Meeting Report

Cancer Research

AACR Special Conference on Epithelial-Mesenchymal Transition and Cancer Progression and Treatment

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Abstract

Epithelial-mesenchymal transition (EMT) is a developmental program implicated in cancer progression and was the subject of the 2010 AACR meeting on the topic of EMT and Cancer Progression and Treatment held on February 28 to March 2 in Arlington, Virginia. A review of the involvement of EMT in gastrulation, organogenesis, carcinogenesis, and metastatic progression elucidated the overlap of EMT in these physiologic and pathologic conditions. Both novel and traditional markers of cells undergoing EMT were discussed and compared with features used to define cancer stem cells. Importantly, these defining characteristics of cells undergoing EMT were discussed in the context of therapeutic and prognostic developments. *Cancer Res; 70(19); 7360–4. ©2010 AACR.*

Introduction

Epithelial-mesenchymal transition (EMT) has been studied in three different contexts: early in development during gastrulation, during organogenesis, and during carcinogenesis and metastatic progression. There is much debate regarding the similarity between these EMT programs, the ability of EMT to direct cell fate versus cell behavior, and how this relates to the context in which EMT occurs (reviewed in refs. 1, 2). In development, EMT is an important mechanism in the control of epithelial plasticity. In cancer, carcinoma cells awaken the EMT program to become motile, stem cell–like, protected from senescence, apoptosis, and immune surveillance, and resistant to conventional and targeted therapeutics. These "EMTed" carcinoma cells are thought to be responsible for seeding distant dissemination, eventually leading to cancer-related mortality. An investigation of common regulatory factors of the various EMT programs in the contexts of development and cancer is essential to our understanding of pathologic EMT.

One of the main goals of this AACR special conference on EMT and cancer progression and treatment was to provide a diverse group of scientists an opportunity to exchange information and ideas leading to a better overall understanding of EMT and its reverse process, termed the mesenchymal-epithelial transition (MET) in normal and cancer development, the signaling pathways driving EMT, the role of EMT in invasion, metastasis, and in the formation of cancer stem cells (CSC), as well as the characterization of novel EMT regulators as potential targets for EMT interference. The conference featured an exciting collection of sessions beginning with keynote presentations by some of the leaders in the EMT field, followed by plenary sessions discussing various novel aspects of EMT, supplemented with ample poster sessions highlighting work within these fields. A list of speakers can be found in the appendix. The following summary provides a brief review of the new insights gained from the work presented at this meeting.

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Integrated Summary of the Findings Presented

Context-dependent regulation of EMT in development and cancer

All adult organs, with the exception of the central nervous system and the epidermis, are the result of the completion of one or several rounds of EMT. Throughout the conference, there was repeated emphasis on the importance of identifying molecular signals that control the initiation and prevention of EMT and understanding how the context of these signals determines the control of cell fate and/or cell behavior (3, 4).

The importance of control of EMT by the transcription factor Snail2 (Slug) has been well studied in both the fields of development and cancer. Angela Nieto (Instituto de Neurociencias, San Juan de Alicante, Spain) presented a mechanism by which EMT is induced by Snail2 in the context of ingression at the primitive streak of the chick embryo. Conversely, EMT is prevented via direct repression of *Snail2* by the transcription factor Sox3 in the context of neural plate development. Traditionally, Snail2 is considered an inducer of mesodermal cell fate, defined by decreased expression of epithelial cell markers and induction of

mesenchymal cell phenotype. However, in this context, Snail2 acts as a mesenchymal inducer by directing cell behavior rather than cell fate.

The topic of context-dependent regulation of EMT was also emphasized by Carole LaBonne (Northwestern University, Evanston, IL). She explained how PPA, an F-box protein/E3 ligase, regulates the expression of *Snail2* through the control of ubiquitination and how the expression of *Snail2* below a certain threshold level leads to the control of cell fate, whereas expression above that threshold affects cell behavior through the control of EMT. The stability of Twist is also regulated by PPA. She also discussed how differences in protein-protein interactions between Snail1 and Snail2 with Ajuba lim factors differentially recruits histone deacetylase 1 and thereby differentially regulates the E-cadherin promoter. Control of EMT in normal physiologic conditions, as described by Drs. Nieto and LaBonne, has also been observed in pathologic conditions, such as cancer and seems to be controlled and carried out in the same manner and under the control of many of the same players. Thus, understanding how EMT is regulated during normal processes may be useful for the development of new, more specifically targeted chemotherapeutic agents for use during malignant progression.

