induce EMT, resulted in a reversion to the epithelial morphology in the E1 cells and an up-regulation of palladin expression. The up-regulation of palladin expression in the U0126-treated E1 cells was associated with a relocalization of cytoplasmic E-cadherin to the cell-cell junction. HCT116 cells transfected with palladin RNAi resulted in a knockdown of palladin expression and a reduction in cell-cell contact. Taken together, the data suggest that palladin is involved in maintaining E-cadherin-catenin-actin interactions in the cytoskeleton that are important in cell-cell adhesion, motility and epithelial cell morphology, and hence play an important role in cell invasion and metastasis. Other genes obtained from the microarray analysis include genes that have been associated with either metastasis, EMT or cytoskeleton remodeling such as BMP4, Jag1, Sox9, DLG5, PDLIM5, TMOD3, Myo6, ABLIM1. The involvement of these genes in the EMT-like phenotype and their potential interactions with the Erk-signaling pathway or with palladin will be explored.

22-POS

**INHIBITION OF TGFβ-MEDIATED EFFECTS BY BMPS ACROSS THE SPECTRUM OF  
BREAST CANCER PROGRESSION**

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Transforming Growth Factor beta (TGFβ) and Bone Morphogenetic Proteins (BMPs) are known to be important effectors in cell differentiation and tumour progression. TGFβ plays a bimodal role in many cancers, ranging from inhibitory in early stages to tumour-enhancing in advanced stages of e.g. breast cancer. To date, the exact role BMPs in tumour progression and metastasis has remained elusive.

The MCF10 panel of cells spans the full spectrum of neoplastic breast cancer progression, including the non-malignant MCF10A1, the premalignant MCF10AT1Kcl2 and the malignant cell lines MCF10CA1h and MCF10CA1a, and has been extensively studied with regard to TGFβ-mediated effects. We have used this panel of cell lines for a systematic study of BMP-mediated signalling effects across this range of cancer progression. In addition, we have studied whether BMPs, in particular BMP 2, 4, 6 and 7, might counteract TGFβ-mediated effects, as has been described for TGFβ and BMP7 in normal kidney epithelium. We measured expression changes on E-cadherin as an epithelial marker and vimentin as a mesenchymal marker induced by TGFβ and BMPs alone or in combination. In parallel, proliferation effects were measured. Finally, we studied SMAD-dependent signalling responses to the various stimuli in the lines using luciferase reporters.

Surprisingly, we observed that TGFβ treatment alone only had a significant effect in one of the malignant cell lines, where it decreased vimentin expression, thus inducing a more epithelial expression pattern in this line. The addition of BMPs usually left the E-cadherin/vimentin ratio unchanged or increased the ratio depending on the specific cell line and BMP, which is consistent with a more epithelial phenotype. When combining TGFβ with BMPs, we observed a similar pattern as with the BMPs alone, indicating that BMP-signalling seemed dominant. TGFβ induced an obvious delay in proliferation in all cell lines. In contrast, BMPs either had no effects or enhanced cell proliferation. In combination with TGFβ, these factors reversed the growth-inhibitory effect of TGFβ. SMAD-dependent signalling reporters for BMPs or TGFβ showed that the cell lines responded to a decreasing degree as they become more malignant.

Although the tested BMPs display differential effects, overall we can conclude that BMPs are indeed able to counteract TGFβ-mediated effects, at least on the expression of two common EMT markers E-cadherin and vimentin and on *in vitro* proliferation. These data are in line with the anti-tumour effects of BMP7 in breast and prostate cancer models of experimental growth and metastasis developed in our laboratory (Buijs et al, Am. J. Pathology 2007, Cancer Research 2007, in press). This suggests that, even in a later stage of disease progression, some BMPs antagonise TGFβ-mediated pro-tumour effects.

## Abstracts

23-POS

### FUNCTIONAL CHARACTERIZATION OF SNAI2 REPRESSION COMPLEX

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The transcription factor Snail2, also called Slug, is a member of the Snail-family of zinc-finger transcription factors that plays a significant role both during development and carcinogenesis, by controlling epithelial-mesenchymal transition (EMT) processes. (1). Snail2 has been also described as a direct transcriptional repression of E-cadherin during EMT being implicated as a prosurvival factor during tumorigenesis (1). Snail1 and Snail2 are highly homologous factors, containing a common N-terminal transrepressor domain (SNAG), a C-terminus DNA binding domain of four (Snail1) or five (Snail2) zinc fingers. Both factors include a divergent central region, which in the case of Snail2 is formed by a unique domain called Slug domain whose function remains to be elucidated (2).

Snail1 repressor activity has been shown to be dependent on SNAG-mediated interaction with a repression complex formed by the corepressor mSin3a and histone deacetylases 1/2 (HDAC1/2)(3). Importantly Snail1 transcription factor is further regulated through phosphorylation by various kinases (4). However, at date little is known about the control of Snail2 repressor activity.

Here, we present interesting data shedding light into the regulation and function of Snail2 as a E-cadherin repressor. For this purpose we have performed ectopic expression of several Snail2 deletion mutants and examined the contribution of the specific domains to protein stability, localization and E-cadherin repressor activity. These data reveal a key role for the `Slug domain' to repress E-cadherin expression. Furthermore, in vivo phosphorylation analysis revealed that specific phosphorylation on Snail2 protein is implicated in Snail2 function as a transcriptional repressor whose functional significance is currently being investigated by analysis of specific point mutations.

1. Peinado H, Olmeda D and Cano A. *Nat Rev Cancer* (2007) 7,415-28
2. Barrallo-Gimeno A, Nieto MA. *Development*. (2005) 132, 3151-61
3. Peinado H, Ballester E, Esteller M, Cano A. *Mol Cell Biol* (2004) 24,306-19
4. Katoh M, Katoh M. *Cancer Biol Ther*. (2006) 5,1059-64

24-POS

### REGULATION OF TRANSCRIPTION FACTOR SNAI1 THROUGH PHOSPHORYLATION.

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Snail1 is one of a family of zinc finger transcription factors demonstrated to repress the cell-cell adhesion molecule, E-cadherin, and acts as a potent inducer of Epithelial to mesenchymal transition (EMT), a process necessary during development for cell migration and with important implications for tumour progression and metastasis. The expression of EMT inducers, such as Snail1, frequently correlates with potency and aggressiveness in tumours.

Snail1 represses E-cadherin through binding to E-box elements within the promoter and recruitment of co-repressors and DNA-modulating enzymes. Such repression correlates with repression of other epithelial markers and expression of those mesenchymal to promote phenotypic changes in the cell.

Post-translational regulation of Snail1 is mediated by the serine/threonine protein kinases GSK3-beta and PAK1, and Lysyl oxidase-like enzyme 2 (LOXL2). Phosphorylation by GSK3-beta at distinct sites within the serine rich regulatory domain (SRD) of Snail1 promotes nuclear export and subsequently proteasomal degradation. Phosphorylation by PAK1 promotes nuclear accumulation and increased Snail1 activity. LOXL2 can modify lysines either side of the SRD promoting Snail1 stability and activity.

Although previous investigations were thorough in their analysis of Snail1/kinase regulation, they lacked in vivo identification of Snail1 phosphorylation sites, relying on mutational analysis and the effects of kinase activity manipulation to pinpoint sites. Therefore, to provide a more complete and accurate view of Snail1 phosphoregulatory sites we have carried out phosphopeptide analysis of in vivo phosphorylated Snail1.

This confirmed the PAK1 phosphorylation site and several, though not all, of the postulated GSK3-beta sites. Furthermore, additional sites were identified including one likely phosphorylated by CK2. We also present evidence of CK2-Snail1 interaction and investigate the effects of such phosphorylation on Snail1 function.

25-POS

### NEW INSIGHTS IN THE REGULATION OF E-CADHERIN AND EMT: THE ROLE OF BHLH FACTORS E2-2 AND THEIR RELATIONSHIP WITH OTHER EMT INDUCERS

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Downregulation of the cell-cell adhesion protein E-cadherin is a key event for epithelial-mesenchymal transition (EMT), which allows cell migration in the developing embryo and tumour invasion. In the last few years, our laboratory and others have isolated and characterized several E-cadherin transcriptional repressors. These include the zinc finger factors Snail (Snai1) and Slug (Snai2), and the bHLH factors E47 and Twist. All are capable of inducing EMT, and are expressed during embryonic development in areas undergoing this transition.

We have identified the class I bHLH transcriptional regulator E2-2 (ITF2/TCF4), in a yeast one-hybrid screen designed to identify repressors interacting with the murine E-cadherin promoter. The E2-2 gene encodes two isoforms, E2-2A and E2-2B (differing in their N-termini), whose specific functions remain unknown.

Here we report that both E2-2A/B repress E-cadherin at the level of transcription, and drive a complete EMT when stably expressed in epithelial MDCK cells. Promoter activity assays indicate a direct action of both isoforms on the E-cadherin promoter, dependent on the integrity of the E-pal element and HDAC activity. RT-PCR analysis shows increased levels of E2-2 transcripts in highly invasive cell lines, and confirms their upregulation in MDCK cells overexpressing Snail, Slug or E47. Furthermore, comparative microarray analysis of MDCK cells expressing E47 or E2-2A/B factors identified common and specific target genes of the bHLH factors. These results support a new role for E2-2A/B in E-cadherin regulation and EMT, and suggest a functional relationship with other E-cadherin repressors.

26-POS

**CHRONIC ALLOGRAFT NEPHROPATHY: CAN TGFβ-EXPRESSING  
INTRAEPITHELIAL T CELLS INDUCE EMT?**

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Tubular epithelial to mesenchymal cell transition (EMT) constitutes a major source of fibroblasts in chronic allograft nephropathy (CAN). In order for this process to contribute to chronic renal injury, the tubular epithelial cells must be spared from cytolysis during periods of acute rejection. Our group has shown previously that intratubular T cells are induced by local TGFβ to express the αE (CD103) β7 integrin for which the only defined ligand is E-cadherin. This study was designed to examine the immunoregulatory phenotype of these intraepithelial T cells and their contribution to graft fibrosis.

Allo-specific T cell lines were stimulated with TGFβ1 and the expression of FOXP3, CD103 and TGFβ were verified by FACS, immunohistochemistry and quantified by real-time RT-PCR. Transplant biopsy sections showing acute and chronic pathology were also labelled by immunohistochemistry to detect antigens associated with EMT and regulatory T cells. Embryonic fibroblasts from TGFβ1<sup>-/-</sup> mice (MFB F11) which secrete SEAP in response to active TGFβ were used to quantify the activity of T cell-surface TGFβ. Co-culture of human tubular epithelial cells and MOLT-16 T cells, which constitutively express CD103 and present LAP-TGFβ complexes, was used as a model for studying the induction of EMT. The MOLT-16 cells were also used in assays to measure the interaction between the αEβ7 integrin and plate bound LAP-TGFβ.

Expression of the regulatory T cell marker FOXP3 (less than 7% +ve resting T cells) and CD103 (less than 10% +ve resting T cells) increased slightly after the activation of allo-specific T cells. Stimulation of activated cells with TGFβ1 increased to 27% and 40% the proportion of cells expressing FOXP3 and CD103 respectively. Stimulation with TGFβ1 also induced autocrine expression of mRNA encoding TGFβ. The co-expression of FOXP3 was observed in 42% of CD103+ T cells. The regulatory potential of cell-sorted CD103+ T cells was confirmed by demonstration of the potential to inhibit primary activation of allo-specific T cells. Importantly, transplant biopsy sections showed CD103 and FOXP3 within intratubular T cells which were colocalised with the EMT marker, S100A4. Interestingly, the αVβ6 integrin was also strongly expressed by tubular epithelial cells in these areas; this integrin is known to activate LAP-TGFβ complexes. Sections with features of CAN showed dense FOXP3 expression in the areas associated with graft fibrosis.

The TGFβ bio-assay revealed that both MOLT-16 and CD103+ T cell lines have the potential to present active TGFβ on their cell surface. Co-culture of these TGFβ-presenting T cells with tubular epithelial cells induced the epithelial cells to develop a mesenchymal morphology with increased expression of the EMT marker, S100A4. Interestingly MOLT-16 T cells were also found to bind exogenous LAP by an αEβ7 integrin-dependent process.

These data suggest that T cells with a regulatory phenotype can persist within renal tubules. Whilst these cells might protect renal tubular epithelial cells from cytolysis during acute rejection, they could also present active TGFβ within the tubules leading to localised EMT and chronic graft failure.

27-POS

**THE TRANSITION OF INTRAHEPATIC BILIARY EPITHELIUM TO MESENCHYMAL  
CELLS DURING CHRONIC INFLAMMATORY LIVER DISEASE.**

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Small intrahepatic bile ducts are lost during chronic inflammatory liver diseases. Recent studies of renal fibrosis have shown that inflammation can induce epithelial to mesenchymal transition (EMT), leading to the loss of tubules as their component epithelial cells develop an invasive fibroblast phenotype. This study was designed to determine whether intrahepatic biliary epithelial cells can undergo a similar transition.

Primary human intrahepatic biliary epithelial cells (HIBEC) and the immortalised human cholangiocyte cell line (MMNK-1) were used for in vitro analysis. HIBEC were cultured and stimulated with various concentrations of TGF-β1 for 48 and 72h. The morphology of treated and resting cells was assessed and immunofluorescence detection of epithelial (CK-7) and mesenchymal markers (S100A4, α-SMA) was carried out. Functional properties of HIBEC were analysed using an invasion assay. The MMNK-1 cell line was used to create a 3-dimensional model of bile duct-like structures by culture in Matrigel. In further studies, liver sections from diseased and normal livers were used for immunohistochemistry (S100A4, α-SMA, CK-7, CK-19) and in situ hybridization (S100A4, TGF-β1).

Biliary epithelium in normal liver and resting HIBEC expressed CK-19 and CK-7 but epithelial cells in neither preparation showed expression of the early and late EMT markers S100A4 and α-SMA respectively. Expression of the mesenchymal markers and the TGF-β-induced signalling molecule pSmad2/3 were noted in both stimulated HIBEC and diseased tissue sections. Additionally, stimulation with 10ng/ml TGF-β1 induced HIBEC to acquire strong migratory and invasive capacities together with typical fibroblastic morphology. In situ hybridization revealed S100A4 and TGF-β mRNAs within bile ducts in tissue sections from diseased livers. Incubation of 3-d cultured MMNK-1 cells with TGF-β1 resulted in disruption and atrophy of the bile duct-like structures.

This study demonstrates that the epithelial cells lining small and medium-sized bile ducts can undergo EMT during chronic inflammatory liver diseases. Furthermore, the process may be driven by TGF-β, and the resulting mesenchymal cells can differentiate to a mature, myofibroblast phenotype. It is tempting to speculate that targeting specifically the profibrogenic TGF-β pathway by application of antagonistic cytokines such as bone morphogenetic proteins or hepatocyte growth factor could inhibit (or reverse) EMT and prevent fibrosis.

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## Abstracts

28-POS

### REGULATION OF EMT DURING AVIAN GASTRULATION BY FGF SIGNALING AND THE RECEPTOR TYROSINE KINASE EPHA1.

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Gastrulation is a critical developmental event that in amniotes (reptiles, birds and mammals) involves extensive cell rearrangements, including epithelial-mesenchymal transition (EMT) in the primitive streak to form the mesoderm and endoderm. FGF signaling has been implicated in regulating EMT during gastrulation, though molecular mechanisms remain unclear. In chicken embryos, Fgf receptors and ERK activity are present in preingression zone epiblast adjacent to the streak and are downregulated as cells undergo EMT, indicating that FGF signaling functions prior to the onset of EMT. To begin investigating the function of FGF signaling upstream of EMT, microarray analyses were performed comparing preingression epiblast from control versus embryos treated with the FGFR inhibitor SU5402. Twenty-nine transcription factors were downregulated, including members of the Ets, bHLH, and TBX families. Wnt8c was also downregulated by SU5402, demonstrating cross regulation between these two signaling pathways. FGF-dependent genes were also identified that may negatively regulate the onset or progression of EMT. The RTK EPHA1, for example, is upregulated in preingression epiblast and downregulated as cells undergo EMT. Expression of a dominant active EphA1 receptor led to accumulation of cells in the epiblast and primitive streak, indicating that downregulation of EphA1 activity is required for proper EMT and cell migration. EPHA1 expression was downregulated by SU5402, the ERK inhibitor U0126 and the PI3-kinase inhibitor LY294002, while GSK3 inhibitors LiCl or SB415286 led to upregulation and lateral expansion of the EPHA1 epiblast expression domain. Surprisingly, forced expression of dominant active B-catenin downregulated EPHA1 expression. These findings indicate that EPHA1 expression requires FGF signaling through FGFR1 via ERK, and a GSK3 dependent pathway that acts through PI3-kinase. Downregulation of EPHA1 during EMT is likely regulated by canonical Wnt signaling. Studies are combining experimental results with microarray and bioinformatic tools to generate a preliminary network model of EMT during gastrulation.

