Meeting Report

IDIBELL Cancer Conference on Metastasis and Angiogenesis

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Abstract

The IDIBELL Cancer Conference (ICC) on Metastasis and Angiogenesis was held in Barcelona, Spain, on May 26–27, 2011. The program content was developed by Dr. Manel Esteller, director of the Cancer Epigenetics and Biology Program (PEBC-IDIBELL), Dr. Oriol Casanovas and Dr. Francesc Viñals Canals of the Catalan Institute of Oncology (ICO-IDIBELL), and Dr. Danny R. Welch from the University of Kansas Cancer Center. The topics discussed during the meeting included the latest advances in epigenetic control of metastasis and tumor cell invasion, and molecular mechanisms of angiogenesis and tumor angiogenesis, and were presented by invited keynote speakers. One issue that recurred throughout the meeting was the increased appreciation of tumor–stromal/microenvironment interactions and how the tumor cells respond to these signals in the cancer dissemination process. Cancer Res; 71(19): 1–5. ©2011 AACR.

Opening Lecture

Robert A. Weinberg (Whitehead Institute for Biomedical Research, Cambridge, MA) gave the distinguished EMBO Lecture in which he focused on the role the epithelial-to-mesenchymal transition (EMT) program plays in inducing dedifferentiation of committed cell populations into stem cell–like cells (SC). Differentiated human mammary epithelial cells can acquire self-renewal capacity upon EMT induction, drawing attention to the connection between EMT and stem cell biology in cancer. Translating these to tumor biology, EMT would be responsible for generating not only more invasive and malignant features but also a stemness state that would support tumor development at distant metastases (1). There are 2 key components in this process. The first is the differentiation stage of the cell of origin, which determines diverse signaling requirements to induce an SC phenotype: fully differentiated human mammary epithelial cells require the expression of Slug transcription factor before entering an EMT program, whereas myoepithelial progenitors only require Sox9 expression to acquire stemness. The second component is the maintenance of this mesenchymal/SC state, which seems to be achieved through coordinated signaling pathways—canonical Wnt, noncanonical Wnt, and TGF-\beta. The surrounding stromal cells appear to be crucial to the stimulation and maintenance of these signaling networks, highlighting the value of an integrative investigation to understand the mechanisms of EMT as an interaction between tumor cells and the environment.

Epigenetic Control of Metastasis

Manel Esteller (Cancer Epigenetics and Biology Program, Bellvitge Biomedical Research Institute) outlined the relevance of epigenetic alterations in monitoring cancer progression and their involvement in the development of metastases, as revealed by the latest technologies for epigenomic profiling. Hypermethylation of the microRNA (miR) 200 family, which are modulators of EMT, was shown to be a determinant in triggering the metastatic cascade. The use of methylation arrays has enabled obtaining of the complete DNA methylation fingerprint of 1,600 human samples (2), and thereby the identification of signatures that can predict metastatic organ tropism of primary tumor cells. It has also revealed specific patterns useful for identifying the tumor type of origin of cancers of unknown primary (CUP), and helps clarify the various DNA methylation gains and losses at gene-promoter regions during tumorigenesis depending on the presence or absence of canonical CpG islands. Dr. Esteller also illustrated the profound differences that exist in the DNA methylation patterns of the colon cancer cell lines HCT-116 and the derived double knockout for DNA methyltransferases, compared with human normal colon samples (3).

Thomas Brabletz (University of Freiburg, Freiburg, Germany) discussed the interplay between miRs and EMT in the generation of cancer stem cells. MiR-200 and ZEB1 regulate each other in a feedback loop that modulates the EMT/MET program and, consequently, influence the motility, stemness, and survival of cancer cells (4). On one hand, ZEB1 is important for tumor initiation and metastasis establishment in pancreatic and colorectal cancer cells; on the other, miR-200, whose
expression is promoted by p53, represses SC properties and stimulates epithelial differentiation by limiting Notch signaling (5). Furthermore, his results have direct clinical implications because it was shown that ZEB1 and miR-200 levels are directly correlated with drug response, thereby offering treatment opportunities for these tumors.