In tumors, a key regulatory pathway contributing to the initiation of EMT is transforming growth factor-β (TGF-β). TGF-β functions both as a tumor suppressor and an oncogenic signal in human cancer. William Schiemann (University of Colorado, Denver, CO) discussed the possibility that EMT may be the underlying switch in the TGF-β function from tumor suppression to oncogenesis. He described a model whereby activated c-Abl interacts with E-cadherin to regulate polarity and to suppress oncogenic signaling by TGF-β. Changes in extracellular matrix rigidity and integrin function may then cause a change in TGF-β function leading to the induction of EMT and inactivation of c-Abl. Importantly, the use of Gleevec in this context could promote tumor progression and thus could be detrimental to patient outcome.

Other important findings regarding the regulation of TGF-β were presented by Harold Chapman (University of California, San Francisco, CA) who, through the study of murine pulmonary fibrosis and human lung cancer, found that direct binding of integrin α-v-β3-1 to E-cadherin and the TGF-β receptor is important for internalization of the receptor, leading to its activation and downstream signaling events. He also provided evidence that α-v-β3-1 is important for tyrosine phosphorylation of β-catenin which leads to the regulation of cell–cell contacts, another key regulatory component of EMT. Lastly, Dr. Chapman discussed that nuclear versus perinuclear localization of β-catenin, often used as a marker of mesenchymal versus epithelial type cells, respectively, is context-dependent. Nuclear localization of β-catenin is dependent on TGF-β signaling, whereas perinuclear localization may be dependent on the combination of signaling by epidermal growth factor (EGF) and TGF-β.

Aristidis Moustakas (Ludwig Institute for Cancer Research, Uppsala, Sweden) shared data showing how HMGA2 regulates *Snail1* and *Twist* expression to orchestrate EMT in a fashion that is also under the control of TGF-β. Additionally, he discussed the promotion of epithelial differentiation by an AMP-regulated kinase, LKB1, required for the activation of SIK1, a potential marker of tumor invasiveness. Finally, he introduced CDC6 and CDt1, factors shown to promote EMT and regulate ZEB1 and Twist in a TGF-β–independent manner.

The presence or absence of signaling molecules controlling EMT are often used as markers of either an epithelial or a mesenchymal state. However, in both development and in cancer, researchers have identified an intermediate EMT phenotype in which the presence of these markers within a cell does not result in the completion of the EMT program. Raghu Kalluri (Beth Israel Deaconess Medical Center, Boston, MA), described gene expression profiling of tumor cells following the ablation of pericytes, one of the mesenchymal cell types in the tumor microenvironment, from a transgenic mouse model. He reported an increase in expression of *Slug*, *Snail1*, and *Twist* in tumor cells following pericyte ablation. Increases in vessel permeability and metastasis were also observed and suggested the promotion of an EMT program. However, he also observed a decrease in tumor growth and angiogenesis, supporting the existence of an intermediate EMT phenotype. Dr. Kalluri also showed that treatment with Gleevec could promote EMT and metastasis unless coupled to knockdown of *Twist*, supportive of the association of the EMT program with metastasis and the findings presented earlier by William Schiemann.

Other regulators of early development have been shown to be associated with cancer such as the Six1 homeoproteins. Heide Ford (University of Colorado Denver School of Medicine, Aurora, CO) reported that inducible overexpression of Six1 homeoproteins led to partial EMT. Tumors from these mice had an epithelial histology with areas of decreased E-cadherin expression and nuclear β-catenin. Additionally, she showed that EMT induction is dependent on TGF-β in Six1-overexpressing cells.

EMT in invasion and metastasis

Metastatic carcinoma cells represent cell populations that have gone through EMT and can be found within many different types of primary tumors prior to dissemination. For this reason, many researchers have turned to the study of EMT to guide the development of better therapeutic interventions and prognostic markers. During his keynote address, Jean Paul Thiery (Institute of Molecular and Cell Biology, Singapore, Singapore) described a new high-throughput EMT assay to identify compounds able to interfere with EMT in carcinoma. This assay also allows the definition of new targeted synergistic drug combinations to alleviate tumor resistance to currently existing targeted drugs such as erlotinib and gefitinib. Additionally, Dr. Thiery described the development of algorithms connecting EMT to survival genes to help determine the genomic alterations driving EMT.

Many researchers continue to uncover the characteristics of EMT adopted by tumor cells to promote malignancy. Eric Neilson (Vanderbilt University, Nashville, TN) discussed how metastatic tumor cells reuse the molecular EMT program

normally employed by fibroblasts via expression of cytoplasmic protein *FSP1* to promote motility and prevent proliferation. Erik Thompson (St. Vincent's Institute of Medical Research, Fitzroy, Victoria, Australia) reviewed the strong correlations between different subtypes of breast cancer cell lines, EMT status, and patient prognosis. He stressed that EMT positively correlates with basal, claudin-low, metaplastic-type breast cancers and is associated with poor prognosis. Dr. Thompson also discussed that follow-up of patients after treatment has shown the persistence of an EMT signature, notably including *ZEB1* expression, in circulating and disseminated tumor cells, suggesting resistance to anoikis.

