### Meeting Report

**Exploiting Tumor Vulnerabilities: Epigenetics, Cancer Metabolism and the mTOR Pathway in the Era of Personalized Medicine**

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**Abstract**

Patient stratification according to drug responses, together with the discovery of novel antitumor targets, is leading to a new era of personalized cancer treatments. With the aim of identifying emerging pathways and the challenges faced by clinicians during clinical trials, the IDIBELL Cancer Conference on Personalized Cancer Medicine took place in Barcelona on December 3–4, 2012. This conference brought together speakers working in different areas of cancer research (epigenetics, metabolism and the mTOR pathway, cell death and the immune system, clinical oncology) to discuss the latest developments in personalized cancer medicine. Cancer Res; 73(14): 4185–9. ©2013 AACR.

**Introduction**

Unresponsiveness to therapy remains a significant problem in cancer treatment. As part of a series of IDIBELL Cancer Conferences, this meeting focused on emerging fields in cancer research: epigenetic drugs, the mTOR pathway and cancer metabolism, the tumor microenvironment, and overcoming resistance to cancer therapeutics. Moreover, clinical researchers shared with the audience some of the challenges encountered in the implementation of clinical trials to test novel drugs, the criteria for patient selection, and their perspectives about how to improve personalized medicine. The conference was organized by Manel Esteller (IDIBELL, Barcelona, Spain), George Thomas (IDIBELL and University of Cincinnati, Cincinnati, OH), Sara Kozma (IDIBELL), and Cristina Muñoz-Pinedo (IDIBELL) and took place in the futuristic Hotel Hesperia Tower, which served as an appropriate venue to discuss the future of anticancer therapies.

**Tumor Metabolism and the mTOR Pathway**

Proliferating cells require synthesis of DNA, RNA, proteins, and lipids at a higher rate than untransformed cells. Because oncogenic transformation leads to "metabolic transformation," analysis of metabolic preferences of cancer cells may permit the development of novel drugs, targeting metabolic processes heavily relied upon by tumors, as novel anticancer agents (1). Lipid synthesis and fatty acid usage through β-oxidation are upregulated in many tumors, which opens the door to treatment of certain tumors with drugs that target this pathway. But what mutations will lead to sensitivity to drugs that inhibit lipid metabolism? Brendan Manning (Harvard School of Public Health, Boston, MA) and Arkaitz Carracedo (bioGUNE) are trying to understand the role of specific oncogenes in the regulation of lipid metabolism. Manning showed how the phosphoinositide 3-kinase or RAS oncogenes promote de novo lipid synthesis in an mTOR-dependent manner. Carracedo discussed the role of the promyelocytic leukemia protein as a prosurvival protein in breast cancer that promotes fatty acid catabolism and PPAR signaling (2). Tak Mak (The Campbell Family Institute for Breast Cancer Research, Toronto, Ontario, Canada) presented preclinical data about the role of carnitine palmitoyltransferase 1C, an enzyme involved in fatty acid oxidation and normally expressed exclusively in the brain, as a prosurvival molecule in breast cancer cells. This suggests that drugging this enzyme with molecules that do not cross the blood–brain barrier could be highly effective (3). Cristina Muñoz-Pinedo has studied the ability of the glycolytic inhibitor 2-deoxyglucose to induce apoptosis in sarcoma. This drug is effective in vitro against alveolar rhabdomyosarcoma (4), but not other soft-tissue sarcomas, which are nevertheless dependent on glucose. Nucleotide biosynthesis is another pathway usually upregulated in tumors. Drugs that inhibit this pathway, such as methotrexate and 5-fluorouracil, have been used for decades in the clinic because tumor cells are more sensitive to inhibition of nucleotide metabolism. Brendan Manning reported that growth factors regulate de novo pyrimidine synthesis through mTOR (5). The mTOR downstream effector S6 kinase, by phosphorylating a key enzyme of the pathway, stimulates nucleotide synthesis, thereby providing a link between the nucleotide pool required for increased RNA and DNA synthesis in growing cells.