Danny R. Welch (University of Kansas Cancer Center, Kansas City, KS) reported the progress made in the study of the metastasis-suppressor gene KISS1, and shed light on its mechanism of action in melanoma colonization of distant metastasis. The expression of KISS1 alters the colonization step without affecting primary tumor growth (6). This is achieved through a paracrine feedback loop acting in stromal alveolar macrophages that responds to tumor cell-secreted KISS1 and inhibits growth of the metastatic cell population by altering the glucose metabolism and oxygen consumption. The explanation behind this selective suppression of lung metastasis but not of the original primary tumor is found in the different macrophage populations present among tissue types and their despair reaction to kiss peptins.

Frank J. Rauscher, III (Wistar Institute Cancer Center, Philadelphia, PA) presented data on translational approaches to therapeutic intervention of cancer metastasis through the transcriptional inhibition of Snail, a central transcriptional repressor promoting EMT. The protein complex formed by Snail and its corepressors AJUBA and PRMT5 cooperates in E-cadherin repression and EMT induction, and this interaction requires binding of 14-3-3 proteins to fulfill its biological function (7). The association of 14-3-3 proteins with this complex is dependent on Snail phosphorylation by PRKG1 kinase, which in turn is inhibited by phospho-Snail binding to E-box sites in a negative feedback loop. As a conclusion, the data presented suggest that kinases that target Snail/Slug are putative targets for treatment initiatives that inhibit Snail-mediated EMT induction, thereby controlling tumor progression.

Tumor Cell Invasion

Gerhard Christofori (University of Basel, Basel, Switzerland) discussed the different roles of transcription factors in the progression from a differentiated tumor to an invasive carcinoma (8, 9). Specifically, Dlx2 expression is critical for switching from TGF-β tumor-suppressive to oncogenic functions. It represses TGF-β–induced cell-cycle arrest by activating phosphoinositide-3-kinase, mitogen-activated protein kinase, and epidermal growth factor receptor signaling, thereby promoting mammary epithelial cell and melanoma cell survival during EMT and tumor progression. Another key player in the process is Sox4, which is upregulated upon TGF-β–induced EMT. This transcription factor regulates EZH2 methyltransferase and consequently produces changes in H3K27 trimethylation levels, inducing gene expression changes that regulate morphogenesis at the early stages of EMT.

Johanna Joyce (Memorial Sloan Kettering Cancer Center, New York, NY) outlined the importance of the tumor microenvironment in cancer progression. In human pancreatic endocrine tumors, cathepsin expression increases with malignancy, and corresponds to infiltrating activated tumor-associated macrophages (TAM) in response to interleukin (IL)-4 stimulation (10). As a result, increased cathepsin expression confers a tumor growth advantage and chemoresistance to tumor cells, increasing metastatic dissemination and reducing response to therapy. Removal of cathepsins B and S reduces tumor burden and invasiveness and promotes encapsulated tumor formation (10). Similarly, deletion of IL-4 reduces cathepsin activity in pancreatic tumors although it does not reduce macrophage infiltration. Furthermore, it was shown that the microenvironment might have a role in tumor rebound by enhancing cell survival, and could thereby be targeted to enhance therapeutic efficacy. Infiltrating TAMs increase after Taxol administration as well as in breast cancer patients after chemotherapy. These macrophages have a protective effect on tumor cells from Taxol-induced cell death, which is reversed following cathepsin inhibition. In this manner, the combination of chemotherapy administration and cathepsin inhibition was shown to reduce the size of breast tumors and metastases significantly.

Baghu Kalluri (Harvard Medical School, Beth Israel Deaconess Medical Center, Boston, MA) discussed the involvement of fibrosis as a precursor event in certain types of cancer. The connection between fibrosis and cancer is supported by a series of observations, including a higher risk of developing lung tumors in patients suffering from idiopathic pulmonary fibrosis, or the fact that increased levels of collagen deposition—a characteristic of fibrosis—leads to higher invasive phenotypes. The epigenetic mechanisms of DNA methylation and histone modifications lead to fibroblast activation in a fibrotic microenvironment. Specifically, RASAL1 promoter hypermethylation produces Ras activation, resulting in increased proliferation, collagen production, and alpha-SMA expression in myofibroblasts (11). In vitro and in vivo experiments showed how cross-talk between Ras-activated myofibroblasts with nontransformed lung epithelial cells leads to tumor formation in a fibrotic microenvironment.

