

Epithelial-Mesenchymal Transition

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Introduction

The meeting was designed to explore the intersections of signalling networks regulating and supporting epithelial-mesenchymal (EMT) and mesenchymal-epithelial transitions (MET) in development, fibrosis, and cancer. Particular emphasis was placed on correlations between tissue histology and molecular drivers and markers of EMT and on the therapeutic implications of EMT.

Integrated Summary of the Findings Presented

Background. Epithelial-mesenchymal transition (EMT) was first described in three-dimensional culture of corneal epithelial cells in the laboratory of Elizabeth Hay in 1982 and has since been implicated in numerous embryonic states and pathologies including fibrotic disease and carcinogenesis. The first meeting of The EMT International Association was held in Australia in 2003 in honor of "Betty" Hay. This year's Cold Spring Harbor EMT meeting opened with a presentation by Raghu Kalluri (Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA) to celebrate the achievements of Betty and commiserate her sad passing in 2007. The field has grown enormously since her first observations of EMT, and her profound interest and wonderful character will be sorely missed. The 2008 CSH meeting mainly focused on cancer EMT, particularly in light of recent controversies (1–3). The link between EMT and cancer plasticity has been championed by Jean-Paul Thiery (Institute of Molecular and Cell Biology, Singapore), who presented a keynote address highlighting the precise differences in the mechanical forces required to break interactions between epithelial and mesenchymal cadherins. Several talks highlighted evidence of EMT in purified breast cancer stem cells, cancers which resist therapies, and in histologically provocative locations such as the invasive front. These leave little doubt about the existence of EMT in cancer and indeed show important ramifications in metastatic competence. New evidence of EMT in breast cancer was provided by Robert Weinberg (Whitehead Institute for Biomedical Research, Cambridge, MA) and Marisa Ponzio (McGill University, Montreal, Canada). Indications for EMT in novel scenarios such as Barrett's esophagus and the human neuroendocrine tumor cell BON were proposed [Rebecca Fitzgerald (Hutchison-Medical Research Council Research Centre, Cambridge, United Kingdom) and Frank Leu (Verto Institute LLC, New Brunswick, NJ)]. The presentations by Jeffrey

Rosen (Baylor College of Medicine, Houston, TX), Stuart Thomson (OSI Pharmaceuticals, Farmingdale, NY), and Steven Dubinett (David Geffen School of Medicine, Los Angeles, CA) highlighted the therapeutic challenges elicited by tumor heterogeneity associated with EMT.

Transcription factors. Many transcription factors driving embryonic EMT directly repress the adherens junction mediator E-cadherin through the E-box promoter elements and serve the same role in cancer. Numerous studies indicating the prognostic power of these factors in different tumor types and provocative localizations at the tumor invasive front are accruing. The definitive role of these factors in EMT regulation, along with associated behaviors of survival, migration, invasion, and resistance to anoikis, makes them prime candidates for cancer prognosis and treatment (summarized by Angela Nieto, Instituto de Neurociencias de Alicante, CSIC-UMH, San Juan de Alicante, Spain).

These areas were well represented at the meeting. Andrei Bakin (Roswell Park Cancer Institute, Buffalo, NY) presented a model of EMT whereby transforming growth factor β (TGF- β) induces EMT but the ensuing metastatic and invasive properties are rendered by oncogenic *H-RasV12* inhibiting the binding of Smad transducers of TGF- β to the promoter of tropomyosin TPM1, leading to nuclear export of Smad4. Low levels of TPM1 lead to destabilization of actin fibers. Aristidis Moustakas (Ludwig Institute for Cancer Research, Uppsala, Sweden) provided a comprehensive overview of how the Smad proteins facilitate the establishment of EMT, and James Woodgett (Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Canada) summarized the roles of glycogen synthase kinase 3 in cell fate determination, especially in relation to Wnt signaling, β -catenin regulation, and nuclear exportation of Snail. Novel aspects of Twist function in developmental and pathologic EMTs were presented by Carole LaBonne (Northwestern University, Evanston, IL) who showed that Twist, a bHLH transcription factor, physically and functionally interacts with numerous regulators of EMT such as Slug, Sox9, and Foxd3, acting in neural crest development as well as in tumor invasion.