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The kinase mTOR is a major driver of tumor metabolism and proliferation of cancer cells, acting downstream of numerous oncogenic pathways. Several drugs targeting the mTOR pathway are being developed and tested in the clinic. David Sabatini (Whitehead Institute, Cambridge, MA) is trying to understand how the mTOR pathway and its activators, the Rag GTPases, regulate metabolism and survival. Transgenic mice with hyperactive Rag GTPases show accelerated postnatal death related to their inability to maintain blood glucose and amino acid levels. This effect of mTOR is related to negative regulation of autophagy (6).

The mTOR pathway also supervises another fundamental anabolic cellular process, ribosome biogenesis, which is also massively elevated in proliferating cells. George Thomas showed that lesions in ribosome biogenesis activate a cell checkpoint controlled by p53 through a unique mechanism involving the ribosomal proteins RPL5, RPL11, and 5S rRNA. These studies open new avenues in understanding the etiopathology of ribosomopathies (7). Another aspect of cell physiology that is regulated by the mTOR pathway is the control of cell size. José Aramburu (Pompeu-Fabra University, Barcelona, Spain) used a model of cellular adaptation to osmotic stress to show that mTOR can maintain cell size and activate adaptive genes during stress responses (8). Altogether, these findings point out the central role of mTOR as a master regulator of cell growth and stress responses.

**Tumor Microenvironment and the Immune System**

The contribution of the tumor stroma to tumorigenesis has gained increasing attention over the past decade. Understanding the basic players that make the stroma permissive to tumor cells is of key importance to target metastasis pharmacologically. In a comprehensive talk, Gregg Semenza (Johns Hopkins, Baltimore, MD), highlighted the reciprocal paracrine cross-talk between breast cancer and mesenchymal stem cells in the process of lymph node and lung metastasis formation (9). Hypoxia-inducible factors (HIF) are the master regulators of this reciprocal signaling, and drugs that target HIF, such as digoxin or acriflavine, were shown to block metastatic spread, shedding light on the importance of HIF-signaling mitigation during breast cancer treatment.

The immune response undoubtedly affects tumor progression. However, there is increasing evidence that cells of the immune system can be subverted and reeducated by tumors to assist rather than destroy the tumor. In this regard, it is now well established that persistent inflammation can generate a supportive environment within tumors that can assist tumor maintenance and tumor progression on several levels. The plenary lecture given by Michael Karin (University of California, San Diego, San Diego, CA) and sponsored by EMBO placed strong emphasis on the tight link between inflammation and obesity, one of the most common high-risk factors of the Western world. Hepatocellular carcinoma induced by high-fat diet and/or by genetic background is dependent on the production of interleukin (IL)-6/TNF and the establishment of a feedback loop between chronic inflammation and the stimulation of STAT3, which drives cancer progression. In another paradigm, a peculiar mechanism involving intestinal microbiota was shown to initiate colorectal carcinoma. Once penetrated in the tumor lesion, gut microbiota elicit an inflammatory response mediated by IL-23/IL-17, two of the major promoters of colorectal carcinoma. Intriguingly, treatment with antibiotics reduces the tumor load in mice, paving the way for a novel conceptual use of over-the-counter drugs (10).

Although growing evidence suggests that immune responses to certain tumors can assist cancer progression in some cases, there is also heartening evidence that immune responses to tumors can assist in tumor eradication. Moreover, recent evidence also indicates that the immune system may be involved in the therapeutic response to certain anticancer drugs. In this regard, Guido Kroemer (INSERM, Paris, France) showed that drug-mediated cell death in vivo may occur with the externalization of specific immunogenic signals, such as calreticulin, on tumor cells. The latter event seems to promote immunogenic cell death, and is followed by the recruitment of immune cells, which also assist in tumor eradication. Hyperploidization is one of the triggers of immunogenicity (11). In the same line, Jean-Ehrland Ricci (INSERM, Nice, France) analyzed the effects of an antiglycolytic drug, 2-deoxyglucose, on the immunogenicity of cell death induced by etoposide. Although etoposide on its own promoted cell death in a mouse model of B-cell lymphoma, this was greatly enhanced when used in combination with 2-deoxyglucose. Rather surprisingly, Ricci showed that 2-deoxyglucose triggered immunogenicity of dying cells, with the combination effects being more obvious in immunocompetent than in immunodeficient mice (12). A lesson we learned from these talks is that rational drug design and screening should consider the involvement of complex mechanisms that are not only limited to the targeted tumor but could also harness the help of the immune system.