Rakesh Kumar (George Washington University, Washington, DC) examined the role of the metastasis-associated protein 1 (MTA1) gene in tumor progression and as a potential target for therapy. Previous reports have linked MTA1 activity with resistance to tamoxifen in breast cancer. EMT induction through the repression of E-cadherin transcription via FoxO/HDAC2 recruitment, and induction of mammary tumors and metastasis. The association of MTA1 with a range of binding partners determines its role as a NuRD-dependent coactivator or as NuRD-independent corepressor, and its further involvement in the formation of different complexes that encompass genome-wide effects promoting cell survival, oncogenesis, and metastasis. For example, MTA1 acts as coactivator upstream of Twist, triggering lung metastasis development. The bidirectional autoregulation of the MTA1-ARF pathway has been described in relation to oncogenesis (12). Given its wide associations and the pivotal role as a master coregulator, modifications of MTA1 are of prognostic value and represent potential therapeutic targets.
Christoph Klein (University of Regensburg, Regensburg, Germany) discussed the dynamics of disseminating cancer cells (DCC) and highlighted the importance of reviewing the current hypotheses to better explain the emerging evidence in cancer biology. On the basis of the disparate profiles of genomic alterations found in primary tumor and disseminated cells, the concept of early dissemination and dynamic and independent genomic evolution of tumor cells is gaining importance. In melanoma patients diagnosed histopathologically as having negative sentinel lymph nodes, early DCCs were identified on immunocytochemical detection, and their molecular characterization revealed different alterations from those present in the primary tumor (13). Furthermore, these DCCs acquire further genetic changes until they achieve metastatic colonization, and their tumor-forming potential is heterogeneous, as shown by metastatic colonization, and their tumor-forming potential is heterogeneous, as shown by in vivo experiments with melanoma DCCs that exhibited different tumor-forming capacities. Further characterization of DCCs may increase our knowledge about systemic cancer progression.

Mechanisms of Angiogenesis

Ralph H. Adams (Max Planck Institute and University of Münster, Münster, Germany) gave the first presentation on mechanisms of angiogenesis. He reported the regulation of angiogenesis modulated by Notch and ephrin signaling (14). In vascular sprouting, tip cell selection is produced from the balance between tissue-derived proangiogenic cues (e.g., VEGF) and signaling interactions with adjacent endothelial cells (EC). Specifically, DI4/Notch (VEGF receptor-2 and -3 repressors) and Jagged1 (Notch signaling repressors) comprise the regulatory network that determines whether levels of VEGF are high or low, and activate or repress angiogenesis. Concomitantly, VEGFR endocytosis is a key determinant of vascular extension, and this process is dependent on ephrin-B2 and -B4 signaling. Ephrin-B2 expression promotes motility in ECs when bound to its partners, Dab2 and PAR3, to accomplish VEGFR endocytosis (14). In fact, mutation of any of these components induces important defects in VEGF receptor endocytosis and angiogenic growth.

Holger Gerhardt (London Research Institute, London, United Kingdom) also discussed the involvement of the regulatory network formed by VEGF/VEGFR and DI4/Notch signaling in controlling blood vessel development, and emphasized the value of computer modeling and imaging techniques for studying angiogenesis in pathologic scenarios. Intense cell competition between ECs results in the acquisition of tip cell phenotypes. A central component of these signaling cascades underlying tip cell selection includes VEGFR2 and VEGFR1 balance in promoting (VEGFR2) or decreasing (VEGFR1) tip cell potential (15). This was shown to determine the angiogenic branching to assist cooperative guidance of the expanding vessels in response to VEGF signals emerging from hypoxic or ischemic tissues.