Many transcription factors driving EMT in development become commandeered by cancers, an observation that preempted the identification of the role of gooseoid in cancerous EMT (R. Weinberg). Exciting new talks were presented on embryonic transcription factors with roles in mammary carcinoma. Heide Ford (University of Colorado Health Sciences Center, CO) identified the homeobox gene *Six1* as a regulator of TGF- β and Wnt signaling in EMT and metastasis in a mouse mammary carcinoma model, whereas Weston Porter (Texas A&M University Health Science Center, TX) showed that loss of *single-minded-2s* in the mouse mammary gland induced EMT associated with up-regulation of Slug.

Mouse models, MET, and "metastable" phenotype. A survey of EMT in models of mouse mammary carcinoma was presented by Robert Cardiff (University of California, Davis, CA) showing that

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spindle cell morphology has been described for years but only recently attributed to EMT (See Supplementary Fig. S1). Not all spindle cell tumors exhibit EMT, and EMT paradoxically does not correlate with metastasis in reported mouse models, possibly due to limitations in downstream plasticity required for colonization. M. Ponzio described the heterogeneity of mouse mammary tumor virus-Met-driven mammary tumors exhibiting either basal or luminal features. Basal type tumors were confirmed by gene arrays to exhibit EMT with Wnt pathway features and to resemble the basal subgroup of human breast cancers, in which c-met is prognostic and Snail and c-met together were highly prognostic.

Mouse mammary tumors bearing EMT features are often not metastatic, which may be due, at least in part, to the scenarios presented by Elizabeth Williams (Monash University, Victoria, Australia; bladder carcinoma) and Thomas Brabletz (University of Freiburg, Germany; colon carcinoma) whereby a reverse transition (MET) may be needed to effect metastatic competence at the site of recolonization. This was suggested further in talks of Sendurai Mani (M. D. Anderson Cancer Center, Houston, TX), H. Ford, W. Porter, and R. Weinberg. A related theme that surfaced repeatedly was the “metastable” or “hybrid” phenotype comprising both epithelial and mesenchymal features. R. Cardiff showed evidence of this in several mouse models, and the predominantly epithelial bladder cancer sublines presented by E. Williams showed increased expression of a set of mesenchymal marker gene products.

Development. Strong parallels between developmental and pathologic EMT provided significant cross-fertilization in talks on various disease studies. Gromoslaw Smolen (Massachusetts General Hospital and Harvard Medical School, Boston, MA) described a Rap GTPase interactor, RADIL, discovered by gene expression analysis of TGF- β -treated NMuMG mouse mammary carcinoma cells, and found to be involved in the EMT mediating migration of enteric neural crest precursors. The debate on the role of EMT in palate fusion was presented by Kathy Svoboda (Texas A&M University Health Science Center, Dallas, TX), followed by comments by A. Nieto, who also described an exciting new role for Snail1 in bone, downstream of FGFR3 signaling.

MicroRNA and EMT. Recent groundbreaking studies established functional associations between noncoding microRNAs and key effectors of EMT occurring in the context of carcinogenesis and embryonic development. Philip Gregory (University of Adelaide, Australia), Sun-Mi Park (University of Chicago, IL), and Otto Schmalhofer (University of Freiburg, Germany) made complementary presentations on how microRNAs miR-200 and miR-205 inhibit repressors of E-cadherin expression δ EF1(ZEB1) and SIP1 (ZEB2) and thus maintain the epithelial cell phenotype. Notably, Gregory and colleagues extended their study to microdissected metaplastic breast carcinoma where they found loss of miR-200 correlating with increased expression of vimentin and decreased E-cadherin in the mesenchymal component. Schmalhofer and colleagues reported that the interactions of δ EF1/ZEB1 and the microRNA-200 family members miR-141 and miR-200c are part of a transcriptional feedforward loop that stabilizes EMT and promotes cancer invasion. Another link between EMT and microRNAs was emphasized by R. Weiberg, showing the EMT regulator Twist to be a positive, direct activator of miR-10b, a microRNA overexpressed in human breast carcinoma, which, acting through translational inhibition of HOXD10, initiates invasion and distal metastases. Jiri Zavadil (New York University School of Medicine, NY) showed that the oncogenic microRNA miR-21, which is consistently up-regulated in many types of carcinomas, conveys the

effects of TGF- β in the context of epithelial injury leading to EMT and fibrosis.