**Novel Antitumor Therapies: Overcoming Apoptotic Resistance**

The aim of most antitumor therapies is to promote cell death in tumor cells while sparing nontransformed cells. However, this is a difficult challenge, especially because tumors frequently overexpress antiapoptotic proteins, such as Bcl-2 family members, or are deficient in proapoptotic proteins, such as p53. Stig Linder (Karolinska Institutet, Stockholm, Sweden) screened for drugs that kill p53-deficient cells and identified a small molecule that blocks the deubiquitinase activity of the proteasome. This molecule, b-AP15, can bypass protection from apoptosis exerted by Bcl-2 and is effective in a number of animal tumor models (13). Another way to bypass apoptotic impairment is to induce nonapoptotic cell death. Doug Green (St. Jude Children’s Research Hospital, Memphis, TN) is studying the mechanisms of necroptosis. This form of cell death is induced by TNF, mediated by the kinases RIPK1 and RIPK3, and suppressed by the proapoptotic protein caspase-8 (14). Deciphering the mechanisms by which this form of cell death proceeds may lead to the identification of specific activators of
the pathway to target tumors overexpressing antiapoptotic proteins.

Epigenetics: A Tool for Personalized Medicine and a Target for Novel Drugs

Epigenetic regulation of gene expression by histone modifications or DNA methylation is now well established to make important contributions to cell differentiation and proliferative states. Disruption of the epigenetic landscape in cancer is a well-known phenomenon. In recent years, it has been shown that numerous compounds that inhibit components of the epigenetic machineries are effective against cancer cells. On the other hand, candidate gene approaches to identify the epigenetic alterations present in cancer have recently been complemented by new whole-genome technologies, which are bringing forward the idea to use epigenetic marks as biomarkers to personalize cancer treatment (15). Using DNA methylation arrays, Manel Esteller was able to classify different types of acute lymphoblastic leukemia, non–small cell lung cancer (NSCLC), and cancers of unknown primary origin (16). Lynnette Fernandez-Cuesta (University of Cologne, Germany) presented a unified pipeline that affords efficient mapping of paired-end RNA-seq reads and downstream analysis of transcribed genes in cancer samples. This approach uses a de novo assembly step to increase the sensitivity and specificity in detecting chimeric transcripts. Because translocations might activate druggable cancer pathways, in-depth study of RNA-seq data might help to provide new specific and personalized treatment to patients.

DNA and histone modifications can be targeted, and several talks covered the use of drugs targeting histone modifications. Nick La Thangue (University of Oxford, Oxford, UK) presented his work on a sensitivity determinant for histone deacetylase (HDAC) inhibitor–induced apoptosis: HR23B. HR23B shuttles ubiquitinated cargo proteins to the proteasome for subsequent degradation. Reduced HR23B expression in biopsy samples was highly associated with lack of HDAC inhibitor therapy response. After conducting the studies with patients treated with HDAC inhibitors other than SAHA, La Thangue suggested HR23B as a pan-biomarker for patient stratification into groups that will gain the most clinical benefit (17). Scott A. Armstrong (Memorial Sloan-Kettering Cancer Center, New York, NY) showed that DOT1L, a histone H3K79 methyltransferase, is required for leukemia maintenance and thus a potential therapeutic target in MLL-rearranged leukemias (18). A selective inhibitor of DOT1L has been developed that blocks H3K79 methylation, inhibits leukemogenic gene expression, and selectively kills cultured cells bearing MLL translocations. Small-molecule DOT1L inhibitors have entered a phase I clinical trial. Christopher Yakov (Cold Spring Harbor Laboratory, New York, NY) identified the acetylated histones reader BET bromodomain protein 4 (BRD4) as being critically required for maintenance of acute myeloid leukemia (19). Use of the small-molecule inhibitor JQ1 phenocopies the genetic suppression of Brd4. The effects of Brd4 suppression are, at least in part, due to its role in sustaining Myc expression to promote aberrant self-renewal. Moreover, myc regulation via long-range enhancement through Brd4 seems to be leukemia/hematopoietic specific. Esteller presented studies on several epigenetic drugs: zebularine, a DNA methylation inhibitor; enoxacin, an enhancer of microRNA production; and CHR-6494, an inhibitor of the histone kinase Haspin (20). Importantly, Esteller also discussed mechanisms of resistance, such as a truncating mutation in HDAC2 conferring resistance to HDAC inhibitors and amplification in DNMT3B conferring resistance to demethylating agents.