The importance of contributions by the tumor microenvironment to EMT and MET as they relate to metastatic progression was highlighted throughout the meeting. John Condeelis (Albert Einstein College of Medicine, Bronx, NY) explained the use of technologies such as multiphoton microscopy, photoconvertible proteins, and an *in vivo* invasion assay to identify and characterize invasive tumor cells within two tumor microenvironments: EMT and the tumor microenvironment of metastasis. He discussed the properties of these tumor cells as nonproliferating, nonapoptotic, chemotherapy- and radiation-resistant, CSC-like, chemotactic to EGF receptor (EGFR) ligands, and EMT-positive. Dr. Condeelis also emphasized the characterization of Mena, a cytoskeletal protein that acts as an amplifier of pathways controlling EMT and the assembly of tumor microenvironment of metastasis.

Frank Gertler (Massachusetts Institute of Technology, Cambridge, MA) stressed the importance of alternative splicing before and after EMT, noting that 1,400 post-EMT alternative splice events make up the largest group of alternatively spliced genes out of all groups studied to date. He also expanded on the importance of the Mena protein through his discussion of two alternatively spliced isoforms, $Mena^{INV}$ and Mena11a, which are found to be differentially regulated in metastatic cancer cells. The upregulation of $Mena^{INV}$ enhances invasion and metastasis in cancer cells by sensitizing cells to signaling by EGF through effects on EGFR dynamics. Expression of Mena11a, downregulated in invasive tumor cells, affects the geometry and assembly of actin networks and is lost following Twist-driven EMT *in vitro*. Splicing factors that control this process have been shown to affect the EMT phenotype and add a new level of complexity to EMT and metastasis. These exciting findings stress the importance of a continued effort to define the role of EMT in invasion and metastasis.

EMT and CSC formation

Multiple presentations addressed the ongoing investigation of overlapping characteristics of normal and CSC with cells undergoing EMT and how these properties promote cancer progression. A functional demonstration of the connection between EMT and stem cell formation was provided by Robert Weinberg (MIT Whitehead Institute for Biomedical Research, Cambridge, MA). He showed that when normal mouse mammary epithelial cells were forced to undergo EMT via tetracycline-inducible expression of Slug, they were able to regenerate a complete mammary ductal tree at rates as much as 100-fold greater in frequency than control cells following implantation into cleared mammary stromal fat pads. He also described the development of high-throughput drug screens designed to uncover agents that preferentially target CSCs. One of the recovered drugs, salinomycin, increased killing of CSCs by 8-fold versus non-CSCs. This creates a hope that other such agents could be identified using this screening method. Another study using the HME-flopc (a floating subpopulation of basal-like normal human mammary epithelial cells) model and HMLER (human mammary epithelial cells overexpressing hTERT, SV40 T/t, and H-RasV12) cancer cell line has shown the ability of spontaneous dedifferentiation of non–stem cells/non-CSCs into new stem cells/CSCs as judged by their changing CD44/CD24 profile. This process seems to be enhanced as cells progressively undergo oncogenic transformation. Such findings illustrate the need for therapeutic strategies directed towards both CSCs and non-CSCs.

These observations are supported by the clinical work of Jenny Chang (Baylor College of Medicine Cancer Center, Houston, TX). She has been investigating whether conventional chemotherapies target only the bulk, non–tumor-initiating subset of cells within the primary tumor, leaving the CSCs unaffected. Biopsies of 35 patients showed increased $CD44^{hi}/CD24^{lo}$ cell populations, mammosphere formation, and xenograft outgrowth in samples taken from patients postchemotherapy. However, treatment with lapatinib, as opposed to letrozole and taxane, reduced not only the levels of $CD44^{hi}/CD24^{lo}$ cells within the tumor but also the formation of mammospheres by tumor cells. Dr. Chang also found a CSC signature enriched in breast cancers classified as claudin-low and identified the involvement of Jak-STAT and Notch pathways in this signature.