Polarity and EMT. Several talks on cytoarchitecture/polarity and EMT were opened by the plenary lecture of Mina Bissell on elegant use of biophysics to illustrate the importance of cell shape change to matrix metalloproteinase (MMP)/Rac1b-induced EMT, and additional modeling studies predicted the role of a diffusible inhibitor on regulating branching morphogenesis. Selective expression of vimentin-GFP was seen at the tips of branching morphogenesis that escaped the inhibitor. Harold Chapman (University of California, San Francisco, CA) described a complex involving α_3 integrin, TGF- β type II receptor, and E-cadherin, essential for internalization of TGF- β type II receptor, and mediating cross talk between pSMAD2 and β -catenin. Several talks presented data to show a role for polarity proteins as mediators or drivers of EMT. Jeffrey Wrana (Samuel Lunenfeld Research Institute, Toronto, Canada) presented evidence to show a role for the polarity protein Par6 during TGF- β -induced EMT. In response to TGF- β stimulation, the Par6 bound to TGF- β type I receptor is phosphorylated by the TGF- β type II receptor. The phosphorylated Par6 facilitates recruitment of the E3 ubiquitin ligase SMURF1 and promotes degradation of the small GTP binding protein RhoA to induce EMT. Wrana used a phospho-specific Par6 antibody to show that this pathway is active in primary breast tumors. Selective loss of RhoA and the GEF NET1 was shown in the basal region of the chick embryo ingressing epiblast, which forms the mesenchyme, where RhoA otherwise mediates clustering of the cells due to basement membrane retention via $\alpha_6\beta_4$ integrin (Guojun Sheng, RIKEN Center for Developmental Biology, Kobe, Japan). Senthil Muthuswamy (Cold Spring Harbor Laboratory, NY) discussed the role of Par6 in erbB2-induced loss of cell polarity in human mammary epithelia. Whereas the ErbB2-Par6 interaction was not sufficient to induce EMT, additional changes in polarity proteins, in particular AF6 and Scribble, promoted EMT, showing that disruption of polarity pathways cooperates with oncogenes such as *ErbB2* to induce a loss of the epithelial phenotype. T. Brabletz presented evidence to show how the polarity proteins are critical mediators of EMT induced by the transcription factor ZEB1. In colorectal cancer-derived cell lines, ZEB1 repressed expression of the polarity protein LGL2. Reexpression of LGL2 suppressed the increase in invasion and metastasis promoted by ZEB1, identifying a polarity protein as a target for the EMT-inducing transcription factor ZEB1. Gregory Longmore (Washington University, St. Louis, MO) described how Ajuba LIM proteins of adherens junctions shuttle to the nucleus and act as nuclear Snail corepressors, binding the NH₂-terminal SNAG domain and regulating neural crest development. John Condeelis (Albert Einstein College of Medicine, Bronx, NY) extended pioneering work on imaging invasive cells with an exposé of actin regulatory machinery including ZBP1, N-Wasp, MENA, and cofilin.