29-POS

### MUC1 AND MUC4 EPITHELIAL MUCINS: ACTORS OF EPITHELIAL-MESENCHYMAL TRANSITION?

**Perrais, M, Aubert, S, Hemon, B, Porchet, N, Leroy, X and Van Seuning, I**

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MUC1 and MUC4 membrane-bound mucins have a very long extracellular domain (from 200 nm to 2.12  $\mu$ m), interact with ErbB family receptors and contribute to the growth and metastatic properties of tumours. Mucin expression is cell- and tissue-specific but this pattern is altered in numerous epithelial cancers. Altogether these elements indicate that cancer-associated membrane mucins play an important role during tumorigenesis. Epithelial-mesenchymal transition (EMT) occurs in late steps of carcinogenesis and is often associated with bad prognostic (metastasis). In this study, we have used three different cell lines: renal carcinomatous ACHN, renal immortalized human proximal tubular HK-2 and oesophageal adenocarcinomatous OE33. In these cellular models, by using RT-PCR, immunofluorescence, and western blotting, we show that EMT can be induced either by TGF-beta treatment or under prolonged hypoxic conditions. A decrease of epithelial markers and an increase or appearance of mesenchymal markers was observed. Surprisingly, during EMT, expression of MUC1 and MUC4 mucins, two epithelial markers, increased both at the mRNA and protein levels. Our aims are (i) to decipher the molecular mechanisms responsible for the altered pattern of expression of these two mucins and (ii) to better understand the role(s) of these mucins during EMT.

30-POS

### OXIDATIVE STRESS DRIVES EPITHELIAL TO MESENCHYMAL TRANSITION (EMT) IN HUMAN LUNG EPITHELIAL CELLS VIA THE TGF-BETA PATHWAY

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**Introduction:** The response of lung epithelium to injury is considered crucial to the pathophysiology of chronic lung diseases like Pulmonary Fibrosis, Asthma, COPD and post-transplant Obliterative Bronchiolitis. Change in epithelial cell phenotype to that of a (myo)fibroblast via EMT may contribute to lung remodelling and excessive connective tissue deposition seen in these diseases. As increased oxidative stress is commonly present in these diseases, we hypothesised that this injury source may drive EMT in the lung.

**Methods:** Lung epithelial cells (A549) were exposed to hydrogen peroxide H<sub>2</sub>O<sub>2</sub> (conc 50 – 400  $\mu$ M) for 1 hour and left for 12 days. Generation of intracellular reactive oxygen species (ROS) was assessed by FACS analysis using DHR and MitoSOX staining. Change in cell morphology was monitored by phase contrast microscopy. Changes in expression of EMT markers were assessed by Western blotting and confocal microscopy. Zymography was used to assess presence of active matrix metalloproteinases (MMPs) in cell supernatants. TGF-beta mRNA level was determined using real-time PCR with GAPDH as endogenous control.

**Results:** Untreated A549 cells show a uniform epithelial morphology with high level expression of tight junction protein, E-Cadherin, with very low levels of the mesenchymal markers collagen type III and vimentin, and no expression of alpha-SMA. Exposure to H<sub>2</sub>O<sub>2</sub> 200  $\mu$ M and 400  $\mu$ M caused a marked change in cell morphology typical of EMT. At concentrations of 400  $\mu$ M cells dramatically increased expression of collagen III (75 fold change), vimentin (5 fold change) and started to express alpha-SMA. Moreover collagen type III and fibronectin, as components of extracellular matrix in mesenchymal tissue, were secreted to extracellular space in response to H<sub>2</sub>O<sub>2</sub> treatment. E-cadherin expression was almost completely abolished. Both 200  $\mu$ M and 400  $\mu$ M H<sub>2</sub>O<sub>2</sub> increased 2 fold activity of MMP-9. Furthermore H<sub>2</sub>O<sub>2</sub> exposure markedly upregulated TGF-beta mRNA after 2 hours post-treatment, which was maintained up to 72 hours suggesting that oxidative stress may stimulate EMT via TGF-beta signalling.

**Conclusion:** Oxidative stress can induce EMT in lung epithelial cells and provides a potential mechanism for fibrogenesis in the lung microenvironment. The most probably TGF-beta signalling plays a key role in this process.

31-POS

**SNAIL AND SMAD ACT AS CO-REPRESSORS OF COXSACKIE- AND ADENOVIRUS RECEPTOR IN TGF-BETA-INDUCED EMT**

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The coxsackie- and adenovirus receptor (CAR) is a tight junction-associated cell adhesion molecule, which is predominantly expressed in epithelial cells in adult tissues. Little is known about the mechanism of CAR regulation in normal and malignant tissues, but a decrease in CAR expression levels is frequently observed in carcinomas. Here, we describe that CAR is suppressed during TGF-beta-induced EMT, a phenomenon that resembles carcinoma progression into a more invasive state. Both CAR protein and mRNA levels are decreased upon TGF-beta treatment of mouse mammary epithelial NMuMG cells in a time dependent fashion. CAR repression correlates with the nuclear translocation of the transcription factors Snail, a repressor of intercellular junction components, and both Smad3 and Smad4, intracellular transducers of TGF-beta mediated signaling. Moreover, we show that these three transcription factors cooperate in an additive manner to repress CAR as demonstrated by promoter activation experiments. Chromatin immunoprecipitation studies suggest that Snail, Smad3 and Smad4 are part of the same transcriptional complex and are associated with the CAR promoter. We conclude that Snail, Smad3 and Smad4 act as co-repressors of CAR in TGF-beta-induced EMT. This represents a novel mechanism of gene repression

32-POS

**MUC1 AND MUC4 EPITHELIAL MUCINS: ACTORS OF EPITHELIAL-MESENCHYMAL TRANSITION?**

**Perrais, M, Aubert, S, Hémon, B, Porchet, N, Leroy, X and Van Seuning, I**

Inserm U837, Place de Verdun, Lille cedex, 59045, FRANCE

MUC1 and MUC4 membrane-bound mucins have a very long extracellular domain (from 200 nm to 2.12  $\mu$ m), interact with ErbB family receptors and contribute to the growth and metastatic properties of tumours. Mucin expression is cell- and tissue-specific but this pattern is altered in numerous epithelial cancers. Altogether these elements indicate that cancer-associated membrane mucins play an important role during tumorigenesis. Epithelial-mesenchymal transition (EMT) occurs in late steps of carcinogenesis and is often associated with bad prognosis (metastasis). In this study, we have used three different cell lines: renal carcinomatous ACHN, renal immortalized human proximal tubular HK-2 and oesophageal adenocarcinomatous OE33. In these cellular models, by using RT-PCR, immunofluorescence, and western blotting, we show that EMT can be induced either by TGF-beta treatment or under prolonged hypoxic conditions. A decrease of epithelial markers and an increase or appearance of mesenchymal markers was observed. Surprisingly, during EMT, expression of MUC1 and MUC4 mucins, two epithelial markers, increased both at the mRNA and protein levels. Our aims are (i) to decipher the molecular mechanisms responsible for the altered pattern of expression of these two mucins and (ii) to better understand the role(s) of these mucins during EMT.

33-POS

**HLH TRANSCRIPTION FACTORS E47 AND ID1 IN E-CADHERIN PROMOTER REPRESSION AND EMT**

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Epithelial cells ectopically expressing E12/E47 adopt a fibroblastic phenotype and acquire migratory/invasive properties, concomitant with the suppression of E-cadherin expression. The class I bHLH E12/E47 factor usually acts as heterodimers with tissue-specific class II bHLH members, and its transcriptional activity can be negatively regulated by its interaction with members of the Id HLH subfamily. In agreement with that, downregulation of Id proteins is required for TGFbeta-induced EMT. However, overexpression of Id1 in epithelial cell lines confers a more invasive phenotype; moreover, an enhanced expression of Id1 has been associated with metastasis and poor prognosis in several carcinomas.

In the present study, we have investigated the involvement of E47 and Id proteins in the regulation of E47 mediated E-cadherin repression and in EMT process. Transient expression of Id proteins abrogates the repression of E-cadherin promoter induced by E47 in epithelial MDCK cells, but they are unable to derepress the basal activity in fibroblastic MDCK cells stably expressing E47 (MDCK-E47). In fact, Id proteins are upregulated and interacting with E47 in MDCK-E47 cells, suggesting that E47 might be acting through an indirect mechanism to maintain E-cadherin repression once EMT has been established. Nevertheless, sustained E47 expression is essential for the maintenance of EMT, since silencing of E47 by stable RNA interference in MDCK-EGFP-E47 induces a complete mesenchymal to epithelial transition (MET), associated to the upregulation of E-cadherin and, significantly, downregulation of Id proteins. In order to get further insights into the function of Id1 in relation to EMT, we have analyzed the effect of Id1 overexpression in MDCK cells. Stable expression of Id1 in MDCK cells leads to the full repression of E-cadherin and triggers a complete EMT, similar to that induced by E47. These results support an important but complex role for E47 and Id factors in E-cadherin regulation and EMT.

## Abstracts

34-POS

### INVOLVEMENT OF NF-kappa B IN EMBRYONIC VASCULAR REMODELLING AND ENDOTHELIAL-MESENCHYMAL TRANSITION PROCESS

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Arterial wall remodelling is a dynamic structural and architectural alterations process that occurs during embryonic and adult vascular development as well as in many vascular pathologies. This process is regulated by hemodynamic stimuli, as well as by environmental and genetic factors. Alterations in hemodynamic conditions have been widely recognized to cause intimal hyperplasia or intimal thickening. At present there is controversy not only regarding the origin or source of vascular smooth muscle cells involved in development and the cells that conform the intimal thickening, but also regarding the mechanisms contributing to their formation. Emerging evidence suggests that intimal cells originate from the endothelium by an endothelial-mesenchymal transition process (EndoMT). The NF-kappaB family members, particularly the activated p50/p65 heterodimer, are expressed in vascular cells during neointimal formation when hemodynamic conditions are altered. Here we report that p50, p65, IkappaB-alpha, and IKK-alpha have different spatial and temporal patterns of expression and distribution during chicken embryo aortic wall remodelling and intimal thickening development. Additionally, we show that both NF-kappaB subunits were present in the nucleus of some mesenchymal cells that expressed alpha-smooth muscle actin which were observed at embryonic day 12-14 (E12-E14) of development when the intimal thickening is evident. We also demonstrate that both activated p50 and p65 subunits were present in monolayers of embryonic endothelial cells growing on fibronectin coated dishes that had been transiently mechanically altered. Specifically, they were found at the nucleus of most cells of the monolayer as well as in some spreading, separating, detaching and migrating cells, and in some of those that had acquired mesenchymal characteristics. As a whole, these results support a role for NF-kappaB in embryonic vascular development and EndoMT process.

35-POS

### EMT OF HEPATOCYTE THROUGH INCREASE OF TGF-B1 AND 2 CONTRIBUTES TO HEPATOCYTE DYSFUNCTION AND LIVER FIBROSIS

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Liver cirrhosis is characterized by hepatic dysfunction with extensive accumulation of fibrous tissue in the liver. We surmised that epithelial-mesenchymal transition (EMT) of hepatocytes might contribute to both hepatocyte dysfunction and liver fibrosis. To address this issue, we analyzed dimethylnitrosamine (DMN)-induced fibrotic rat livers. Indeed, hepatocytes showing cytosolic E-cadherin, a sign of EMT, significantly increased in fibrotic liver, albeit the expression level of the protein hardly changed. In addition, we could observe  $\alpha$ -SMA (+) and vimentin (+) hepatocytes located inside or near the fibrotic bridges by immunohistochemistry, which further supports the presence of EMT of hepatocytes in this model. Regarding the hepatocyte function, hepatocytes isolated from fibrotic liver revealed a significant decrease in their ammonia-metabolizing activity comparing to normal hepatocytes. Furthermore, human liver cirrhotic tissues also revealed the increase of hepatocytes showing cytosolic E-cadherin staining as well as the presence of  $\alpha$ -SMA (+) and vimentin (+) hepatocytes inside or near the fibrotic bridges. Type I collagen staining was also increased in hepatocytes inside or near fibrotic lesions. Incubation of primary rat hepatocytes with TGF-b1 or 2, the central cytokine in fibrogenesis, induced decrease of epithelial cell markers such as cytokeratin18 and ZO-1, cytosolic translocation of E-cadherin, but increase of mesenchymal cell markers such as N-cadherin, vimentin and F-actin. In addition,  $\alpha$ -SMA and type I collagen expression was also induced by TGF-b treatment, evidencing that both TGF-b1 and 2 effectively induces EMT of cultured hepatocytes. Interestingly, immunohistochemistry with fibrotic rat liver revealed the expression of TGF-b from Ito cells located inside fibrotic bands along with the expression of TGF-b2 from hepatocytes near the bands. Since treatment of hepatocytes with TGF-b1 increased TGF-b2 secretion into the culture medium, we suggest that TGF-b1 secreted from Ito cell induces TGF-b2 secretion from hepatocytes, and both TGF-b1 and 2 contribute together in the process of EMT. Taken together, EMT of hepatocytes via TGF-b1 and 2 contribute to both the hepatocyte dysfunction and liver fibrosis.

36-POS

### DISSECTING REGULATORY NETWORKS CONTROLLING EMT AND MESODERM FORMATION BY IN VIVO RNAI

**Morkel M, Brouwer-Lehmitz A, Liu F, Leushacke M, Werber M and Herrmann BG**

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Mesoderm formation is a key process in the establishment of the vertebrate body. It depends on epithelial-mesenchymal transition (EMT), the conversion of an epithelial to a mesenchymal (motile) cell type. EMT during mouse trunk and tail development is mainly controlled by the Wnt, FGF and BMP signalling pathways and their downstream effectors. We have started to dissect the role of these signalling pathways in EMT during trunk development of the mouse embryo, using systematic RNAi knockdown of key factors involved in mesoderm formation. Since loss-of-function of key signalling factors can result in early embryonic lethality, we established a vector system enabling spatial and temporal control of siRNA expression. For that purpose, we employed the miR system, allowing expression under control of RNA Pol-II promoters. We have successfully utilized the mesoderm-specific Brachyury promoter for knockdown of Brachyury activity in vivo specifically in early embryonic mesoderm cells. Temporal control is presently being introduced into the system by virtue of the TetR repressor. Currently, approx. 100 hairpins directed against 30 genes with potential crucial functions in EMT and mesoderm formation are tested in order to isolate highly effective sequences for in vivo knockdown. Thus, the stage is set for a systematic analysis of EMT and mesoderm formation in embryogenesis.

37-POS

**THE MUTUAL REPRESSION BETWEEN SNAIL2 AND SOX3 REGULATES THE EMT AT GASTRULATION****Acloque, H, Ocaña, O, Nieto, MA****Instituto de Neurociencias de Alicante CSIC-UMH, 03550 San Juan de Alicante, Spain**

Snail genes are key factors controlling epithelial plasticity and EMT during embryonic development and in the adult. At gastrulation stages, the primitive streak (PS) forms at the posterior end of the embryo from which mesendodermal precursors delaminate upon undergoing EMT. Epithelial and neural markers are concomitantly repressed at the PS. Loss of function experiments have shown that Snail1 in the mouse and Snail2 in the chicken are crucial for the EMT process at the PS. Through gain of function experiments and analysis of cell movements with time-lapse confocal microscopy we have observed that (1) Snail2 overexpression is able to induce an ectopic EMT in territories that would otherwise give rises to epidermal or neural cells. (2) Snail2 overexpression represses the neural marker Sox3 and the epithelial marker B-cadherin in the presumptive neural and ectodermal territories respectively. (3) Conversely, Sox3 overexpression in the PS area represses Snail2 expression and blocks EMT while expression of a dominant-negative form of Snail2 expands Sox3 expression up to the embryonic midline also blocking EMT. Thus, the antagonistic roles of Snail and Sox proteins define the neural and mesendodermal territories in the gastrulating embryo, with Snail2 inducing EMT and Sox3 maintaining epithelial integrity by preventing Snail2 expression.

**38-POS IDENTIFICATION OF NOVEL GENES AND SIGNALING NETWORKS INVOLVED IN TUMOR ASSOCIATED EMT****Marc Leushacke\*, Ralf Spoerle\*, Lorenz Neidhardt\*, Anne-Kristin Heninger", Mirko Theis", Frank Buchholz", Bernhard G. Herrmann\* and Markus Morkel\*****\* Max Planck Institute for Molecular Genetics, Ihnestr. 73, Berlin 14195, Germany; " Max-Planck-Institute of Molecular Cell Biology and Genetics, Pfotenhauerstr. 108, Dresden 01307, Germany**

Epithelial-to-mesenchymal transitions (EMT) are key events in embryogenesis, and take place at multiple timepoints during normal development. Increasing evidence suggests that the main signalling networks that govern EMT in the embryo, the Wnt, Fgf and BMP signalling pathways, also modulate the metastatic spread of tumours. Thus, EMT in the embryo can serve as an /in vivo /model of mechanisms that are involved in tumour progression and metastasis. We have previously, using high-throughput in-situ hybridisation, identified a genome-wide set of candidate genes for EMT in the mouse embryo. We now utilized esiRNA technology to assess the impact of these (approx. 400) candidate genes on EMT in human colon cancer SW480 cells, which are of epithelial origin, but display a mesenchymal phenotype. We identified approx. 20 novel EMT effector genes whose knock-down results in mesenchymal-to-epithelial reversion, and thus, loss of characteristics of motile and potentially metastatic tumor cells. Many of the novel EMT effector genes are activated selectively in carcinoma, suggesting important roles during tumor progression. We now systematically analyse the roles of our effector genes in EMT signalling networks. Our approach allows for the first time to screen a large number of EMT candidate genes in a simple functional assay and thus to define important regulators of EMT and metastasis and potential drug targets that may modulate progression of human tumors.