Elisabetta Dejana (FIRC Institute and University of Milan, Milan, Italy) examined the influence of VE- and N-cadherins in vascular remodeling. These adhesion molecules exhibit different distribution patterns in ECs and, despite their similarity, have opposing functions. It was shown that VE-cadherin regulates N-cadherin expression and localization at the transcriptional and posttranscriptional levels. Cadherin levels modulate the rate of cell proliferation and apoptosis, and have opposing effects on cell migration: N-cadherin upregulates genes involved in motility, which are necessary for vessel elongation, whereas VE-cadherin reduces cell migration. Dr. Dejana showed that a correct balance between cadherin levels is required for the correct development of vascular system (16).

Tumoral Angiogenesis

Kari Alitalo (University of Helsinki, Helsinki, Finland) opened the session on tumoral angiogenesis by telling the audience about the VEGF/VEGFR signaling networks involved in lymphangiogenesis (17). Molecular mechanisms regulating lymph vessel development differ from those controlling blood vessels, but cross-talk is also evident. Lymphangiogenesis is fostered by VEGFR3 activation in lymphatic ECs, by VEGF-C and VEGF-D signals secreted by tumor cells and associated macrophages. In fact, over-expression of these molecules results in enhanced tumor lymphangiogenesis and tumor dissemination. Conversely, when using blocking antibodies that target VEGF-C or VEGFR3, tumor lymphangiogenesis was inhibited, and lymph node metastasis and angiogenic vessel sprouting were strongly suppressed. Therefore, VEGF-C/VEGFR3 provide new targets for therapeutic intervention, to support and enhance the efficacy of current antiangiogenic treatments targeted at VEGF/VEGFR2 (18).

Francesc Viñals (Catalan Institute of Oncology-IDIBELL) presented findings with regard to the role of the TGF-β signaling network in tumor angiogenesis. In particular, filamin-B is an actin-binding protein that acts as a transmitter of TGF-β signaling, stimulating the expression of genes involved in EC migration and angiogenesis. Blocking filamin-B function inhibits TGF-β signaling and exerts an antiangiogenic effect on tumor vascularization, as observed in ovarian cancer (19).

Oriol Casanovas (Catalan Institute of Oncology-IDIBELL) discussed the effect of current antiangiogenic therapies on tumor malignization. Angiogenesis inhibitors targeting the VEGF signaling pathway frequently result in hypoxia-driven tumor adaptation and relapse with a more aggressive phenotype (20). Alternative therapeutic strategies to avoid adaptation operate by modulating antivascular potency or finding other non-VEGF/VEGFR targets. In particular, Casanovas presented data on the inhibition of semaphorin-4B as an alternative VEGF-independent pathway strategy. Results show different branching patterns of vasculature and no hypoxia, causing an overall reduction in tumor volume and survival benefit.

William Cruz-Muñoz (Sunnybrook Research Institute, Toronto, ON, Canada), on behalf of Robert Kerbel, presented some recent observations on brain metastasis affecting melanoma patients. Studying metastatic variants of a human melanoma cell line led to the identification of the EDNRB gene as a brain metastasis promoter gene in
melanoma. Overexpression experiments resulted in higher lung and brain metastases and enhanced proliferative rates, although its effect on local invasion capacities remains to be investigated. In addition, this model of spontaneous brain metastasis served to identify the benefits of combined vinblastine and cyclophosphamide therapy in reducing the incidence of brain metastasis in melanoma patients (21).

Eva Gonzalez-Suarez (Cancer Epigenetics and Biology Program, Bellvitge Biomedical Research Institute) presented recent findings with regard to the contribution of the RANK/RANKL pathway to tumorigenesis. Experiments manipulating RANK/RANKL signaling showed increased proliferation and induces tumour adaptation — relapse.

Overall, the latest findings about EMT biology, tumor microenvironment contribution to tumor progression, and mechanisms of angiogenesis were discussed at great length during the meeting, and many potential targets for treatment improvement were proposed (Fig. 1).

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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References