EMT and endothelial-mesenchymal transition in fibrosis. In addition to cancer progression, EMT contributes to chronic epithelial injury, leading to tissue fibrosis and organ failure, as has been experimentally shown in the models of renal, pulmonary, and hepatic fibrogenesis. The cellular source of fibrosis is activated fibroblasts and/or myofibroblasts, the principal collagen-producing cells on activation. R. Kalluri described how myofibroblasts arise from residing endogenous mesenchymal cells, an observation supported by J. Zavadil's presentation of aristolochic acid-associated nephropathy and fibrosis modeled in the mouse,

from circulating fibrocytes originating from bone marrow stem cells and from epithelial and endothelial cells by EMT or endothelial-mesenchymal transition in cardiac fibrosis. The EMT-undergoing cells may compose up to 36% of epithelial cells as shown in the kidney, using a key EMT marker, FSP1 (calcium-binding protein S100A4). The renal epithelium capable of undergoing EMT has a distinct embryonic origin from mesenchymal cells, and in the conditions of injury it maintains the developmental capacity of reversible cellular plasticity. The EMT, endothelial-mesenchymal transition, and activation of fibroblasts are typically driven by inflammatory cytokines such as TGF- β , and both processes are inhibited by the opposing action of TGF- β /BMP family member BMP7, a soluble molecule with a promising therapeutic potential. Many parallels that can be drawn between EMT in fibrosis and cancer were summarized by Eric Neilson (Vanderbilt University School of Medicine, Nashville, TN) in his concluding presentation. He drew from his published works in both areas and presented elegant new work on the transcriptional regulation of FSP-1. Microenvironmental effects on cellular function were highlighted by Valerie Weaver (University of California, San Francisco, CA), with EMT-related lysyl oxidase stiffening matrix at the invasive front, promoting durotaxis of cells up a gradient of increasing stiffness. In this area, Stephen Weiss (University of Michigan, Ann Arbor, MI) illustrated the benefits of basement membrane for studies on the role(s) of EMT-related expression of MT1-MMP in breast cancer invasion and spread.

EMT in cancer stem cells. One of the strongest evidence for EMT in the clinical scenario is the loss of epithelial features in colon cancer cells at the invasive front, typified by nuclear β -catenin, lack of E-cadherin, and vimentin expression, as presented by T. Brabletz. Microarray analysis of these cells isolated by laser capture microdissection revealed "migrating stem cell" phenotypes. In a captivating opening plenary, R. Weinberg summarized recent works highlighting EMT from his laboratory, culminating with recent data showing that CD44^{hi}/CD24^{lo} cells purified from normal and malignant mammary tissues exhibited EMT features, and that human mammary cells induced to undergo EMT exhibited stem cell characteristics and increased mammospheric and malignant potential. S. Mani later presented a more detailed view of this work. Erik Thompson (St. Vincent's Institute, Fitzroy, Australia) presented supporting work confirming overlap between the gene expression profile distinguishing mesenchymal breast cancer cell lines from luminal or basal lines and that which distinguishes purified CD44^{hi}/CD24^{lo} cells from CD44^{hi}/CD24^{lo} counterparts depleted of malignant potential. J. Rosen continued this theme to show that tumors resistant to lapatinib showed higher ratios of BCSC phenotype and EMT-like signatures. Parallels between the attributes of cancer stem cells (also called cancer initiating cells) and EMT were drawn, including enhanced survival, resistance to anoikis, and resistance to chemotherapy.

Clinical relevance of EMT. Therapeutic consequences of EMT also apply to recently developed targeted therapies, wherein sensitivity to epidermal growth factor receptor (EGFR) and insulin-like growth factor-I (IGF-I) receptor inhibitors correlates with receptor usage and an epithelial cell phenotype. Paradoxically, receptor tyrosine kinases (RTK) such as EGFR, c-Met, IGF-I receptor, fibroblast growth factor receptors, and the non-RTK c-Src induce phosphorylation of E-cadherin and associated catenins, resulting in their degradation, providing a link between oncogenic

activation of these kinases and induction of EMT. S. Thomson showed that signaling from EMT promoters EGF and IGF-I was attenuated on occurrence of EMT, and other RTKs become dominant. The loss of EGFR inhibitor sensitivity associated with EMT-like transitions has been described for non-small-cell lung cancer, pancreas, head and neck squamous cell cancer, colorectal cancer, bladder, and breast tumor cells. EMT-like transitions promote the novel acquisition of alternate RTK autocrine and paracrine loops (S. Thomson), such as platelet-derived growth factor receptor, which can exert proliferative and antiapoptotic actions.