**39-POS THE SMALL GTPASE RHOV IS AN ESSENTIAL REGULATOR OF NEURAL CREST INDUCTION IN XENOPUS****Signal, E., Guemar, L., de Santa Barbara, P., Donnay, JM., Fort, P. and Faure, S.****CRBM, CNRS UMR 5237, 1919 Route de Mende, Montpellier cedex 5, 34293, France**

In vertebrates, the Rho family of GTPases is made of 20 members, which regulate a variety of cellular functions, including actin cytoskeleton dynamics, cell adhesion and motility, cell growth and survival, gene transcription and membrane trafficking. To get a comprehensive view of Rho implication in physiological epithelial-mesenchymal transition, we carried out an in situ hybridization-based screen to identify Rho members expressed in *Xenopus* neural crest cells, in which we previously reported RhoB expression at the migrating stage. In the present study, we identify RhoV as an early expressed neural crest marker and provide evidence that its activity is essential for neural crest cell induction. RhoV mRNA is maternally expressed and accumulates shortly after gastrulation in the neural crest forming region. Using antisense morpholino injection, we show that RhoV depletion causes at the neurula stage a dramatic inhibition of neural crest markers except Snail, a pivotal transcription factor implicated in EMT in many biological situations. At the tailbud stage, RhoV depletion induced a loss of the cranial structures derived from the neural crest. These defects are rescued by ectopic wild-type RhoV, whose overexpression expands the neural crest territory. Finally, we show that changes in the level of RhoV expression have opposite effects on neural crest and neural plate territories, without affecting cell proliferation or apoptosis. Our findings that RhoV regulates neural crest gene expression during early development disclose an unprecedented Rho function in tissue-specific cell commitment.

## Abstracts

### 40-POS PHOSPHOPROTEOMIC IDENTIFICATION OF EFFECTORS OF THE BREAST TUMOUR SUPPRESSOR SYK

**Larive, RM, Mascré, G, Poncet, J\*, Urbach, S\*, Jouin, P\*, Mangeat, PH, Coopman, PJ and Bettache, N**  
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The cytoplasmic Spleen tyrosine Kinase (Syk) has mainly been studied in haematopoietic cells in which it is involved in coupling activated immunoreceptors to downstream signalling events. We evidenced for the first time that Syk is also expressed in mammary epithelial cells and is a potential breast tumour suppressor. Ever since, this property has been also demonstrated in melanocytes. Syk is present in normal breast tissue, benign breast lesions and low tumorigenic breast cancer cell lines. However, Syk protein is low or undetectable in invasive breast carcinoma tissue and cell lines. Most of these invasive breast cancer cell lines display fibroblastic differentiation and are E-cadherin-negative and vimentin-positive. The reintroduction of Syk in Syk-negative breast cancer cells reverses the invasive morphology of cell colonies in Matrigel. In order to elucidate the mechanism by which Syk exerts its tumour-suppressive function, we identified its downstream signalling effectors in breast cancer cells by a quantitative phosphoproteomic approach.

Stable Isotope Labelling in Cell culture (SILAC) was used to quantify by mass spectrometry tyrosine-phosphorylated proteins from Syk-positive breast cancer cells treated or not with piceatannol, a pharmacologic inhibitor of Syk activity and comparison was performed with Syk-negative breast cancer cell lines. Among 350 phosphoproteins quantified, 130 had a higher level of tyrosine phosphorylation in Syk non-inhibited conditions, 41 being more highly phosphorylated in Syk expressing cell lines. We selected these latter proteins as potential effectors of Syk tumour-suppressive function in breast cancer cells. Ten of them are implicated in cell adhesion and epithelium polarization. Our current efforts are concentrated on verifying the hypothesis that these proteins could mediate the effects of Syk on epithelial-mesenchymal transition of breast cancer cells.

### 41-POS DISTINCT BONE MORPHOGENETIC PROTEIN EXPRESSION PROFILES AND SMAD PATHWAY ACTIVATION IN DIFFERENT PHENOTYPES OF EXPERIMENTAL CANINE MAMMARY TUMORS

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Bone morphogenetic proteins (BMP) are expressed in breast cancer and have been associated with tumor invasion. We have investigated the BMP pathway activation and specific BMP mRNA expression profiles in clones from different types of canine mammary tumors: a spindle cell tumor, an osteosarcoma and a scirrhous carcinoma. Clones from the spindle cell tumor and the osteosarcoma expressed high levels of various BMP mRNAs, in particular BMP-2, -4 and -6, although variable expression was seen in different clones. Clones from the carcinoma expressed much lower BMP levels. The various clones gave rise to different tumor types in nude mice but only clones that expressed high levels of BMP-6 gave rise to bone formation. Phosphorylated Smad-1/5, located in the nucleus, was detected in tumors derived from clones expressing high levels of BMPs, indicating an active BMP signaling pathway. In contrast, tumors derived from scirrhous carcinoma clones, i.e. low BMP-producers, were only weakly positive. Further, all of the tumors formed from the various clones were positive for BMP-6 protein. We conclude that the specific BMP expression repertoire differs substantially between different types of mammary tumors and that the BMP pathway activation is correlated with the level of BMP expression. Future studies of expression array data from primary canine mammary osteosarcomas, fibrosarcomas and simple carcinomas may give information on the link between BMP expression and tumor invasion. The first round of analysis show that BMP-2, BMP-4 and BMP-6 are expressed in the same tumors as the EMT inducing gene gooseoid.

### 43-POS OVEREXPRESSION OF THIOREDOXIN REDUCTASE 1 INHIBITS PROTEIN KINASE C-DEPENDENT INDUCTION OF PHENOTYPIC TRANSITION AND MOTILITY OF HEK-293 CELLS

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Thioredoxin reductase (TrxR), apart from protecting against oxidative stress, plays a role in the redox regulation of intracellular signalling pathways controlling, among others, cell proliferation and apoptosis. The aim of this study was to determine whether TrxR1 is involved in the regulation of cell morphology, migration and gap junctional intercellular communication. Stable transfected HEK-293 cells that overexpress cytosolic TrxR1 (HEK-TrxR15) were used in this study. We found that the stimulation of epithelial-mesenchymal transition, reorganization of the F-actin cytoskeleton and cell motility induced by protein kinase C (PKC) activators, 12-O-tetradecanoylphorbol-13-acetate (TPA) and diphenyltin (DPhT) were significantly inhibited in the HEK-TrxR15 cells compared to control cells. In addition, the selective activation of PKC-delta by DPhT was inhibited in cells that overexpress cytosolic TrxR1 cells, whereas rottlerin, a selective inhibitor of PKC delta, and PKC-delta siRNA, suppressed the morphological changes induced by DPhT in the control cells. This indicates the specific role of PKC-delta in the observed processes. On the other hand, we also found that TPA and DPhT inhibited gap junctional intercellular communication in HEK-293 but not in HEK-TrxR15 cell populations. Our results suggest that TrxR1 may be involved in the PKC-dependent epithelial-mesenchymal transition, cell migration and cell-cell communication.

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44-POS

**SMAD3 IS A KEY MEDIATOR OF TGF $\beta$ -INDUCED TRANSCRIPTIONAL RESPONSES  
AND EMT IN MOUSE MAMMARY EPITHELIAL CELLS**

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Smad2 and 3 proteins are intracellular mediators of TGF $\beta$  signaling, and emerging data from analysis of several cell types indicate that these proteins have distinct functions in conveying cellular responses to this ligand. Using mouse mammary epithelial cells as a model system, we have investigated the role of Smad2 and 3 in mediating TGF $\beta$ -induced transcriptional responses and epithelial to mesenchymal transition (EMT). We have used a transient RNaseH mediated strategy to successfully deplete cells from Smad2 and 3 and examined the effect on hallmark processes of EMT such as dissolution of epithelial junctions, stress fibers and focal adhesions formation, activation of MMP's and transcriptional regulation of acknowledged target genes. We have extended this investigation using microarray analysis and identified a lot of target genes that strictly depend on Smad3 to be transcriptionally regulated by TGF $\beta$ . In contrast, we identified only a few genes of which transcriptional modulation induced by TGF $\beta$  was dependent on Smad2. Moreover, absence of Smad2 only partially affected the transcriptional response of these genes to TGF $\beta$ . Our results identify Smad3 as a key mediator of TGF $\beta$  regulated transcriptional responses and EMT in mouse mammary gland epithelial cells.

45-POS

**SNAI1 AND SNAI2 SILENCING EFFECTIVELY SUPPRESSES TUMOR GROWTH AND INVASIVENESS.**

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The Snail family of transcription factors has been recently proposed as important mediators of tumor invasion because of its role in downregulation of E-cadherin and induction of epithelial-mesenchymal transitions (EMT). This behavior has led to the consideration of Snail factors as a potential therapeutic target to block tumor progression. We have investigated this hypothesis by stable silencing Snai1 (Snail) and Snai2 (Slug) in MDCK-Snail and MDCK-Slug cells. Specific silencing of Snai1 or Snai2 in the corresponding cell line induce a complete mesenchymal to epithelial transition (MET), associated to the upregulation of E-cadherin, downregulation of mesenchymal markers and inhibition of invasion. More importantly, stable interference of endogenous Snai1 and Snai2 in two independent carcinoma cell lines leads to a dramatic reduction of in vivo tumor growth and a significant decrease in MMP-9 expression and in vitro invasiveness. These results indicate that use of RNA interference can be an effective tool for blocking Snai1 and Snai2 function, opening the way for its application in new anti-invasive therapies.

46-POS

**THE E-CADHERIN-REPRESSED hNANOS1 GENE INDUCES TUMOR CELL INVASION BY UPREGULATING MT1-MMP EXPRESSION**

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Tumor cell invasion requires modifications of cell-cell adhesion properties and remodelling of extracellular matrix. Previous studies have shown that downregulation of E-cadherin is associated with overexpression of matrix metalloproteinases (MMPs) including MT1-MMP during this process. In this study, we examined the role of the E-cadherin-repressed gene hNanos1 in tumor invasion process. First, our in vivo study revealed that hNanos1 mRNAs were overexpressed in invasive lung carcinomas. Moreover, hNanos1 mRNAs and proteins were localized in invasive lung tumor cells. Using an inducible Tet-on system, we showed that induction of hNanos1 expression in DLD1 cells increased their migratory and invasive abilities in a 3D migration and in a modified Boyden chamber assays. Accordingly, we demonstrated that hNanos1 up-regulated MT1-MMP expression at the mRNA and protein levels. Inversely, to evaluate the effect of hNanos1 downregulation on MT1-MMP expression, we used an RNA interference strategy to inhibit hNanos1 expression in invasive Hs578T, BT549 and BZR cancer cells. We observed that transfection of hNanos1 siRNA down-regulates MT1-MMP mRNAs and proteins and concomitantly decreases the invasive capacities of tumor cells in a modified Boyden chamber assay. Taken together, our results demonstrate that hNanos1, by regulating MT1-MMP expression, plays an important role in the acquisition of invasive properties by epithelial tumor cells.

## Abstracts

47-POS

### REGULATION OF CXCL8/IL-8 BY ZONULA OCCLUDENS-1 DURING BREAST TUMOR-ASSOCIATED EPITHELIAL-TO-MESENCHYMAL TRANSITIONS

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Accumulating data suggest that ZO-1 can be implicated in the regulation of tumor promoting genes, once delocalized from tight junctions. More precisely, cytoplasmic accumulation of ZO-1 has also been shown to activate the b-catenin pathway and epithelial-to-mesenchymal transition-associated processes.

Using GeneArray analysis to compare chemokine expression in breast tumor cells transfected with a siRNA against ZO-1, we here identified CXCL8/IL-8 as a major potential target of ZO-1 signalling. We accordingly demonstrated that CXCL8/IL-8 expression correlates with a relocalization of ZO-1 in breast cancer cell lines. Indeed, CXCL8/IL-8 is overexpressed only in invasive breast cancer cells that display a rather cytoplasmic localization of ZO-1. Non-invasive cell lines displaying a membrane-associated staining of ZO-1 do not express CXCL8/IL-8. Moreover, CXCL8/IL-8 is downregulated in invasive BT549 cells transfected with 3 different ZO-1 siRNA and overexpressed in non invasive BT20 and SKBR3 cells transfected with vectors expressing full length ZO-1 or with a NH2-terminal fragment of ZO-1 comprising the PDZ domains 1-3. We also showed an activation of the CXCL8/IL-8 promoter by ZO-1. Finally, we demonstrated that this regulation of CXCL8/IL-8 by ZO-1 is independent of the b-catenin pathway.

Our results thus clearly demonstrated an implication of ZO-1 in CXCL8/IL-8 regulation. Because of the major implications of CXCL8/IL-8 in tumor invasion, such a regulation could play an important role in breast cancer progression.

48-POS

### POSITIVE AND NEGATIVE COOPERATION OF P38 MAPK WITH TGF-BETA IN EPITHELIAL TO MESENCHYMAL TRANSITION (EMT)

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Epithelial to mesenchymal transition (EMT) has been recognized as the cellular mechanism for tumor progression towards invasion and metastasis. TGF-beta signaling is associated with induction and maintenance of EMT while p38 MAPK is reportedly required for TGF-beta-mediated EMT. To investigate the cooperation of p38 MAPK with TGF-beta at different stages of EMT, we established cell lines inducible expressing the dominant-negative (DN) MKK3 and MKK6 in MDCK and Ras-transformed MDCK cells. We found that activation of p38 MAPK by anisomycin resulted in morphological changes and the disruption of the adherens junction, but not the tight junction. Interestingly, TGF-beta-induced slow partial disruption of adherens and tight junctions was p38 MAPK independent. Cooperation of p38 MAPK with TGF-beta had a synergistic effect in disruption of cell polarity. However, the cooperation was not sufficient to down-regulate proteins involved in cell-cell junctions, or to increase cell migration and invasion in vitro. In contrast to the positive effects of p38 MAPK on the initiation of EMT, inhibition of p38 MAPK by induction of DN MKK3 or MKK6 enhanced cell migration and invasion of Ras-transformed mesenchymal MDCK cells in vitro. Moreover, synergistic effects were observed between induction of DN MKK3 or MKK6 and TGF-beta, indicating a negative cooperation of p38 MAPK with TGF-beta signaling in advancing mesenchymal functions. These results were further confirmed by using p38 inhibitor, SB203580, in both the MDCK and Ras-transformed MDCK cells. Thus, p38 MAPK cooperates positively with TGF-beta signaling in initiating early stages of EMT while negatively in advancing late stages of EMT.

49-POS

### EPITHELIAL-MESENCHYMAL TRANSITION INDUCES MIGRATORY/INVASIVE PHENOTYPE, WHILE INHIBITING TUMOR CELL GROWTH, TO PROMOTE METASTASIS IN LUNG CANCER.

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Metastatic cancer cells are known to be migratory and invasive; they are also thought to be less proliferative and less apoptotic. However, the regulatory factors and the mechanism(s) that induce such a phenotype in tumor cells are not clear. TGF-beta promotes tumor progression and metastasis by its pleiotropic activities on both cancer cells and the surrounding stroma. In several epithelial cancer cells, TGF-beta induces epithelial to mesenchymal transition (EMT), a process that confers migratory and invasive abilities to cells. We recently showed that stimulation of A549 lung adenocarcinoma cells with TGF-beta induces EMT. During EMT, A549 cells acquire fibroblastoid morphology, down-regulate epithelial-specific proteins (E-cadherin), express mesenchymal-cell proteins (vimentin and N-cadherin), and acquire a migratory/invasive phenotype as assessed by in-vitro migration and invasion assays. Here we report that Smad3 is essential for EMT as evidenced by siRNA approach; in contrast, Smad2 is dispensable for TGF-beta-induced EMT. The role of other TGF-beta-activated signaling pathways, including Erk1/2, p38 MAPK, JNK, PI3-K/Akt and Src kinase(s) pathways are not required for EMT as evidenced by pharmacological and siRNA approaches. However, gamma-secretase inhibitor blocks TGF-beta-induced EMT, demonstrating a role for Jagged1/Notch signaling. Consistently, we also observed an increase in time-dependent JAG1 expression upon TGF-beta stimulation. Next, we assessed the in-vivo effect of EMT on metastatic potential and primary tumor growth of A549 xenografts in SCID mice. A549 cells induced to undergo EMT by TGF-beta, demonstrated higher rates of spontaneous metastasis in this xenograft model. Metastatic potential was further confirmed in an experimental metastasis assay using SCID mice; a 5-fold increase in the number of metastatic colonies in the lung was observed in cells treated with TGF-beta compared to control. Induction of EMT simultaneously inhibited the growth of primary A549 xenografts in SCID mice. Cell-cycle analysis of cells undergoing EMT by flow cytometry showed a significant decrease in number of cells in 'S' phase and an increase in the number of cells in G0/G1 phase, suggesting a G0/G1 arrest. Induction of p21 and p27 expression during EMT further confirmed G0/G1 arrest. Transcriptional profiling of cells undergoing EMT showed activation of genetic programs that regulate cell migration and invasion, along with the activation of growth-inhibitory pathways. Together, our data demonstrate that TGF-beta, by induction of EMT confers a less proliferative, but more migratory and invasive phenotype to tumor cells leading to metastasis.