MicroRNAs (miRNA) have been shown to be important in stem cell maintenance in both normal tissue and cancer. The connection between the miRNA expression pattern of breast CSC and normal stem cells is being studied by Michael Clarke (Stanford University School of Medicine, Palo Alto, CA) and Thomas Brabletz (University of Freiburg, Freiburg, Germany). Dr. Clarke described how three clusters of miRNAs (miR200c-141, miR-200b-200a-429, and miR-183-96-182) are downregulated in human breast CSCs, normal human and murine mammary stem/progenitor cells, and in embryonal carcinoma cells relative to nontumorigenic cancer cells. He showed that miR200c strongly suppressed the ability of normal mammary stem cells to form mammary ducts and also suppressed the tumor formation by BSCSs *in vivo*. Furthermore, he illustrated how miR200c negatively regulates BMI1, a molecule involved in stem cell self-renewal. EMT regulators ZEB1 and ZEB2 were shown to be downregulated by miR200c, providing a molecular link between EMT, breast CSCs, and normal stem cells. Dr. Clarke identified miRNA 142, 150, and 223 as being overexpressed in breast CSCs. Dr. Brabletz provided evidence demonstrating the role of miR200 family in antagonizing ZEB1 expression in colon cancer cells and the resulting mutually inhibitory regulation of ZEB1 and miR200 family. Downregulation of ZEB1 leads to impaired tumor formation, almost-total ablation of metastasis and a downregulation of

several molecules involved in the maintenance of stem cells, such as BMI1, SOX2, and p63. Dr. Brabletz's work proposes that Zeb1 links EMT activation and stemness maintenance by suppressing stemness-inhibiting miRNAs and as such is a promoter of mobile, migrating CSCs. This provocative topic was discussed throughout the meeting and is indicative of the direction in which the field is heading.

Development of an EMT signature and models used to study EMT

A recurring theme throughout the meeting was the development of an EMT signature for potential use in drug development, prognostic testing, and prediction of treatment outcomes. Low E-cadherin, high vimentin, and N-cadherin expression are traditional markers currently used to identify cells that have undergone an EMT and have recently been identified as well in circulating tumor cells. Andrew Armstrong (Duke University Medical Center, Durham, NC) presented results from the Dunning breast and PC3 prostate cancer models showing an association of EMT marker expression in circulating tumor cells with prognosis. Although the expression of mesenchymal markers was variable, this study provides evidence that use of EMT markers could be considered for direct selection of malignant cells.

Discussion of traditional EMT markers as powerful predictive markers was led by David Sternberg (OSI Pharmaceuticals, Inc., Farmingdale, NY) who presented findings from the Tribute and BR.21 trials. These have shown that E-cadherin score, obtained via immunohistochemistry staining of samples from patients with non small cell lung cancer (NSCLC), had favorable progression-free survival following therapy with the EGFR tyrosine kinase inhibitor (erlotinib). Increased vimentin within the tumor mass was also correlated with EGFR mutation and with sensitivity to EGFR tyrosine kinase inhibitor.

Robert Goldman (Northwestern University, Chicago, IL) discussed the findings that vimentin is more than just a marker of EMT, but instead seems also capable, on its own, of causing some of the phenotypes associated with EMT. He described how upregulation of vimentin in epithelial cells causes a reversible change in shape from tall and round to oval and flat. He also provided evidence that forced expression of vimentin in MCF7 cells increases motility, promotes elimination of cell adhesions via internalization of desmoplakin, and enhances focal adhesion dynamics by 400-fold. Dr. Goldman suggested that these dynamics are regulated by phosphorylation of Rac. He also emphasized that the ability of vimentin to adapt its function in response to stress as compared with actin and microtubule proteins contributes to its critical role in physiologic and pathologic EMT.

There was a wide consensus that there is a need to identify other markers of EMT beyond those currently used. David Spencer (Baylor College of Medicine, Houston, TX) discussed how inducible expression of FGFR-1, which normally leads to the expression of Snail1 in gastrulation, led to a mesenchymal-like phenotype in prostate cancer models. He suggested that the FGF signaling axis is linked to prostate cancer etiology and plays a role in driving stemness and EMT in tumor cells. However, the frequently observed coexpression of E-cadherin and vimentin in transitional cells within tumor samples begs for improved, additional markers of EMT.

Maryland Franklin (OSI Pharmaceuticals, Inc., Boulder, CO) discussed the establishment of an 88-gene EMT signature by DNA microarray and real-time PCR profiling, useful in defining EMT in laser capture microdissected patient tissues. Although tremendous progress has been made to validate new and old EMT markers, there was wide agreement that there is a need to establish a list of markers acceptable for use in varied models of cancer and for use on human samples.