The link between inflammation, cancer progression, and EMT was highlighted in the work of S. Dubinett and coworkers. Elevated COX-2 and its distal product prostaglandin PGE₂, together with the loss of 15-PGDH, responsible for the degradation of PGE₂, were correlated with a mesenchymal phenotype deriving from EMT-like transitions in non-small-cell lung cancer. The adherens junction mediator and tumor suppressor protein E-cadherin was shown to be negatively regulated by COX-2 activation and PGE₂ production, and other COX-2 target genes have been implicated in EMT and metastasis (*CD44*, *MMP2*, *Snail*, and *Zeb1*), immunoregulation (*FOX-P3*, *IL10*, and *IL12*), angiogenesis (*CXCL5*, *CXCL8*, and *VEGF*), and apoptosis (*survivin*, *IGFBP3*, and *IL6*). Many of these findings could be recapitulated in Snail-transfected HBEC cells, which were functionally shown to be more invasive *in vitro* and metastatic *in vivo*. Taken together, the data support the concepts underlying recent clinical studies combining COX-2 and EGFR inhibitors, whose initial data suggest improved patient outcome.

Recommendations for future research. Several major areas emerged during the meeting that were consolidated and emphasized as needing urgent further work, in addition to the important work ongoing in existing paradigms. These included (a) understanding the integration and redundancy of regulatory pathways converging on E-cadherin repression; (b) understanding the pathways that specifically regulate mesenchymal gene expression rather than epithelial repression, such as FoxC2; (c) emphasis on the so-called hybrid or metastable phenotype, which is particularly evident in carcinomas and seems to be more lethal than the purely mesenchymal phenotype; (d), perhaps related to (c), the possible requirement for reversal of EMT by MET to allow successful colonization at the metastatic site, with implications for tumor dormancy and relapse after systemic chemotherapy and/or radiotherapy; (e) the new associations between EMT attributes and the phenotypes seen in purified breast cancer stem cells, with obvious implications for metastatic competence and therapy resistance; (f), again in conjunction with (e), the therapy resistance associated with tumors showing EMT features, extended from lung carcinoma to lapatinib-resistant breast carcinoma; (g) extracellular signals from the microenvironment, which regulate EMT with clear therapeutic consequences in relation to both EMT and MET regulation; (h) production of tumor microenvironment through EMT and endothelial-mesenchymal transition, with clear links to a plethora of fibrotic conditions; and (i) EMT-targeted therapies such as BMP7 currently in trial in kidney fibrosis.

Part of the solution to these problems and new therapeutic opportunities will come from the application of novel high-throughput technologies to the EMT and MET processes. Suzanne Brady (Vanderbilt University Medical Center, Nashville, TN) reported the results of a high-throughput screen to identify small

molecules that restore E-cadherin expression in metastatic colorectal cancer cells. Aleksandra Nita-Lazar (Massachusetts Institute of Technology, Cambridge, MA) presented quantitative proteomic analysis of the Met signaling network, and Daniel Peeper (Netherlands Cancer Institute, Amsterdam, the Netherlands) identified the neurotrophic tyrosine kinase receptor TrkB in anoikis suppression, EMT, and metastasis using high-throughput screening. Although somewhat preliminary, the presentation of Anna Roschke (National Cancer Institute, NIH, Bethesda, MD) using array-based comparative genomic hybridization technology to search for associations between chromosomal instability and EMT in cancer was thought-provoking. Similar approaches to the transcriptomics, epigenomics, metabolomics, and genomics of epithelial-mesenchymal plasticity are ongoing in many laboratories

and may well provide important new leads into the understanding and rational targeting of these processes.

Disclosure of Potential Conflicts of Interest

J. Haley is an employee of and shareholder in OSI Pharmaceuticals Inc., a biopharmaceutical company with an active research interest in the biology and pharmacology of EMT. The other authors disclosed no potential conflicts of interest.

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