50-POS

**MESENCHYMAL TO EPITHELIAL TRANSITION IN THE ESTABLISHMENT OF SECONDARY TUMOURS: INSIGHTS FROM A BLADDER CARCINOMA PROGRESSION SERIES**

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Tumour metastasis remains the primary cause of morbidity and mortality for cancer sufferers. A better understanding of the molecular process is required to facilitate the development of new therapeutic strategies. The breakdown of epithelial cell homeostasis leading to aggressive cancer progression has been correlated with the loss of epithelial characteristics and the acquisition of a migratory phenotype. This phenomenon, referred to as epithelial to mesenchymal transition (EMT), has been identified as a crucial event in the development of metastasis. Furthermore, it has been recognized that in order to form tumours at secondary sites, it is likely that the tumour cells must re-activate certain epithelial properties, i.e. undergo a mesenchymal to epithelial transition (MET).

We have selected the TSU-Pr1 bladder carcinoma cell line *in vivo* for increased metastatic ability following intracardiac inoculation, generating a series of 3 related cell lines. A striking feature associated with increasing metastatic ability was an increase in a number of epithelial markers, suggestive of a MET. This was supported by phenotypic changes in the *in vitro* characteristics of the cells, including altered appearance of the cells from a 'fibroblastic' to an epithelial, cobblestone morphology, decreased migration and invasion abilities, and a diminished ability to form colonies under conditions of anchorage independent growth.

Despite the overriding epithelial nature of the metastatic cells, mesenchymal characteristics, such as elevated expression of various matrix metalloprotease family members and continued expression of vimentin, was also observed. This supports the notion of tumour cell plasticity and suggests that tumour cells may exhibit a combination of epithelial and mesenchymal traits which is likely to be important in determining metastatic ability.

Coordinated alteration in the fibroblast growth factor (FGF)/FGF receptor axis indicated that this pathway may be driving the MET, reminiscent of embryological processes. Indeed, knockdown of the FGF receptor 2 using RNA interference reversed the epithelial phenotype (appearance and functional assays) of the most metastatic cell line *in vitro*, an effect that was related to the degree of receptor knockdown. *In vivo*, FGF receptor 2 knock down decreased the formation of tumours following intracardiac inoculation and increased survival.

Thus FGF receptor-mediated MET at the distant site contributes to the development of secondary tumours. Understanding how tumour cells use both epithelial and mesenchymal characteristics to disseminate and form secondary tumours is likely to provide potential new targets for intervention.

51-POS

**PTEN REPRESSION: A NOVEL MECHANISM INVOLVED IN SNAIL1 INDUCED RESISTANCE TO APOPTOSIS**

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The product of Snail1 gene is a transcriptional repressor required for triggering the epithelial-to-mesenchymal transition (EMT). Furthermore, ectopic expression of Snail1 in epithelial cells promotes resistance to apoptosis. In this study we demonstrate that inhibition of the expression of PTEN phosphatase by Snail1 accounts for this higher resistance to gamma radiation-induced apoptosis in MDCK cells. PTEN mRNA levels are induced after irradiation of this cell line and over-expression of Snail1 prevents this upregulation. Snail1 represses PTEN promoter activity, an effect that is dependent on the integrity of a 5-'CACCTG-3' box present in this promoter. Association of Snail1 to PTEN promoter is detected by chromatin immunoprecipitation experiments, performed either with endogenous or with ectopic Snail1. This association is increased after gamma radiation, correlating with the stabilization of Snail1 protein. Besides, Snail1 and PTEN show an inverse distribution in embryos at early stages of development. Depletion of Snail1 in these embryos alters the pattern of PTEN expression. These results indicate that Snail1 is a key effector of apoptosis through the repression of PTEN transcription.

52-POS

**A NATURAL ANTISENSE TRANSCRIPT REGULATES ZEB2/SIP1 GENE EXPRESSION DURING SNAIL1-INDUCED EPITHELIAL-MESENCHYMAL TRANSITION**

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Snail1 transcriptional factor is essential for triggering epithelial-mesenchymal transition. During this process, Snail1 represses the expression of E-cadherin gene through its direct binding to this promoter. In this work we demonstrate that Snail1 also increases the synthesis of Zeb2, another transcriptional repressor of E-cadherin. Snail1 does not affect Zeb2 mRNA synthesis but the alternative splicing of the 5'UTR of this transcript: in cells transfected with Snail1 a large intron located in this 5'UTR is not processed. This intron contains an Internal Ribosomal Entry Site (IRES) necessary for the expression of Zeb2. Maintenance of 5'UTR Zeb2 intron is dependent on the expression of a natural antisense transcript (NAT) that overlaps the donor site in the intron. Ectopic over-expression of this NAT in epithelial cells prevents Zeb2 5'UTR splicing and increases the levels of Zeb2 protein. Consequently, cells stably transfected with this NAT present lower levels of E-cadherin mRNA and protein. These results indicate that Snail1 up-regulates the expression of the E-cadherin repressor Zeb2 through the synthesis of a NAT and provides the first example of an antisense transcript controlling the function of an IRES with a physiological relevance.

## Abstracts

53-POS

### SNAIL1 AND 1ALPHA,25-DIHYDROXYVITAMIN D3 HAVE OPPOSITE EFFECTS ON WNT/BETA-CATENIN SIGNALING AND GENE EXPRESSION PROFILE IN HUMAN COLON CANCER CELLS

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Epidemiologic and preclinical data indicate that the active vitamin D metabolite 1alpha,25-dihydroxyvitamin D3 (1,25(OH)2D3) has anticancer activity. We have previously reported that 1,25(OH)2D3 inhibits proliferation and promotes epithelial differentiation of SW480-ADH human colon cancer cells. These effects are mainly due to the induction of E-cadherin expression and to the blockade of Wnt/beta-catenin signaling (J. Cell Biol. 154:369-388, 2001). We also found that the EMT inducer Snail1 represses vitamin D receptor (VDR) expression and inhibits some 1,25(OH)2D3 effects in SW480-ADH cells (Nat. Med. 10:917-919, 2004). Now, we demonstrate that Snail1 abolishes the beta-catenin nuclear export induced by 1,25(OH)2D3 in cultured cells and in xenografted mice, and also blocks the inhibition exerted by 1,25(OH)2D3 on the expression of beta-catenin target genes. Moreover, Snail1 abrogates the inhibitory effect of 1,25(OH)2D3 on cell proliferation and migration. Remarkably, the introduction of an exogenous VDR gene in cells over-expressing Snail1 normalizes the gene regulatory activity of 1,25(OH)2D3. However, exogenous VDR failed to fully restore 1,25(OH)2D3 effects on Wnt/beta-catenin signaling, as reflected in the expression of beta-catenin target genes and on cell proliferation and migration. These results suggest that Snail1 is a positive regulator of Wnt/beta-catenin pathway in part through the abrogation of the inhibitory action of 1,25(OH)2D3. In addition, we examined the effects of 1,25(OH)2D3 and Snail1 on the gene expression profile of SW480-ADH cells. 1,25(OH)2D3 and Snail1 change the expression of 128 and 181 genes, respectively. The majority of 1,25(OH)2D3 regulated genes (72%) were induced, while that of Snail1 target genes (69%) were repressed. 1,25(OH)2D3 and Snail1 modulate in an opposite way the expression of genes involved in the same cellular functions and, in some cases, the same gene or genes of the same family are regulated inversely. These results are in agreement with the contrary effect exerted by 1,25(OH)2D3 and Snail1 on the phenotype of SW480-ADH cells.

54-POS

### THE ROLE OF INTERLEUKIN-LIKE EMT INDUCER (ILEI) IN LIVER CARCINOMA PROGRESSION

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The synergy of transforming growth factor (TGF)-beta with constitutive active Ras causes an epithelial-to-mesenchymal-transition (EMT) during liver and breast cancer progression. A novel key regulator of malignant epithelial plasticity, referred to as Interleukin-like EMT Inducer (ILEI), has been recently identified during EMT of neoplastic mammary epithelial cells. ILEI has been demonstrated to be sufficient for malignancy and tumor progression of breast carcinoma cells. In this study, we employed a model of liver cancer in order to investigate the role of ILEI in hepatocellular carcinoma (HCC) progression. We show that ILEI in collaboration with oncogenic Ras is capable to (i) induce and maintain EMT in vitro involving the activation of the MAPK/Erk pathway, (ii) accelerate the migratory behaviour of malignant hepatocytes, (iii) augment tumor formation and tumor dissemination including distal metastasis, and (iv) activate nuclear beta-catenin accumulation as well as phosphorylation of Stat3 in vivo. Further analyses focused on human HCC samples and aimed at examining the cellular distribution of ILEI in neoplastic tissues of different stagings. ILEI has been localized in granular compartments of normal liver tissues and differentiated tumors, whereas a more cytoplasmic distribution has been detected in poorly differentiated HCCs. These data indicate that ILEI represents a crucial regulator of hepatocellular EMT and liver carcinoma progression that acts independently of TGF-beta.

55-POS

### MODULATION OF EMT AND TGFbeta SIGNAL BY EXTRACELLULAR MATRIX COMPONENTS IN VIVO AND IN VITRO

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Purpose. To evaluate the roles of extracellular matrix (ECM) components, lumican and osteopontin (OPN), in the process of epithelial-mesenchymal transition (EMT) and fibroblast-myofibroblast conversion (FMC) in mouse lens and cornea in vivo and in cultured mouse cells.

Methods. Puncture injury in a mouse lens induces EMT in lens epithelial cells. We investigated the roles of lumican and OPN on this EMT by using mice lacking lumican or OPN gene. Immunohistochemistry was used to study Smad2/3 activation. In cultured ocular fibroblasts effects of lacking such ECM component on TGFbeta1 signal and FMC by using immunocytochemistry and western blotting. In vivo roles of these ECM molecules were investigated by using cornea injury model in KO mice.

Results. Lacking these ECM components perturbed injury-induced EMT. In vivo immunohistochemistry showed delayed activation of Smad2/3 signal in the epithelial cells. In cultured ocular fibroblasts lacking such ECM component suppressed Smad2 and p38 signal and also perturbed FMC in association with decreased expression of collagen I and TGFbeta1 mRNAs. In vivo healing mouse cornea lacking OPN inhibited connective tissue healing after incision injury.

Conclusion. ECM molecules, i.e., lumican or OPN, affects TGFbeta signal and modulate EMT or FMC during tissue repair.

**56-POS STUDY OF GENE ACTIVATION MEDIATED BY SNAIL1: REQUIREMENTS OF THE FIBRONECTIN PROMOTER****Porta de la Riva, M and Agusti, C and Baulida, J****Unitat de Biologia Cel·lular i Molecular, Institut Municipal d'Investigacio Medica, Doctor Aiguader 88, Barcelona, 08003, Spain**

Snail1 is a transcription factor from the Zn finger family of transcription factors that is involved in Epithelial to Mesenchymal Transition (EMT). Accordingly, forced Snail1 expression in epithelial cells results in an EMT which includes both repression of epithelial genes and expression of mesenchymal marker genes such as Fibronectin. It has been described that the repression is mediated by direct binding of the Snail1 protein to E boxes existing in the promoters and subsequent recruitment of histone deacetylases; however, little is known about the activation mechanism. Here we describe some molecular requirements for the Snail1 activation mechanism concerning the Fibronectin promoter. We observed that, although Snail1 and beta-catenin are part of a complex on the Fibronectin promoter in vivo, their interaction was independent of their canonical binding sites, E and TCF boxes respectively. Furthermore, we show the involvement of Rel A, member of the NF $\kappa$ B complex, as well as the ubiquitous protein LSF. Preliminary experiments also indicate that these proteins associate to different complexes on the promoter.

**57-POS****ORAL ADMINISTRATION OF GW788388, A KINASE INHIBITOR OF THE TGF-beta TYPE I AND TYPE II RECEPTORS, REDUCES RENAL FIBROSIS IN DB/DB MICE****Maj Petersen****Molecular Cell Biology, LUMC, Einthovenweg 20, Leiden, 2333 RC, The Netherlands**

Diabetic nephropathy precedes end-stage renal failure in up to a third of patients with diabetes mellitus. Elevated levels of transforming growth factor beta, in the kidneys of patients, are proposed to underlie the progression of chronic renal disease, by promoting deposition of extracellular matrix and epithelial-mesenchymal transition. Disrupting TGF-beta signaling could provide a therapeutic approach for treating fibrosis. TGF-beta signals via specific type I and II receptor serine/threonine kinases and its intracellular effector proteins Smads, which regulate gene transcription. We studied the effects of the small molecule GW788388 in vitro and in vivo. Db/db mice were used as a model for diabetic nephropathy. GW788388 was orally administered at 2 mg/kg/day for 5 weeks. GW788388 potently inhibits both the TGF-beta type I and type II receptor kinase activities but not the related bone morphogenic protein type II receptor. It blocked TGF-beta-induced Smad activation and target gene expression and abrogated the TGF-beta-induced epithelial mesenchymal transition and fibrotic response. In db/db mice, GW788388 significantly reduced nephropathy and decreased the mRNA levels of key mediators of extracellular matrix deposition in kidneys. GW788388 is a potent and selective inhibitor of TGF-beta signaling in vitro and renal fibrosis in vivo. Our study supports an effort to identify therapeutics which targets TGF-beta signalling in fibrotic diseases.

**58-POS****GENIPIN SUPPRESSES FIBROGENIC BEHAVIORS OF ALPHA-TN4 LENS EPITHELIAL CELL LINE.****Ai Kitano, Osamu Yamanaka, Yuka Okada, Kumi Shirai and Shizuya Saika****Ophthalmology, Wakayama Medical University School of Medicine, 811-1 Kimiidera, Wakayama, 641-0012, Japan**

**Purpose.** We determined in a lens epithelial cell line, alpha-TN4, if genipin, an intestinal metabolite component of the herbal medicine, Inchin-ko-to, suppresses profibrogenic myofibroblast generation and up-regulation of fibrogenic cytokines. These reactions potentially cause posterior capsular opacification (PCO), a major post-cataract surgery complication that impairs post-operative patients' vision.

**Methods.** Alpha-TN4 cell proliferation, migration and expression of alpha-smooth muscle actin (alphaSMA), the hallmark of EMT or myofibroblast generation, were assayed with a colorimetric assay, scratch wound assay, immunohistochemistry and western blot. Expression of TGFbeta1 and CTGF was characterized with real-time RT-PCR. Signaling of MAP kinase, p38MAP kinase, ERK and Smad was evaluated by western blotting and immunohistochemistry. Cytotoxicity of genipin was evaluated by using a colorimetric assay for nuclear matrix protein 41/7 (NMP41/7) in culture medium.

**Results.** Genipin suppressed cell proliferation and migration in association with inhibition of Smad and p38 MAPK activation although ERK signaling was enhanced. Genipin suppressed expression of TGFbeta1 and CTGF. Cytoplasmic fiber formation declined based on less intense alpha-SMA immunocytochemical staining. Treatment of the cells with genipin for 48 hrs did not increase the release of NMP41/7 to the medium, indicating no cytotoxicity of this compound.

**Conclusion.** Since genipin suppresses alpha-TN4 lens cell fibrogenic behaviors, it may be of therapeutic value to suppress PCO.

## **Abstracts**

**59-POS**

**NF-KB MEDIATES MATRIX METALLOPROTEINASE-INDUCED EMT IN MAMMARY EPITHELIAL CELLS**

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Matrix metalloproteinases (MMPs) are upregulated in nearly every tumor type, acting to facilitate tumor invasion, angiogenesis, and metastasis. Previous studies with transgenic mice showed that expression of MMPs in mammary epithelial cells disrupted tissue structure, stimulated cell proliferation, and caused malignant transformation; histological examination of the resultant carcinoma cells revealed a spindle-shaped, mesenchymal morphology. These effects were dissected using cell culture models in which mouse mammary epithelial cells were exposed to matrix metalloproteinase-3/stromelysin-1 (MMP-3). Cells exposed to MMP-3 underwent epithelial-mesenchymal transition (EMT), a phenotypic alteration in which epithelial cells adopt mesenchymal characteristics. EMT occurs during embryonic development, and there is increasing evidence to suggest that EMT-related processes are also associated with tumor progression. We have now found that MMP-3-induced EMT is mediated by the transcription factor NF-kappa B: cells exposed to MMP-3 produce increased levels of reactive oxygen species (ROS), which activate NF-kappaB, which in turn increases transcription of Snail, a key mediator of physiological and pathological EMT. Snail is expressed in human breast cancers and causes tumor development when expressed in mammary epithelial cells of transgenic mice. These results illuminate tumor-promoting processes downstream of MMPs that could serve as potential targets for therapeutic interventions.