Targeting EMT to prevent cancer progression

A number of presentations throughout the meeting discussed the therapeutic implications of EMT and revealed promising developments, particularly for inhibition of invasion, for possible use in the clinic. Margaret Frame (University of Edinburgh, Edinburgh, United Kingdom) is seeking to understand the molecular processes by which the adhesion-regulated kinase Src and its substrate, focal adhesion kinase (FAK), promote malignancy. Her studies show that the FERM domain of FAK interacts with RACK1, a protein important in the establishment of cell polarity. Both conditional deletion of FAK in the skin of mice, as well as the expression of the FAK mutant E139D140, which is unable to bind RACK1, show impairment in polarization and invasion into Matrigel. She showed that the Src inhibitors dasatinib and AZD0530 affect migration rather than proliferation of cells and inhibit invasion *in vivo*. Lastly, the use of intravital imaging enabled Dr. Frame to investigate the use of inhibitors of adhesion regulators for therapeutic use.

Another receptor tyrosine kinase, MET has been shown to promote invasive growth and is a potential candidate for targeted therapeutic intervention. Paolo Comoglio [Institute for Cancer Research and Treatment, Candiolo (Torino), Italy] showed that the *MET* oncogene, which encodes receptor for hepatocyte growth factor (scatter factor), is overexpressed in certain gastric and colorectal tumors. Oncogenic MET is induced by hypoxia, and indeed, the promoter of the *MET* gene contains six response elements recognized by hypoxia-inducible factor 1α. Ionizing radiation could also induce MET expression. The potential use of a small molecule inhibitor to inhibit the growth of "addicted" MET tumors (tumors whose continuous growth is dependent on the expression of the MET oncogene) was discussed. Dr. Comoglio described the development of therapeutic MET targeting antibody DN30, which causes, via the activation of matrix metalloproteinase, MET shedding—removal of the MET ectodomain followed by intracellular cleavage of the receptor by gamma-secretase and proteasome-mediated cleavage of the receptor (5). The use of this antibody in a xenograft model led to the disappearance of MET "addicted" tumors.

Another current therapeutic class under investigation is angiogenesis inhibitors. These inhibitors were expected to limit tumor growth through the limitation of vascular proliferation. However, unexpectedly EMT, invasiveness, and metastatic potential were increased by these drugs. Donald McDonald (UCSF Helen Diller Family Comprehensive Cancer

Center, San Francisco, CA) reviewed his studies of vascular endothelial growth factor receptor inhibitors and their ability not only to block vessel growth but also their ability to promote, via the induction of hypoxia signaling pathways, tumor invasiveness and upregulation of MET receptor. He found that anti–vascular endothelial growth factor receptor antibody leads to decreased E-cadherin and a 3-fold induction in *MET* mRNA levels within a tumor. He discussed the use of multitargeted receptor tyrosine kinase inhibitors that block both angiogenesis and invasion. Treatment of tumors in Rip-Tag2 mice with the dual vascular endothelial growth factor receptor/MET inhibitor, XL184, caused encapsulation of the primary tumor and inhibition of invasive growth, leading to markedly improved survival.

John Haley (OSI Pharmaceuticals, Inc., Farmingdale, NY) used a systems profiling approach to define signaling network changes in metastable and epigenetically fixed NSCLC models of EMT. A marked attenuation of autocrine EGFR, IGF1R, and *Met* signaling, together with a loss of cell junction and polarity complexes, was observed. During EMT, signal network switching resulted in a heterogeneous, compensatory gain in autocrine $\alpha5\beta1$ integrin, gp130-JAK, TGFβR, FGFR, PDGFR, and Axl signals in a cell-specific manner. Targeting autocrine FGFR and PDGFR with specific inhibitors blocked mesenchymal tumor cell growth. Investigation of targeting EMT with existing therapeutics as well as the development of new therapeutics is important for the expansion of treatment options to include metastatic prevention and has the potential to diminish distant recurrence in patients with cancer.

Summary and recommendations for future research

There are two major themes that emerged from this conference: the role of EMT in cancer progression and EMT as a target in treatment. The role of EMT in cancer progression seems to involve the reawakening of developmental and wound-healing programs by carcinoma cells to acquire stem cell–like properties and to disseminate to distant organs. Identification and understanding of regulatory factors involved in this process is crucial for the development of successful therapeutic interventions. Additionally, in the future, the identification of an EMT signature will provide clinicians with a tool to help direct therapy and identify patients who might benefit from these therapies.

Upcoming Meeting Information

February–March 2012.

Disclosure of Potential Conflicts of Interest

Dr. John Haley and Dr. David Epstein are employees of and shareholders in OSI Pharmaceuticals, Inc., a biopharmaceutical company with an active research interest in the biology and pharmacology of EMT. The other presenters disclosed no potential conflicts of interest.

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