**60-POS**

**EPITHELIAL TO MESENCHYMAL TRANSITION OF MESOTHELIAL CELLS: PATHOLOGIC SIGNIFICANCE IN PERITONEAL DIALYSIS PATIENTS AND POTENTIAL THERAPEUTIC INTERVENTIONS**

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Peritoneal dialysis (PD) is a form of renal replacement and is based on the use of the peritoneum as a semi-permeable membrane across which ultrafiltration and diffusion take place. Nevertheless, continuous exposure to bio-incompatible PD solutions and episodes of peritonitis cause acute and chronic inflammation and injury to the peritoneal membrane, which progressively undergoes fibrosis and angiogenesis and, ultimately, ultrafiltration failure. Resident fibroblasts and infiltrating inflammatory cells have been classically considered the main responsible of structural and functional alterations of the peritoneum. More recently, by using ex vivo cultures of PD effluent-derived mesothelial cells (MC) and immunohistochemical analysis of peritoneal biopsies from PD patients, we have demonstrated that new fibroblastic cells may arise from local conversion of MC by epithelial to mesenchymal transition (EMT) during the inflammatory and repair responses, and that trans-differentiated MC are protagonists of peritoneal membrane deterioration. From clinical nephrologists points of view, the identification of the EMT of MC as a key event in peritoneal membrane failure provides future hope for long-term preservation of peritoneal membrane because the EMT process can be manipulated with a wide range of agents and pharmaceutical products. The therapeutic strategies may be designed either to prevent or reverse the EMT itself or to treat its effects such as cellular invasion, ECM accumulation or angiogenesis factors synthesis. However, the design of new therapeutic strategies still requires extensive analysis, in vitro and in animal models, of the different drugs and factors with potential effects on EMT and its deleterious consequences, prior to starting with clinical trials.

**61-POS**

**ANALYSIS OF THE BRCA1 BREAST TUMORS IDENTIFIES A NOVEL ONCOGENE AND EMT INDUCER, YAP.**

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Cancer arises when a cell sustains an initial genetic lesion, followed by the accumulation of multiple genetic hits during tumor progression. Secondary genetic events are thought to be particularly critical in cancer models triggered by global genomic instability, such as those linked to inactivation of the BRCA1 breast cancer susceptibility gene. In our analysis of gene copy number aberrations in mouse Brca1-driven breast tumors, we discovered a selective amplification of a small region of mouse chromosome 9, syntenic with the 11q22 amplicon commonly observed in human cancers. The mouse amplicon contained only one known gene, Yap, a transcriptional coactivator related to Drosophila Yorkie (Yki). Yki was recently characterized as a downstream effector of the Hippo (Hpo)-Salvador (Sav)-Warts (Wts) signaling cascade that regulates cellular proliferation and apoptosis. Interestingly, YAP also induced EMT phenotypes in the MCF10A cell line (nontransformed mammary epithelial cell line), such as induction of migratory ability, loss of epithelial markers, acquisition of mesenchymal markers, and protection from anoikis. Furthermore, YAP displayed such classical oncogenic properties as the ability to confer growth factor-independent proliferation and anchorage-independent growth in soft agar. Together, these observations point to the oncogenic role of YAP in 11q22-amplified human cancers and suggest a fundamental connection between EMT phenotypes and the process of tumorigenesis.

62-POS

**TRIP6, A NOVEL MOLECULAR PARTNER OF THE MAGI-1 SCAFFOLDING MOLECULE, PROMOTES INVASIVENESS****Larissa Kotelevets<sup>1</sup>, Alexey Kruglov<sup>1</sup>, Erik Bruyneel<sup>2</sup>, Marc Bracke<sup>2</sup>, Yolande Di Gioia<sup>1</sup>, Mary C Beckerle<sup>3</sup>, Frans van Roy<sup>4,5</sup> and Eric Chastre<sup>1</sup>****1** INSERM, U773, Centre de Recherche Biomedicale Bichat Beaujon CRB3, BP 416, F-75018, Paris, the Université Paris 7 Denis Diderot, site Bichat, BP 416, F-75018, Paris, France; **2**Laboratory of Experimental Cancerology, Ghent University Hospital, B-9000 Ghent; **3**Huntsman Cancer Institute, Departments of Biology and Oncological Sciences, University of Utah, Salt Lake City, Utah 84102, USA; **4**Department for Molecular Biomedical Research, VIB, B-9052 Ghent, Belgium; **5**Department of Molecular Biology, Ghent University, B-9052 Ghent, Belgium

We recently established the critical role of the PTEN/MAGI-1b signalosome in stabilization of cell-cell contacts and suppression of invasiveness. The PTEN tumor suppressor is recruited to E-cadherin junctional complexes through the binding to the 2nd PDZ domain of the MAGI-1b scaffolding molecule, whereas beta-catenin interacts with the 5th PDZ domain (Kotelevets et al, J. Cell. Biol. 155: 1129-1135, 2001; FASEB J. 19: 115-117, 2005). To identify additional effectors of this signalosome, we used yeast two-hybrid screening. Among the clones identified, we focused on TRIP6, which belongs to the zyxin family of proteins. We demonstrated that TRIP6 interacted directly with MAGI-1b by binding to its 5th PDZ domain. Ectopic expression of TRIP6 induced invasiveness in the epithelial MDCK and MDCKts-src cells in a PI3-kinase and a NF- $\kappa$ B dependent manner, and reverted E-cadherin-dependent cell-cell aggregation. TRIP6 effects were associated with induction of NF- $\kappa$ B and Akt activities and decrease in active Rac1-GTP. The TRIP6Stop473 mutant, which lacks the PDZ binding motif, was unable to promote cell invasiveness and to interfere with cell-cell aggregation. Intracellular delivery of competing peptides corresponding to TRIP6 or beta-catenin C-terminus restored invasive properties in MDCKts-srcTRIP6Stop473 cells. These results indicate that TRIP6 binding to MAGI-1b exerts a permissive role on invasiveness by destabilizing E-cadherin junctional complexes

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**FOLLOWING SNAIL TRAILS: A SCREEN FOR EMT REGULATORS IN VIVO****Mary Y.W. Wu and Caroline S. Hill****Developmental Signalling Laboratory, Cancer Research UK, 44 Lincoln's Inn Fields, London, WC2A 3PX, UK**

Neural crest cells give rise to a multitude of adult cell types. They maintain pluripotency until late in embryonic development when they delaminate from the surrounding epithelium and migrate to their sites of final differentiation. This process of delamination-migration, also known as epithelial to mesenchymal transition (EMT), is unique to neural crest cells in vertebrates and is reminiscent of metastasis in many cancers of epithelial origin. Key molecular events of EMT include the down or mis-regulation of E-cadherin and upregulation of many transcription factors, including Snail and Slug. While multiple signalling pathways are known to affect the expression of these transcription factors, and thus the maintenance of neural crest cell identity and EMT, the molecular details of how these signals regulate Snail and Slug expression are unknown. We have carried out an overexpression screen in *Xenopus laevis* embryos to look for factors affecting Snail and Slug transcription; changes in transcript levels were detected by Whole-mount In Situ Hybridisation (WISH). We have identified several candidates of interest, as well as already known effectors, which validates the screen. Our goal is to characterise candidates from this screen in order to elucidate detailed molecular events governing EMT in vivo, which should provide further insight into both normal embryonic development as well as the progression of cancers.

64-POS

**CONTROL OF TGF $\beta$  SINGALLING BY SMAD4 UBIQUITINATION****Mamidi, A, Dupont, S, Morsut, L, Cordenonsi, M and Piccolo, S****Department of Medical Biotechnologies - University of Padua - Italy**

We recently described Ectoderm/Tif1 $\gamma$  as an E3 ubiquitin-ligase for Smad4 (Dupont et al., Cell 2005). Here we show that the inhibitory effect of Ecto requires its E3 ubiquitin-ligase activity: point mutations in the RING-finger domain (CAmut) render the protein inactive or a dominant negative. Inhibition of TGF- $\beta$  signals is independent of simple binding to the Smads, as Ecto/Smad2 or Ecto/Smad4 interactions are preserved in the CAmut protein. Moreover, the endogenous ubiquitination pattern of Smad4 in human cells depends on Ecto, as both the ubiquitination pattern of Smad4 is strongly downregulated in cells lacking Ecto, and this effect is rescued by adding-back siRNA-insensitive Ecto. This parallels with biological responses as TGF- $\beta$ -dependent activation of synthetic sensors of the canonical Smad pathway, as well as endogenous genes (i.e. p21Waf1) is greatly enhanced by lack of Ecto/Tif1 $\gamma$ .

## Abstracts

65-POS

### ILEI, A NOVEL CYTOKINE ESSENTIAL FOR TUMOR PROGRESSION: DOES PROTEOLYTIC PROCESSING MATTER?

**Csiszar, A, Goepfert, A, Waerner, T, Alacakaptan, M, Gal, A and Beug, H**

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ILEI (Interleukin-like EMT Inducer) is essential for tumor formation and progression in a murine mammary epithelial cell model (Eph4/EpRas). Stable expression of ILEI in Eph4 and EpRas cells caused EMT, tumor growth and metastasis. RNAi-mediated knock-down of ILEI in EpRas cells before and after EMT (EpRas-XT) prevented and reverted TGFbeta-dependent EMT, also abrogating metastasis formation.

ILEI (FAM3C) belongs to the FAM3 protein family of secreted cytokines. Therefore, the effects of ILEI overexpression in epithelial cells are most probably the consequence of an autocrine action of the secreted protein. However, it was very difficult so far to show this with purified recombinant ILEI. Our aim is to understand the way of ILEI action and thereby find possibilities for potential therapeutic interference with the pathway.

Initially, Western Blot analysis showed that the secreted form of the ILEI protein is smaller in size than intracellular ILEI. Mass spectrometric data confirmed the lack of 17 amino acids at the N-terminus in addition to the signal peptide sequence, giving a strong indication for additional proteolytic processing of ILEI.

Many secreted factors are expressed as precursor proteins, often harbouring N-terminal propeptides, which have to be proteolytically removed in order to get the mature, active form of the protein. On the one hand, such a cleavage can occur by peptidases along the secretory pathway. Mutational analyses of several factors as well as naturally occurring mutations indicate that the lack of proteolytic recognition site for these peptidases inhibits secretion and thus, the action of the cytokine. Alternatively, proteolytic activation can occur extracellularly by secreted or membrane tethered proteases. In this latter case, cytokine action does, but secretion does not depend on proteolytic processing. However, proteolytic cleavage in the extracellular space is also used (e.g. in case of chemokines) to inactivate secreted factors.

To investigate the role of ILEI processing, we generated a series of mutant ILEI forms with the hope being defective in proteolytic cleavage. Using these mutants in overexpression studies we could identify essential amino acids for proteolytic cleavage. In addition, we found that proteolytic cleavage is not essential for ILEI secretion, indicating the possibility of extracellular processing. Surprisingly, all overexpressed non-cleavable ILEI forms showed biological activity. This might indicate that proteolytic processing is not required for ILEI action. The option of inactivating cleavage is currently under investigation as well as protease inhibitor studies are ongoing to narrow down the group of proteases involved in ILEI processing.

66-POS

### ILEI, A NOVEL CYTOKINE ESSENTIAL FOR TUMOR PROGRESSION: AN INDUCIBLE SYSTEM TO STUDY GENE FUNCTION IN EMT AND TUMOR PROGRESSION - ON THE EXAMPLE OF ILEI OVEREXPRESSION

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A common way to study gene function in vitro is to generate stable cell lines with altered levels of gene expression. However, these systems have the risk to select for clonal effects and primary effects often can not be distinguished from downstream events or might be masked by compensatory mechanisms.

Choosing inducible systems for the manipulation of gene expression levels can avoid all this problems. In addition, this approach makes it possible to investigate the kinetics of cellular responses after the induction of gene overexpression or knock down.

Although there are some inducible tumor cell lines available to study EMT, there is not a comprehensive model system which would allow studying several aspects of tumor formation and progression.

We have chosen the Eph4 cell system and generated Tet-ON (T-Rex, Invitrogen) inducible Eph4 (mouse mammary epithelial cells) EpRas (oncogenic Ras transformed Eph4 cells) and EpC40 (Eph4 cells transformed with oncogenic Ras bearing in addition the effector loop mutation C40) cell lines. Through this, we have a series of cell lines representing non-tumorigenic (Eph4), tumorigenic but non-metastatic (EpC40) and metastatic (EpRas) stages. All the generated cell lines maintained the characteristics of the parental cells.

Our gene of interest is ILEI (Interleukine-like EMT Inducer), a secreted cytokine, with an important role in EMT.

Stable overexpression of ILEI in Eph4, EpRas and EpC40 cells caused EMT, tumor growth and metastasis, independent of TGFbeta receptor signalling. However the molecular mechanism of ILEI action is still not known and the stable overexpressing cells are not suitable to answer this question. Therefore, we generated inducible ILEI overexpressing cell lines.

This poster summarizes the in vitro and in vivo characterization of the inducible ILEI overexpressing cell lines and compares it with the findings of the stable ILEI overexpressing cell lines.

67-POS

### ILEI, A NOVEL CYTOKINE ESSENTIAL FOR TUMOR PROGRESSION: CHEMOKINES: KEY PLAYERS IN FUNCION OF THE INTERLEUKINE-LIKE EMT INDUCER (ILEI)?

**Gal, A, Alacakaptan, A, Csiszar, A and Beug, H**

**Radiation Oncology and Biology, Radiobiology Research Institute, Churchill Hospital, Headington, Oxford, OX3 7LJ, United Kingdom**

The Interleukin-like EMT Inducer (ILEI) was identified as an EMT-specific gene in expression profiling for epithelial to mesenchymal transition. ILEI is a secreted protein upregulated exclusively at the translational level by TGF-beta.

Stable ILEI-overexpressing mammary epithelial sublines, Eph4-ILEI and EpRAS-ILEI, proved to have undergone EMT, were found tumorigenic and showed aspects of metastasis (Waerner et al. 2006, Cancer Cell), while NMuMG-ILEI cells showed disruption of epithelial polarity and increased motility.

In search for ILEI-regulated genes by expression profiling, several CC and CXC chemokines were found to be upregulated. These chemokines were proven to significantly facilitate motility and migration of epithelial cells. In 3D collagen culture, chemokine receptor antagonists were able to interfere with the EMT and migratory phenotype of ILEI-overexpressing cells. Investigating signaling pathways downstream of the chemokine receptors, we found Stat3, PI3-kinase and MAPK pathways involved.

In our current working model, TGF-beta induces ILEI which leads to the induction/ rearrangement of the chemokine profile. The finely tuned chemokines play an important role in the effect of ILEI to promote EMT, cell migration and invasive properties.

68-INV

**SMAD PROTEINS OF THE TGF-BETA PATHWAY INSTRUCT A NETWORK OF TRANSCRIPTION FACTORS FOR THE ESTABLISHMENT OF EMT****Moustakas, A, Thuault, S, Vanlandewijck, M, Tan, E-J, Raja, E and Heldin, C-H****Ludwig Institute for Cancer Research, Box 595, BioMedical Center, Uppsala University, Uppsala SE-751 24, Sweden.**

Signal transduction by transforming growth factor (TGF-beta) coordinates many physiological responses in diverse cell types. TGF-beta signals via plasma membrane serine-threonine kinase receptors, the type II and type I TGF-beta receptors, and intracellular signaling effectors, the Smad proteins. TGF-beta inhibits cell proliferation and acts as tumor suppressor during neoplastic growth, but also promotes tumor cell invasiveness and metastasis. Epithelial-mesenchymal transition (EMT) is a differentiation switch that plays important roles during early development and organogenesis, but also assists tumor cell invasiveness and metastasis. Our goal has been the identification of novel gene targets of TGF-beta that critically support execution of the cytostatic and EMT programs.

We have recently reported that the nuclear protein high mobility group A2 (HMGA2), is required for the induction of EMT by TGF-beta (Thuault et al. *J. Cell Biol.* 174:175-83, 2006). HMGA2 regulates a cohort of transcriptional regulators of E-cadherin gene expression and of the EMT process overall, such as Snail and Twist. HMGA2, together with the highly related protein HMGA1, constitute the HMGA family of non-histone architectural nuclear factors that bind to AT-rich DNA sequences. Their expression is high during embryonic development and becomes silent in normal adult tissues. However, HMGA factors are abundantly expressed by oncogenically transformed cells or tumors of mesenchymal and epithelial origin. We will present data on the role of HMGA2 in cell migration of breast carcinoma cells that are highly metastatic.

We have additionally investigated the mechanism by which HMGA2 regulates Snail gene expression. Here, we report that HMGA2 is required for full induction of Snail by TGF-beta. In fact, HMGA2 synergizes with the heteromeric Smad3/Smad4 complex to activate the Snail promoter. The mechanism behind this cooperation involves interaction between these factors leading to an increased binding of Smads to the Snail promoter. Thus, Smad signaling induces HMGA2 protein levels, and subsequently Smads bind to HMGA2 to regulate Snail expression. Following the same combinatorial logic of signal transduction, Snail protein then binds to Smads in order to repress potently expression of E-cadherin. Our model proposes that TGF-beta instructs de novo synthesis of various transcription factors that cooperate with Smads in order to execute the full EMT program and destroy the epithelial phenotype. Whether a similar mode of action operates during the induction of the mesenchymal phenotype remains to be examined.

In addition to Smad signaling, TGF-beta receptors activate several well-established cascades of intracellular serine-threonine kinases, including mitogen activated protein kinases (MAPK). Our current efforts aim at delineating the contribution of specific kinases that become rapidly activated by TGF-beta and regulate components of the polarity complex and of adherens and tight junction disassembly. The final goal is to exploit such enzymes as targets for therapy against carcinoma cell invasiveness and possibly metastasis.

69-INV

**SEARCHING FOR NOVEL REGULATORS OF EMT****Wu, MY, Daly, A and Hill, CS****Developmental Signalling Laboratory, London Research Institute, Lincoln's Inn Fields Laboratories, 44 Lincoln's Inn Fields, London, WC2A 3PX, UK**

Xenopus neural crest cells are multipotent cells that arise at the border of the neural plate and epidermis and which undergo EMT before migrating to their sites of final differentiation. They are readily visualized by staining for markers of EMT, Snail and Slug. In an attempt to discover novel regulators of EMT, we have undertaken a high throughput expression screen in *Xenopus laevis* embryos using an arrayed and normalized library of 7600 full length *Xenopus tropicalis* cDNAs. 160 pools, each containing 48 synthetic mRNAs were injected into single cell embryos and their effect on neural crest development was assayed by staining for Snail expression at the neurula stage. After deconvolution of the positive pools we have now identified 4 new regulators of neural crest development. Three of these act negatively when overexpressed and one acts positively. Their potential role in EMT regulation will be discussed. We have also succeeded in culturing *Xenopus* neural crest cells in vitro and can visualize their delamination and migration. Comparing their behaviour with epithelial cells from the same embryos is allowing us to assess what signaling pathways are required for neural crest differentiation and migration. In addition to the *Xenopus* model we are also studying the regulation of EMT in the context of cancer and are using the Eph4/EpRas model tissue culture system. I will discuss the role played by the TGF $\beta$  signal transducer, Smad3 in determining whether these cells undergo growth arrest in response to TGF $\beta$  or EMT.

## **Abstracts**

**70-SYMP**

### **PDGF LINKS TGF-BETA SIGNALING TO NUCLEAR BETA-CATENIN ACCUMULATION IN HEPATOCELLULAR CARCINOMA PROGRESSION**

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The cooperation of Ras - Erk/MAPK and transforming growth factor (TGF)-beta signaling provokes an epithelial to mesenchymal transition (EMT) of differentiated p19ARF null hepatocytes, which is accompanied by a shift in malignancy and gain of metastatic properties. Upon EMT, TGF-beta induces the secretion and autocrine regulation of platelet-derived growth factor (PDGF) by upregulation of PDGF-A and both PDGF receptors. Here we demonstrate by loss-of-function analyses that PDGF provides adhesive and migratory properties in vitro as well as proliferative stimuli during tumor formation. PDGF signaling resulted in the activation of PI3K, and furthermore associated with nuclear beta-catenin accumulation upon EMT. Hepatocytes expressing constitutively active beta-catenin or its negative regulator axin were employed to study the impact of nuclear beta-catenin. Unexpectedly, active beta-catenin failed to accelerate proliferation during tumor formation, but in contrast, correlated with growth arrest. Nuclear localization of beta-catenin was accompanied by strong expression of the Cdk inhibitor p16INK4A and the concomitant induction of the beta-catenin target genes cyclin D1 and c-myc. In addition, active beta-catenin revealed protection of malignant hepatocytes against anoikis which provides a prerequisite for the dissemination of carcinoma. From these data we conclude that TGF-beta acts tumor-progressive by induction of PDGF signaling and subsequent activation of beta-catenin which endows a subpopulation of neoplastic hepatocytes with features of cancer stem cells.

**71-SYMP**

### **THE TRANSCRIPTION FACTOR SNAIL REPRESSES CRUMBS3 EXPRESSION AND DISRUPTS APICO-BASAL POLARITY COMPLEXES**

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Cell polarization results in the asymmetric distribution of proteins, lipids, and carbohydrates. A specialized intercellular junctional complex in epithelial tissues, termed the tight junction, divides the plasma membrane into distinct apical and basolateral domains. The tight junction also mediates adhesion between adjacent cells and prevents free diffusion across the epithelium. Polarization is essential for epithelial cell function, and cell polarity is lost during the epithelial to mesenchymal transition (EMT), a program of events characterized not only by loss of cell polarity, but also by enhanced cell motility and increased cell invasion. Among several apically localized protein complexes, the Crumbs and Par protein complexes have pivotal roles in control of epithelial polarity and apical membrane formation. Here, we demonstrate that the Snail transcriptional repressor antagonizes expression of the Crumbs polarity complex. We show that Snail abolishes localization of the Crumbs and Par complexes to the tight junction, decreases Crumbs complex protein levels, and suppresses Crumbs3 transcription. Evidence that Snail acts directly to antagonize Crumbs3 transcription is presented. Our findings provide new insights into the links between the transcriptional repression function of Snail and its role in antagonizing epithelial cell polarity factors during EMT.

**72-INV**

### **THE PAR/TIAM1 POLARITY COMPLEX CONTROLS EPITHELIAL AND MESENCHYMAL PROPERTIES OF EPITHELIAL CELLS.**

**Collard, J**

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Invasion and metastasis of carcinomas is often associated with reduced or loss of E-cadherin-mediated cell-cell adhesions. A balance between invasion-inhibitory cell-cell interactions and invasion-promoting cell-substrate interactions determines epithelial-mesenchymal transition (EMT) and the degree of invasion and migration of epithelial cells. Rac and Rho small GTPases play antagonistic roles in epithelial-mesenchymal transition. Activation of Rac promotes cell spreading and firm cadherin-based cell-cell adhesions whereas activation of Rho leads to cell contraction and a mesenchymal migratory phenotype.

Recently we found that the conserved Par-polarity complex (Par3, Par6 and PKCz) in conjunction with the Rac activator Tiam1 controls apical-basal cell polarity in contacting epidermal keratinocytes by regulating Tight Junctions (TJ) formation. Interfering in Tiam1- or Par signaling leads to EMT but little is known on the function of Tiam1 and the Par polarity complex in migrating epithelial cells. Intriguingly, we found that keratinocytes rapidly migrate in a linear and persistent polarized fashion. In contrast, keratinocytes lacking Tiam1 or Par3 are impaired in sustaining front-rear polarization and hence migrate in a non-linear (random) fashion. Thus, the Par3/Tiam1 complex appears not to be required for migration per se but specifically controls the directionality of migrating cells. Consistent with this observation we found that keratinocytes lacking Tiam1 or Par3 do not migrate towards a chemotactic gradient.

We conclude that the Par/Tiam1 complex stabilizes front-rear polarization of non-contacting migratory keratinocytes thereby stimulating persistent and chemotactic migration while in contacting keratinocytes the same complex controls the establishment of long-lasting apical-basal polarity. These findings underscore a remarkable flexibility of the Par polarity complex that depending on the biological context, controls distinct forms of cellular polarity.

73-INV

## REGULATION OF EMT AND WNT SIGNALING BY INTEGRIN-LINKED KINASE

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Previous studies have shown that overexpression of Integrin-Linked Kinase (ILK), in epithelial cells, results in EMT. In addition, ILK plays a central role in EMT induced by a several growth factors and cytokines such as endothelin-1 and TGF-beta. ILK over expression also promotes the nuclear stabilization of beta-catenin and stimulation of Tcf-mediated gene transcription. We have now explored whether ILK directly modulates Wnt-mediated signaling using both in vitro and in vivo models. We find that ILK is required for Wnt 3a mediated inhibition of beta-catenin phosphorylation and nuclear accumulation, and that ILK accelerates Wnt-1 mediated mammary tumor formation in mice. Using SILAC/Mass spectrometry based proteomics, as well as DNA microarray analysis we have now identified novel interactors of ILK, which also modulate Wnt signaling, and we have also identified genes that are the targets of combined Wnt/ILK signaling in the promotion of mouse mammary tumors.

These novel data will be discussed, along with data suggesting an important role of ILK in EMT associated resistance of non small cell lung cancer and hepatocarcinoma cells to EGFR inhibitors.

74-SYMP

## EMT AND ONCOGENIC SIGNALING BY TGF-BETA IN MAMMARY EPITHELIAL CELLS

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TGF- $\beta$  regulates all stages of mammary gland development and suppresses breast cancer formation by preventing cell cycle progression in mammary epithelial cells (MECs). However, genetic and epigenetic events often negate the cytostatic function of TGF- $\beta$  in MECs, an event that ultimately enables malignant MECs proliferate, invade, and metastasize when stimulated by TGF- $\beta$ . The molecular mechanisms underlying this phenotypic conversion of TGF- $\beta$  function during mammary tumorigenesis remain poorly defined. We recently discovered that the expression and activity of  $\alpha\beta$ 3 integrin and Src are essential for TGF- $\beta$  stimulation of MEC proliferation, invasion, and epithelial-mesenchymal transition (EMT). Mechanistically,  $\beta$ 3 integrin interacts physically with the TGF- $\beta$  type II receptor (T $\beta$ R-II), leading to its Tyr phosphorylation by Src, and to its activation of MAP kinases, particularly that of p38 MAPK. Accordingly, expression of wild-type or inactive  $\beta$ 3 integrins enhances or abolishes, respectively, the ability of TGF- $\beta$  to induce invasion in normal and malignant MECs. Thus, we have defined a novel  $\alpha\beta$ 3 integrin:SRC:T $\beta$ R-II signaling axis that promotes oncogenic signaling by TGF- $\beta$  in MECs. We further show that oncogenic messages transduced via this signaling axis requires Src to phosphorylate T $\beta$ R-II on Tyr284. Indeed, although expression of Y284F-T $\beta$ R-II mutants in breast cancer cells has no effect on TGF- $\beta$  stimulation of Smad2/3 signaling, this T $\beta$ R-II mutant completely abrogates p38 MAPK activation by TGF- $\beta$ . Moreover, Src-mediated phosphorylation of Y284 coordinates the docking of the SH2 domains of Grb2 and Shc to T $\beta$ R-II, thereby identifying a novel signaling specificity determinant in T $\beta$ R-II that dissociates the ability of TGF- $\beta$  to activate Smad2/3 from that of MAP kinases. Accordingly, Grb2-deficient MECs fail to activate p38 MAPK, and consequently, to undergo EMT and invasion when stimulated by  $\alpha\beta$ 3 integrin and TGF- $\beta$ . Similarly, expression of Y284F-T $\beta$ R-II mutants also abrogates breast cancer cell invasion induced by  $\alpha\beta$ 3 integrin and TGF- $\beta$ , as well as partially restores their cytostatic response to TGF- $\beta$ . Finally, we find that TGF- $\beta$  stimulation of breast cancer growth and their metastasis to the lungs in mice requires phosphorylation of Y284F in T $\beta$ R-II. Collectively, our findings have identified a novel  $\alpha\beta$ 3 integrin:Src:phosphoY284F-T $\beta$ R-II:Grb2 signaling axis that mediates oncogenic signaling by TGF- $\beta$  in MECs, and suggest that antagonizing this signaling axis may one day prove beneficial in treating patients with metastatic breast cancer.

75-INV

## INVASIVE GROWTH: A MET-DRIVEN GENETIC PROGRAMME FOR CANCER AND STEM CELLS

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Metastasis follows the inappropriate activation of a genetic programme termed Invasive Growth, which is a physiological process –also known as Epithelial Mesenchymal Transition- that occurs during embryonic development and post-natal organ regeneration. Burgeoning evidence indicates that invasive growth is also executed by stem and progenitor cells, and is usurped by cancer stem cells. The MET proto-oncogene, which is expressed in both stem and cancer cells, is a key regulator of invasive growth. Recent findings indicate that the MET tyrosine-kinase receptor is a sensor of adverse microenvironmental conditions (such as hypoxia) and drives cell invasion and metastasis through the transcriptional activation of a set of genes that control blood coagulation.

## Abstracts

76-INV

### EMT IN THE NEURAL CREST MODEL: DOES FUNCTIONAL MANIPULATION OF ADHESION AND CYTOSKELETAL SYSTEMS RESET THE SENSITIVITY TO PRO-EMT GROWTH FACTORS?

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The neural tube epithelium undergoes an archetypal EMT to generate migratory neural crest cells. This is normally induced by exposure to growth factors (FGFs, Wnts, BMPs). A number of transcription factors such as members of the Snail and SoxE families are induced and when expressed in vivo these can induce EMT, indicating that they trigger and coordinate the many downstream effects of EMT. These downstream effects in the nascent neural crest cells include a reduction of N-cadherin based cell-cell adhesions, an alteration in the spectrum of integrins expressed and lowered adhesion to ECM, and a loss of apico-basal and circumferential F-actin cytoskeletal distribution. In addition, there are changes in ECM synthesis, assembly and degradation. This EMT can be replicated in detail in simple tissue culture of avian neural epithelium. Cells emigrating distantly from avian embryonic epithelial neural tube explants remain epithelial, but slowly change from N-cadherin+/vimentin+ to an E-cadherin+/cytokeratin+ state, indicating transdifferentiation to epidermal ectoderm: these cells are then refractory to EMT stimuli. In contrast cells emigrating proximal to the neural tube explant undergo spontaneous EMT with SoxE and Snail2 expression. The proportion of emigrant cells undergoing EMT is amplified by exogenous inducer BMP4 and reduced by exogenous BMP4 inhibitor Noggin, suggesting that the original explant is a source of BMP4 sufficient to drive EMT in near but not distant cells. Experimental manipulation of downstream effectors by i) reduction of cadherin based cell-cell adhesions by transient EGTA, or ii) reduction of adhesion to ECM by anti-integrin antibodies and peptides, or iii) dissociation of F-actin bundles by aPKC or Rho inhibition (staurosporine, C3, Y-27632), all induce EMT. This EMT includes changes in all downstream effectors and also in expression of nominally upstream transcription factors. These results are consistent with EMT-like alterations of individual downstream EMT effector systems increasing the sensitivity to pro-EMT growth factors such as BMP4.

77-SYMP

### THE MICRORNA-200 FAMILY REGULATES THE E-CADHERIN REPRESSORS, ZEB1/DELTAEF-1 AND SIP1/ZEB2 AND EMT

**Philip A Gregory, Andrew G Bert, Emily L Paterson, Anna Tsykin, Mathew A Vadas, Yeessim Khew-Goodall and Gregory J Goodall**  
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The MDCK kidney epithelial cell line undergoes EMT in response to TGF- $\beta$  treatment, or overexpression of the protein tyrosine phosphatase, Pez. We used microRNA microarrays to identify microRNAs whose expression is regulated when MDCK cells undergo EMT. We found the 5 members of the miR-200 family, which are highly related in sequence, and are clustered at two sites in the genome, are drastically down-regulated in cells that have undergone EMT. MicroRNA-205, which is unrelated to the miR-200 family, was also drastically down-regulated. We show that the miR-200 family microRNAs repress expression of the E-Box-binding transcription repressors, ZEB1 and SIP1, while miR-205 further represses ZEB1. Down-regulation of the microRNAs in response to Pez or TGF- $\beta$  alleviates repression of ZEB1 and SIP1, leading to repression of E-cadherin, induction of fibronectin, and EMT, including changes in cell shape, motility and invasiveness. Ectopic expression of miRNA-200b, alone or in conjunction with miR-200a and miR-205, reverts invasive mesenchymal MDCK-Pez cells and MDA-MD-231 breast cancer cells towards a non-invasive, epithelial phenotype. Conversely, inhibition of the microRNAs drives epithelial MDCK cells into EMT. Our data indicate that the epithelial microRNAs miR-205 and the miR-200 family act as reinforcers of the epithelial phenotype, and their down-regulation is necessary, and in certain cells, sufficient, to drive cells into EMT. This finding has implications for controlling tumour metastasis.

78-INV

### DISTINCT MECHANISMS OF TUMOR INVASION AND METASTASIS

**Lehembre, F, Wicki, A, Yilmaz, M, Grotegut, S, Kren, A, Fantozzi, A, Achermann, C and Christofori, G**

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Tumor cell invasion into the surrounding tissue can exhibit a phenotype of either single cell or collective cell migration. Single cell migration is mainly dependent on signaling pathways within migrating cells themselves and is usually accompanied by an epithelial-mesenchymal transition (EMT), which involves several genetic and epigenetic alterations including the loss of E-cadherin and the gain of N-cadherin expression (the cadherin switch). Gene expression profiling experiments together with biochemical analysis reveal that the distinct stages of EMT are tightly regulated by epistatic cascades of transcriptional control circuits involving activation and repression of a large number of genes that modulate the migratory and invasive behavior of tumor cells.

In contrast, collective cell migration requires the maintenance of cell-cell adhesion and a certain multicellular organization of the tumor tissue. Recently, we have shown that the expression of podoplanin, a small mucin-like protein, is upregulated in a number of human carcinomas, in particular squamous cell carcinomas. We have investigated podoplanin function in cultured human breast cancer cells, in a mouse model of pancreatic beta cell carcinogenesis, and in human cancer biopsies. Podoplanin induces tumor cell spreading, migration and invasion in vitro and in vivo by a novel molecular pathway that does not involve the loss of E-cadherin function or EMT. Furthermore, it induces actin cytoskeleton rearrangement and the formation of filopodia by modulating the activities of Rho-family GTPases, which ultimately leads to collective cell invasion, a phenotype often observed in human squamous cell carcinomas.

Finally, we propose a third type of metastatic tumor cell dissemination which involves an upregulated expression of lymphangiogenic factors, such as vascular endothelial growth factors C and D, and the subsequent induction of lymphangiogenesis. Increased lymphatic vessel density within and around expanding tumors leads to lymph node metastasis, most likely by a passive washing out and trapping of tumor cell clusters in regional lymph nodes. In conclusion, we propose the existence of at least three distinct mechanisms of metastatic tumor cell invasion and dissemination: single cell invasion involving EMT, collective cell migration in the absence of EMT, and the lymphogenic dissemination to local lymph nodes.



## Abstracts

82-SYMP

### THE TRANSCRIPTION FACTOR ZEB1 PROMOTES TUMOR PROGRESSION BY REPRESSING MASTER REGULATORS OF EPITHELIAL DIFFERENTIATION

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For most carcinomas, progression toward malignancy is accompanied by loss of epithelial differentiation and a shift towards a motile and invasive mesenchymal phenotype. This process, referred to as epithelial to mesenchymal transition (EMT), is likely caused by a pathological activation of transcription factors regulating EMT in embryonic development.

The zinc finger and homeobox containing transcriptional regulator ZEB1 has previously been shown to possess pleiotropic functions in embryonic development and adult tissue homeostasis including T-cell development, skeletal patterning, myogenesis, neurogenesis, corneal physiology and tumor progression. To analyze ZEB1's role in tumor cell plasticity and EMT we have identified transcriptional targets of ZEB1 in human cancer cells. We show that ZEB1 directly repressed the expression of the major intercellular adhesion molecule E-cadherin as well as many additional key determinants of epithelial cell-cell adhesion and differentiation, including the cell polarity genes Crumbs3, LGL2 and PATJ. ZEB1 associated with their endogenous promoters *in vivo*, and strongly repressed promoter activities in reporter gene assays. ZEB1 downregulation in undifferentiated cancer cells by RNA interference was sufficient to upregulate the expression of these epithelial-specific genes on the RNA and protein level. As a consequence affected cancer cells re-established epithelial features and exhibited impaired cell motility *in vitro*.

In human colorectal cancer specimen, ZEB1 expression was limited to the tumor-host interface and was accompanied by loss of intercellular adhesion and tumor cell invasion. In invasive ductal and lobular breast cancer samples upregulation of ZEB1 was stringently coupled to cancer cell dedifferentiation. In summary our data show that ZEB1 represents a key player in pathologic EMTs associated with tumor progression.

83-INV

### CELL MOTILITY AT THE INVASIVE FRONT OF TUMORS: THE ROLE OF NOVEL WNT/ $\beta$ -CATENIN TARGET GENES

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Aberrant  $\beta$ -catenin signaling is prevalent in most colorectal cancer (CRC) patients.  $\beta$ -catenin, an important cell-cell adhesion component, binding cadherin receptors to the cytoskeleton, is also a key co-transcriptional activator of target genes (in complex with LEF/TCF factors) in the nucleus. Hyperactive  $\beta$ -catenin induces genes regulating the cell cycle and the invasion and metastasis of cancer cells. We investigated the coordination between cell-cell adhesion and signaling by the E-cadherin/ $\beta$ -catenin molecular complex in CRC development. We found that sparse cell cultures mimic cells at the invasive front of tumors displaying low levels of E-cadherin, but highly active nuclear  $\beta$ -catenin that transactivates Slug, a negative transcriptional regulator of E-cadherin. Slug is also induced during EMT. In sparse cells, we found high levels of ErbB1/2 and activated ERK. In contrast, dense cultures had distinct membranous E-cadherin and  $\beta$ -catenin, only limited  $\beta$ -catenin signaling, no Slug, and therefore high E-cadherin levels, but low levels of ErbB1/2 and MAPK signaling. This cell density-regulated phenotypic conversion is reminiscent of the plasticity in  $\beta$ -catenin and E-cadherin in the invasive versus differentiated areas of CRC tissue. We identified two members of the L1 immunoglobulin-like cell adhesion receptors (normally expressed in nerve cells), as novel target genes of  $\beta$ -catenin in CRC. We found these proteins exclusively localized at the invasive front of CRC tissue. Forced expression of L1 and Nr-CAM in fibroblasts induced motility, transformation, and conferred tumorigenesis in mice and, in colon cancer cells, caused liver metastasis. Suppression of L1, and of Nr-CAM, in cancer cells, reduced their motility and invasion. The ectodomain of Nr-CAM was sufficient for conferring growth in low serum by constitutively activating MAPK/ERK and AKT, and stable expression of the shed Nr-CAM ectodomain transformed NIH3T3 cells that also formed tumors in mice. We identified L1 and the metalloproteinase ADAM10 (a novel  $\beta$ -catenin target gene) that cleaves its ectodomain at the invasive front of CRC tissue and showed that their co-expression enhances metastasis to the liver. We suggest that colon cancer cells exploit opportunistically these "neuronal" cell adhesion molecules, whose transcription is aberrantly activated by  $\beta$ -catenin - to promote metastasis. Since the ectodomains of L1 and Nr-CAM are often shed by metalloproteinases, they could serve as diagnostic markers and anticancer therapy targets.

84-INV

### PDGF-DEPENDENT CANCER FIBROBLASTS AND PERICYTES AS NOVEL CANCER DRUG TARGETS

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Tumor growth and metastasis depend on interactions between malignant cells and the tumor stroma. PDGF-receptors are commonly expressed on tumor fibroblasts and pericytes. PDGF stimulation of pericytes increases tumor vessel pericyte coverage, which improves vessel function, and increases tumor growth. Furthermore, inhibition of stromal PDGF receptors alters leads to increased tumor drug uptake, presumably caused by the PDGF-antagonist-induced reduction in tumor interstitial fluid pressure. To facilitate clinical development of these findings a novel method for monitoring PDGF receptor phosphorylation *in situ* was developed.

To further explore the therapeutic potential of cancer fibroblast-derived signaling a project was initiated which combines laser-capture microdissection of normal and tumor fibroblasts, RNA amplification, array analyses and functional evaluation of differentially expressed genes. Three novel candidate secreted prostate cancer drug targets was identified. Among these CXCL14 was shown to promote tumor growth by multi-modal mechanisms involving direct stimulation of fibroblasts, as well as enhancement of the ability of fibroblasts to promote migration of the malignant cells and to enhance angiogenesis.

References: Heldin et al., Nat Rev Cancer, 4, 806-813 (2004); Östman and Heldin, Adv Cancer Res, 97, 247-274 (2007); Micke et al., J Invest Derm, 127, 1515-1523, (2007); Jarvius, Mol Cell Prot, e-pub ahead-of -print, (2007)

85-SYMP

**BETA-ARRESTIN-1 AS MESSENGER OF ENDOTHELIN A RECEPTOR-DRIVEN BETA-CATENIN SIGNALING PATHWAY AND EPITHELIAL TO MESENCHYMAL TRANSITION: IMPLICATION FOR AN EFFECTIVE COMBINED THERAPY IN OVARIAN CANCER**

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The endothelin A receptor (ETAR)/endothelin-1 (ET-1) axis has a key role in the pathophysiology of ovarian carcinoma. Among the intracellular pathways activated by ET-1, the epidermal growth factor receptor (EGFR) transactivation, represents a downstream signaling in driving ovarian cancer progression. Recently, it has become evident that beta-arrestin-1 promotes signal transduction by G-protein-coupled receptors acting as multifunctional adaptor protein. Here, we examined a new functional role of beta-arrestin-1 in the cross-communication between ETAR and EGFR to regulate beta-catenin signaling and EMT in ovarian cancer. We found that in ovarian cancer cells, ET-1 induced the membrane translocation of beta-arrestin-1, leading to an ETAR/beta-arrestin-1/Src signaling complex ("signalplex") formation. Using WT- or mutant beta-arrestin 1 constructs, we showed that this signalplex was crucial for EGFR transactivation, that, in turn, controls beta-catenin protein stabilization through its tyrosine phosphorylation. This phospho beta-catenin can translocate to the nucleus and bind the TCF4 transcription factor, thus representing a transcriptional active pool. Silencing of beta-arrestin-1 with siRNA reduced beta-catenin transcriptional activity and cell invasion, indicating that beta-arrestin-1 is a necessary component for ET-1-induced for beta-catenin signaling and enhanced aggressive behavior. Moreover, both ETAR antagonists and ETAR siRNA prevented the signalplex formation, causing cytosolic retention of unphosphorylated beta-catenin and its impaired transcription. In human ovarian carcinoma xenografts, ETAR blockade by specific ETAR antagonist, ZD4054, significantly inhibited tumor growth, peritoneal dissemination and expression of EMT effectors. The in vitro results provided the basis to test combined targeting of ETAR by ZD4054, and of EGFR, by the EGFR inhibitor gefitinib. The coadministration of ZD4054 and gefitinib led to partial or complete tumor regression and inhibition of EMT and metastatic potential on HEY ovarian carcinoma xenografts, indicating new effective therapeutic opportunities for ovarian cancer patients. Supported by AIRC, Italian Ministry of Health.

86-SYMP

**LOXL2 AS A PLAYER IN EPIDERMAL HOMEOSTASIS AND EARLY MARKER OF SQUAMOUS CELL CARCINOMAS**

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We have recently described that Lysyl-oxidase like 2 (LOXL2), a member of the lysyl-oxidase gene family, interacts and cooperates with Snail1 to downregulate E-cadherin expression, indicating a functional cooperation between LOXL2 and Snail1 in the invasive behaviour and tumour cell malignancy. To further investigate the function of LOXL2 in tumour progression we performed organotypic cultures in vitro and in vivo using highly malignant mouse epidermal keratinocytes (HaCa4 cells) in which LOXL2 was interfered by shRNA. Silencing of LOXL2 in HaCa4 cells promotes a dramatic reversion from an epithelioid to a fully differentiated epithelial cell phenotype with the ability to reproduce a "skin-like" multilayered organization in three-dimensional cultures. Analysis of LOXL2 expression in a cohort of mouse skin carcinogenesis derived tumours indicates a positive correlation between LOXL2 and Snail1 expression associated to malignant transformation and tumour progression. These data indicate a main role of LOXL2 in the regulation of keratinocyte differentiation and homeostasis and strongly support that LOXL2 could be considered as a malignancy marker in mouse skin carcinogenesis. Moreover, analysis of LOXL2 expression in a series of human oral squamous cell carcinomas (OSCC) (n=250) demonstrates that LOXL2 could be considered as an early marker in human squamous cell carcinomas and as a new potential anticancer target.

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87-INV

**THE EFFECTS OF BMP-7 ON ENDMT AND CARDIAC FIBROSIS**

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Cardiac fibrosis, associated with a decreased extent of microvasculature and with disruption of normal myocardial structures, results from excessive deposition of extracellular matrix, which is mediated by the recruitment of fibroblasts. The source of these fibroblasts is unclear and specific anti-fibrotic therapies are not currently available. Here we show that cardiac fibrosis is associated with the emergence of fibroblasts originating from endothelial cells, suggesting an endothelial-mesenchymal transition (EndMT) similar to events that occur during formation of the atrioventricular cushion in the embryonic heart. Transforming growth factor-beta1 (TGF-beta1) induced endothelial cells to undergo EndMT, whereas bone morphogenic protein 7 (BMP-7) preserved the endothelial phenotype. The systemic administration of recombinant human BMP-7 (rhBMP-7) significantly inhibited EndMT and the progression of cardiac fibrosis in mouse models of pressure overload and chronic allograft rejection. Our findings show that EndMT contributes to the progression of cardiac fibrosis and that rhBMP-7 can be used to inhibit EndMT and to intervene in the progression of chronic heart disease associated with fibrosis.

## Abstracts

88-INV

### SLUG REGULATES EARLY EPITHELIAL DIFFERENTIATION BY MAINTAINING A METASTABLE PHENOTYPE

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It has become clearer in the recent years that epithelial cells differentiation is mediated from progenitor cells linked to elusive stem cells, progressively identified in several organs. Progenitor cells are already partially committed and can participate to organ morphogenesis through migration and proliferation. Such epithelial precursors adopt an intermediate metastable phenotype along a partial epithelial-mesenchymal transition (EMT) characterized by the maintenance of cell-cell adhesion structures that do not preclude active migration and further differentiation. Several clues point to a causal role for transcription factor Slug during early differentiation process. 1) We found Slug to be located in precursor basal keratinocytes during early phases of skin stem cell expansion, and loss of Slug in knockout mouse provokes aberrant wound healing reepithelialization, a process requiring basal keratinocyte migration. 2) We also found Slug to be expressed by epithelial cell sub-populations involved in mammary tubulogenesis. Accordingly, we found that Slug deprived mice display a deficient mammary tubulogenesis. Recently, we found that this phenotype modulation could reflect specific pathways such as Slug control by EGF/Erk5 pathway and specific targets for Slug, such as P-cadherin in epithelial cells. P-cadherin is known to maintain a basal epithelial phenotype necessary for normal tubulogenesis.

89-SYMP

### GENE REGULATION NETWORK ANALYSIS SUGGESTS EPIGENETIC MECHANISM FOR SILENCING OF TIMP3 IN EMT

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We study EMT-related gene regulation programs using in vitro and in vivo models of epithelial injury and fibrogenesis, and high-capacity profiling of mRNA, microRNA and protein expression patterns. First, we report a TGF- $\beta$ -specific microRNA signature that includes miR-21 and miR-145, both upregulated in several models of EMT/fibrogenesis. Oncogenic microRNA miR-21 has been previously reported as consistently upregulated in human carcinomas and in other tumor types such as human uterine leiomyomas. Next, we show by chromatin immunoprecipitation that the miR-21 promoter is directly targeted by TGF- $\beta$ /Smad signaling and that miR-21 may contribute to cancer progression by targeting tissue inhibitor of metalloproteinase-3 (TIMP3), leading in turn to increased matrix metalloproteinase-mediated degradation of extracellular matrix. Furthermore, hyper-methylation of a CpG island within the TIMP3 promoter is frequently associated with advanced tumorigenesis. We present data to support a working model of a multistep silencing of TIMP3 in an EMT context involving TGF- $\beta$ , miR-21 and DNA methyltransferase-1 activity (DNMT1). Our approach of comprehensive identification of the EMT/fibrogenesis gene regulatory networks may provide molecular targets - such as tumor suppressor genes and regulators of migration and/or motility - for the future therapeutic approaches against renal epithelial injury, carcinoma progression and fibrosis.

90-INV

### REGULATION OF EMT IN THE ATRIOVENTRICULAR CANAL OF THE DEVELOPING HEART

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Transition of endothelia in the developing heart to form progenitors of the mitral and tricuspid valves is a prototypical EMT. Adjacent ventricular endothelia do not undergo EMT but are capable after expression of specific TGF $\beta$  receptors. Atrioventricular myocardium secretes an inductive signal that includes hyaluronan, neuregulin, HGF, transferrin, BMP2/4 and both TGF $\beta$ 2 and TGF $\beta$ 3. We have focused on TGF $\beta$  superfamily members in regulating this EMT. We found that TGF $\beta$ 2 acts as an activation signal to upregulate slug, inhibit ve-cadherin and initiate cellular hypertrophy and cell separation. TGF $\beta$ 3 subsequently acts on activated endothelia to regulate invasion via upregulation of transcription factors, meox1, paraxis and runx2 and others. Using these markers, we confirmed the independence of the TGF $\beta$  isoforms. Recent findings suggest that LTBP-1 is required for activation of both TGF $\beta$ s within the ECM. Further, BMP2/4 and TGF $\beta$ 3 are redundant in regulation of cell invasion. Studies with TGF $\beta$  receptors place T $\beta$ RIII as a receptor for TGF $\beta$ 2 and T $\beta$ RII as a receptor for TGF $\beta$ 3. T $\beta$ RII interacts with Alk-2 to signal via the Smad1/5 pathway during the invasion step of EMT. Another type I receptor, Alk-5, appears to interact with T $\beta$ RIII as loss of Alk-5 blocks the separation/activation step of EMT. Though we have not directly shown that Smad2/3 is required for this step, loss of Sara as a cofactor of Smad2 signaling blocks activation. Loss of endoglin, an additional TGF $\beta$  receptor in endothelium, blocks both activation and invasion steps of EMT. Recently, others showed that Notch receptors regulate EMT in the heart. Notch2 has a regional distribution consistent with regulation of EMT and loss of this Notch by siRNA disrupts TGF $\beta$  ligand and receptor expression. We hypothesize that Notch functions early in heart development to provide regional specification of TGF $\beta$  ligands and receptors to differentiate atrioventricular and ventricular endothelia and enable regional EMT.

91-SYMP

**DELINEATION OF GTPASE PATHWAYS IN EMT: A ROLE FOR EVERY RHO?****Hutchison, N, Hendry, BM and Sharpe, CC****Renal Medicine, King's College London, Bessemer Road, London, SE5 9PJ, UK**

Decline in renal function correlates closely with tubular-interstitial fibrosis, a process driven by the prevalence and activity of myofibroblasts. Mounting evidence suggests these cells derive in part from a mesenchymal transition of proximal tubular epithelial cells. Rho/Rho kinase has been previously implicated in the pathogenesis of EMT so we have used small interfering RNA molecules to delineate the specific roles of the three Rho isoforms, RhoA, RhoB and RhoC.

Using a model of human proximal tubular epithelial cells, we have induced EMT with a combination of TGF-beta1 and EGF resulting in a loss of E-cadherin and an upregulation of alpha-SMA by day 5. By silencing the expression of RhoA, this loss of E-cadherin can be accelerated with no detectable protein or mRNA expression by 48 hours. Interestingly, silencing RhoC results in an equally rapid loss of E-cadherin expression at the protein level whilst maintaining expression at the RNA level. RhoA is also necessary for the induction of alpha-SMA whereas RhoC and RhoB are not. RhoC (not RhoA), is primarily responsible for the reorganization of the actin cytoskeleton into stress fibers. Silencing the expression of RhoB (not RhoA or RhoC) causes a 3-fold increase in apoptosis as measured by Hoechst staining, annexin V binding and activation of caspase3 (p less than 0.05). On a functional level, silencing both RhoC and RhoA reduces TGF-beta1-stimulated cell migration by 66% (p less than 0.01) and 47% (p less than 0.05) respectively. Similarly RhoC siRNA reduces cell proliferation by 30 % (p less than 0.01) compared to 20% with the RhoA siRNA.

We conclude that there is a complex interaction between Rho species and the various facets of EMT in renal fibrosis. The different isoforms of Rho are functionally distinct and may be key foci in the overall pathogenesis of this process. Delineating the differences between these isoforms will increase the understanding of the complex nature of the control of cell phenotype and can ultimately aid in the targeted-design of new therapeutics.

92-SYMP

**NEW CONCEPTS IN DESMOSOMAL ADHESION AND THEIR IMPLICATIONS FOR EMT****Garrod DR, Kimura TE, Nie Z and Merritt AJ****Faculty of Life Sciences, University of Manchester, Michael Smith Building, Oxford Road, Manchester, M139PT, UK**

We have developed a new concept in cell adhesion. Hyper-adhesion refers to the very strong adhesion that is necessary to maintain the integrity of tissues such as epidermis and cardiac muscle that are continually subjected to potentially disruptive mechanical forces. It is a characteristic of the intercellular junctions known as desmosomes and, as far as we are aware, distinguishes them from all other types of intercellular junction. Desmosomes can adopt two different adhesive states. In one they are relatively weakly adhesive, their adhesion resembling that found in adherens junctions; in the other, the hyper-adhesive state, they are more strongly adhesive and this is characteristic of the majority and perhaps all desmosomes in epithelial cell sheets, both in culture and in vivo. New evidence relating to the acquisition of hyper-adhesiveness during mouse early development and epidermis, and its regulation by protein kinase C will be presented. In addition, new data showing that the molecular mechanism of desmosomal adhesion in epithelial cells involves homophilic, isoform-specific binding by the desmosomal cadherins will be described. The implications of these findings for the behaviour of epithelial cells, wound healing and epithelial-mesenchymal transition will be discussed.

93-INV

**THE ROLE OF IGF/AKT PATHWAYS IN THE INDUCTION OF EPITHELIUM MESENCHYME TRANSITION****Lionel Larue****Institut Curie, Bat 110, Orsay, Paris, 91405, France**

Carcinoma progression is associated with the loss of epithelial features, and the acquisition of mesenchymal characteristics and invasive properties by tumour cells. The loss of cell-cell contacts may be the first step of the epithelium mesenchyme transition (EMT) and involves the functional inactivation of the cell-cell adhesion molecule E-cadherin. Repression of E-cadherin expression by the transcription factor Snail is a central event during the loss of epithelial phenotype. Akt kinase activation is frequent in human carcinomas and Akt regulates various cellular mechanisms including EMT. Here, we show that Snail activation and consequent repression of E-cadherin may depend on AKT-mediated NF-kB activation, and that NF-kB induces Snail expression. Expression of the NF-kB subunit p65 is sufficient for EMT induction, validating this signalling module during EMT. NF-kB pathway activation is associated with tumour progression and metastasis of several human tumour types; E-cadherin acts as a metastasis suppressor protein. Thus, this signalling and transcriptional network linking AKT, NF-kB, Snail and E-cadherin during EMT is a potential target for antimetastatic therapeutics.

## Abstracts

94-INV

### THE ADHESION JUNCTION PROTEIN E-CADHERIN PLAYS A CENTRAL ROLE IN THE PROCESS OF EPITHELIAL MORPHOGENESIS

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The adhesion junction protein E-cadherin plays a central role in the process of epithelial morphogenesis. Function of this protein is controlled by the expression of the transcriptional repressor Snail1 that binds to three E-boxes present in E-cadherin promoter. Snail1 overexpression in epithelial cell lines induces an epithelial-mesenchymal transition (EMT), down-regulating E-cadherin and other epithelial genes, and increasing the expression of mesenchymal genes, such as Fibronectin, LEF1 or Zeb1, another transcriptional repressor capable to block E-cadherin expression. We show here that Snail1 also up-regulates the levels of Zeb2, another transcriptional repressor of E-cadherin, by a complex mechanism requiring the different splicing of Zeb2 mRNA.

The activation of mesenchymal genes by Snail1 is dependent on the down-regulation of E-cadherin; over-expression of this protein blocks Fibronectin, LEF1 or Zeb1 expression. Our results show that E-cadherin blocks the transcriptional activity of NFkB and beta-catenin, two factors involved in the expression these mesenchymal genes. Similarly to beta-catenin, NFkB is also detected associated to the E-cadherin-junctional complex; disruption of this complex increases the transcriptional activity of NFkB.

Snail1 expression in epithelial cells increases the resistance to several types of apoptosis. We have identified the apoptosis-related gene PTEN as a target of Snail1-transcriptional repression. Snail1 binds to PTEN promoter and prevents the expression of this gene induced by p53 after gamma irradiation. Our results suggest also that Snail1 protein is stabilized and up-regulated after irradiation

95-INV

### FUNCTIONAL ANALYSIS OF EMT-INDUCING TRANSCRIPTION FACTORS IN INVASION AND METASTASIS

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Recent research has shown a role for the Snail and deltaEF1 family of transcription factors in mediating epithelial mesenchymal transitions (EMT), migration and cell survival. During EMT, epithelial cell layers lose polarity and cell-cell contacts, undergo extensive cytoskeletal reorganization, express mesenchymal features, and manifest a migratory phenotype. EMT has been attracting increasing attention as a determinant of the progression of epithelial malignancies, which fits very well with the action spectrum of Snail and deltaEF1 family members. Furthermore, the identification of Snail, Slug, SIP1 and deltaEF1 as transcriptional repressors of E-cadherin, and in vitro cellular analysis, indicate a potential role in tumor progression. To study the implications of these EMT-inducing transcription factors in breast tumor progression we developed conditional overexpression and knockdown systems for deltaEF1 and Snail family members in different human epithelial cell lines. In vitro functional analysis revealed that conditional expression results in loss of cell adhesion, increased invasion and migration. Comparative expression analysis using microarray transcriptome profiling revealed different gene expression signatures caused by expression of Snail, Slug, deltaEF1 and SIP1 in human epithelial cells. These EMT expression signatures were compared with expression signatures obtained from primary human cancer samples. The specific target genes, which were confirmed by quantitative RT-PCR, will be validated by chromatin immunoprecipitation (ChIP) analysis and transcriptional reporter gene assays. To address the in vivo role of these EMT-inducing transcription factors on invasion and metastatic disease, we employed xenograft studies and used an EMT transgenic model.

96-POS

### CHARACTERIZATION OF SIGNAL TRANSDUCTION PATHWAYS REGULATING SNAIL IN PRIMARY HUMAN ENDOMETRIAL CARCINOMA

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In previous studies it was proposed that the transcription factor and E-cadherin repressor Snail plays a role in tumour progression. The signalling pathways regulating Snail protein expression in different human primary cancers, however, have not been determined. We asked whether the p38-mitogen activated protein kinase (MAPK) and/or the Akt/glycogen synthase kinase-3beta (GSK-3beta) pathways are involved in regulating the epithelial-mesenchymal-transition regulator in primary human tumours. As a first cancer type to study, we chose endometrial carcinomas. In the developed countries endometrial cancer is the most common cancer of the female genital tract and in North America the eighth commonest cause of death from cancer in the female population.

Stimulation of an estrogen receptor negative variant of the endometrial carcinoma cell line Ishikawa with epidermal growth factor (EGF) leads to an increase of Snail protein expression and activation of p38-MAPK but does not increase phosphorylation of Akt and GSK-3beta.

Subsequently, we screened a series of 17 formalin-fixed endometrioid endometrial carcinomas for activation and expression of potential Snail regulating pathways using quantitative protein lysate microarrays. In the tumours analysed expression of activated EGFR (epidermal growth factor receptor, Tyr1086) and p38-MAPK (Thr180/Tyr182) correlated with increased levels of Snail protein as it was seen in the cell culture model. Moreover, activation of AKT did not correlate with Snail expression. However, the inactive (phosphorylated) form of GSK-3beta (Ser9) was associated with expression of Snail. In addition, we observed a statistically significant inverse correlation between Snail and E-cadherin protein levels in these tumours.

Taken together, our data suggest that EGFR and p38-MAPK activation but also GSK-3beta inactivation may be involved in the stabilisation/over-expression of Snail protein in primary endometrial cancers, possibly resulting in down-regulation of E-cadherin.

97-POS

**HA-RAS INDUCED EMT IN HUMAN COLON CELLS: MICROARRAY ANALYSIS AND  
TRANSCRIPTIONAL REGULATION OF VIMENTIN AND S100A4 GENES**

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Colorectal cancer arises after a series of mutational events in the colon epithelia and is often used as a model of the multi-step progression of tumorigenesis. In order to examine their distinctive functions in cancer development in the colon, we introduced constitutively active mutant Ki- and Ha-Ras genes into an intermediate stage colon adenoma cell line (Caco-2). We found that mutant active Ha-RasV12 was very efficient at transforming these colon epithelial cells as assessed by anchorage-independent growth, tumour formation in SCID mice and the development of mesenchymal morphology compared to transformation by Ki-RasV12. We conducted microarray analysis in an attempt to reveal the genes whose aberrant expression is a direct result of overexpression of either Ki-RasV12 or Ha-RasV12. Initially we used arrays of 2,000 (1) and then arrays of more than 25,000 genes. Ha-RasV12 mainly regulated genes involved in controlling cell morphology, correlating to an epithelial-mesenchymal transition only observed in these cells.

The process of epithelial mesenchymal transition, whereby cells acquire molecular alterations and fibroblastoid features, is a fundamental process of embryogenesis and cancer invasion/metastasis. We showed that vimentin, a key molecule of epithelial mesenchymal transition, was differentially regulated between Ha-RAS and Ki-RAS leading to Ha-RAS specific induction of migrative phenotype and eventually epithelial to mesenchymal transition. We demonstrated that the AP-1 sites in vimentin promoter could be involved in this regulation. A potential role of FRA-1 in the regulation of vimentin during the Ha-RAS induced epithelial to mesenchymal transition associated with colon cell migration was suggested. Our results therefore proposed that in colon cells the induction of epithelial mesenchymal transition by oncogenic Ha-RAS could occur through the overexpression of proteins like FRA-1 and vimentin (2). Detailed analysis of vimentin as well as S100A4 gene regulation in this system will be provided

1. Roberts, M., Drosopoulos, K., Vasileiou, G., Stricker, M., Taoufik E., Maercker, C., Guialis, A., Alexis, MN. and Pintzas, A. (2006). Microarray analysis of the differential transformation mediated by kirsten and harvey ras oncogenes in a human colon adenocarcinoma cell line. *Int. J. Cancer* 118, 616–627.

2. Andreolas, C., Kalogeropoulou, M., Voulgari, A. and Pintzas, A. (2007). Fra-1 Regulates Vimentin During Ha-RAS Induced Epithelial Mesenchymal Transition In Human Colon Carcinoma Cells. Under Revision.

98-POS

**A SNAIL-SMAD TRANSCRIPTIONAL REPRESSOR COMPLEX PROMOTES  
TGF-BETA-MEDIATED EPITHELIAL-MESENCHYMAL TRANSITION**

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Epithelial-mesenchymal transition (EMT) is a fundamental process of development and triggered in carcinoma progression into an invasive state. Transforming growth factor-beta (TGFbeta) acts in a cooperative fashion together with Ras and Wnt signaling pathways to induce EMT, both during organogenesis and in tumour progression. The molecular mechanisms of these cooperative actions are incompletely understood. Here, we report that the transcriptional repressor Snail, an inducer of EMT, which is activated by Ras and Wnt pathways, is a cofactor for Smads, intracellular transducers of TGF-beta-mediated signaling. Snail and Smads interacted and formed a transcriptional repressor complex, which repressed the coxsackie- and adenovirus receptor (CAR), occludin and E-cadherin in TGF-beta-induced EMT. Simultaneous inhibition of GSK-3beta, a negative regulator of Snail activity, resulted in more potent repression of CAR and E-cadherin in response to TGF-beta and thus further supported cooperative actions of Snail and Smads. Finally, we observed that Snail and Smad colocalize in vivo in the cell nucleus in a subset of tumor cells in a breast cancer mouse model. Our results reveal a novel mechanism of gene repression and suggest that Snail activation represents a molecular switch, which allows a Snail-Smad repressor complex to drive induction of EMT in response to TGF-beta.

## **Abstracts**

**99-POS**

### **P53 IS A NOVEL REGULATOR OF EMT**

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The p53 gene is mutated in about 50% of human cancers by missense mutations that mainly reside in the region coding for the DNA binding domain. The p53 tumour suppressor gene encodes a transcriptional regulator and plays a critical role in cell cycle progression, senescence and apoptosis. However, p53 may also have cell cycle-independent functions. In particular, we have previously reported that inactivation of p53 function favours cell spreading and cell migration, processes involved in tumour metastasis (Gadea, EMBO J 2002). Metastasis entails a transdifferentiation process, the epithelium-mesenchyme transition (EMT) that transforms cells of epithelial origin into fibroblasts, allowing cells to dissociate from the epithelial structures from which they originate and migrate to metastasize to distant sites. E-cadherin is a key component of adherens junctions, structure that play crucial roles in the maintenance of epithelial integrity. Loss of tumour cell adhesion and gain of mesenchymal markers like vimentin are important factors in this process. Although EMT may contribute to the early steps of invasion and metastasis of carcinoma cells, the mechanisms of induction and regulation of EMT are largely undefined. To study the effects of p53 and p21 (a transcriptional effector of p53) loss on EMT, we comparatively analysed the Hct116 human colon carcinoma cell line and derivatives that were engineered to lack both alleles of the p53 (Hctp53<sup>-/-</sup>) and p21 gene (Hctp21<sup>-/-</sup>) (Bunz, Science 1998 and Waldman, Cancer Res 1995). We found that wild-type and mutant p53 strongly induced EMT and enhanced motility in p21 or p53 deficient Hct116 cells, by analyzing changes in cell morphology, epithelial and mesenchymal marker proteins, and using cell wound healing assay. Chromatin immunoprecipitations and gene-reporter assays showed that p53 directly bound and repressed the promoter of E-cadherin. In addition we showed that re-expression of p21 cDNA by retroviral gene transfer delivery into Hct116p21<sup>-/-</sup> counteracted the repressive function of p53 and restored partially the epithelial phenotype.

This work identifies p53 as a novel regulator of EMT and offers new perspectives in the comprehension of EMT regulation during tumorigenesis.

1) Gadea G and al (2002) Regulation of Cdc42-mediated morphological effects: a novel function for p53, EMBO J 21, 2373-82.

2) Bunz and al (1998) Requirement for p53 and p21 to sustain G2 arrest after DNA damage, Science 282, 1497-501.

3) Waldman T (1995) p21 is necessary for the p53-mediated G1 arrest in human cancer cells, Cancer Res 55, 5187-90.

**100-POS**

### **CONTROL OF RHO/ROCK SIGNALLING BY P53: CONSEQUENCES ON CELL MIGRATION AND INVASION.**

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Much remains to be learned about how cancer cells acquire the properties of migration and invasiveness. We have previously presented evidence that the contribution of the tumour suppressor p53 to the control of tumorigenesis is not restricted to its anti-proliferative activities, but is extended to the modulation of cell migration. Identification of the mechanisms by which p53 modulates cell migration will be important to understand how invasive cells arise. Here we show that p53 deficiency induces a switch in mouse embryonic fibroblasts cultured in a 3D matrices from an elongated, spindle-shaped morphology to a markedly spherical and flexible one associated with highly dynamic membrane blebs that strikingly resembles amoeboid movement. This transition is characterised by a polarised distribution of integrin b1 and ezrin in the direction of movement and requires the RhoA/ROCK pathway. These rounded, motile cells show higher velocity and a significant increase in invasive properties. This type of p53-mediated transition is also observed in melanoma A375P cancer cells. Our data demonstrate that p53 is an important determinant of the mode of cell motility. This connects the regulation of proliferation to the control of cell migration and defines a new concept of p53 function as a tumour suppressor gene, suggesting that the genetic alterations of p53 in tumours are sufficient to promote motility and invasion, thereby contributing to metastasis.

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