Personalized Cancer Medicine: Impact on Clinical Translation

This session brought together basic, translational, and clinical scientists to discuss strategies for the selection of patients who will most likely benefit from a particular therapy. Understanding the mechanisms that contribute to primary or acquired resistance will yield biomarkers that predict how individual patients will respond to drugs, and the development of drugs that can act in synergy with established treatments or overcome drug resistance. Rene Bernards (The Netherlands Cancer Institute, Amsterdam, the Netherlands) uses genome-wide loss of function genetic screens (short hairpin interference libraries) in cancer cell lines to identify genes related to drug response. In this way, it was found that blockade of the EGF receptor (EGFR) shows strong synergism with BRAF (V600E) inhibition in patients with colon cancer (21). These results have led to a clinical trial initiated only 8 months after manuscript publication. This strategy also led to the identification of MED12 as responsible for resistance to tyrosine kinase inhibitors (TKI) in lung cancer. Moreover, MED12 may constitute a multitarget drug resistance beyond TKI, as suggested by Bernard’s data (22).

The laboratories of Manuel Hidalgo, Alberto Bardelli, and Alberto Villanueva have established patient-derived xenografts in mice (PDX) as a resource geared toward establishing a connection between the particular genetic and histopathogenic properties of the tumor and the best therapeutic options. M. Hidalgo (Spanish National Cancer Research Center, Madrid, Spain) and colleagues have used pancreatic PDX to study patient response to gemcitabine, extracting signatures and identifying biomarkers that can be implemented in clinical trials contributing to the classification of pancreatic tumors (23). Bardelli (IRCC Candido and University of Turin, Turin, Italy) showed that it is feasible to study primary and secondary resistance to anti-EGFR antibodies using colorectal PDX derived from patients. These PDX models prospectively recapitulated biomarker-based case stratification and identified KRAS, BRAF, NRAS, and HER2 as predictors of resistance to anti-EGFR blockade. Bardelli also showed how the emergence of KRAS mutations in the plasma of patients undergoing anti-EGFR therapy can be used to detect early relapse and guide further therapies (24). Villanueva (Catalan Institute of Oncology-IDIBELL, Barcelona, Spain) has generated extensive and representative OrthoXenoBanks (OXB) of epithelial ovarian and colorectal tumors generated by orthotopic implantation (i.e., tumors with the same organ of origin) that mimic immunohistologically and genetically the heterogeneity of both
diseases. OXIs are being used to explore the best patient treatment (25).

Clinical researchers Rafael Rosell, Josep Tabernero, and Ramón Salazar shared with the audience their concerns about how intertumor heterogeneity may complicate clinical trials and how the current knowledge on patient stratification is applied in the day-to-day clinical practice. Rosell (Catalan Institute of Oncology, Badalona, Spain) emphasized that targeting cancer with pairs of synthetic lethal drugs may reduce the emergence of resistance. Results from the EURTAC clinical trial (26) support the assessment of two novel markers, BIM expression and EGFR T790M mutation before initiation of treatment with EGFR inhibitors in NSCLC. Moreover, combined inhibition of EGFR and AXL can prevent or overcome resistance to EGFR inhibitors. Salazar (Catalan Institute of Oncology, Barcelona, Spain) talked about the clinical management of EGFR pathway resistance in colorectal carcinomas and highlighted the need to improve the sensitivity of mutation detection to increase prediction of response (27). Nanofluidic digital PCR provides a robust, quantitative measure of the proportion of KRAS-mutant alleles allowing a better classification of tumors. Tabernero (Vall d’Hebron Institute of Oncology, Barcelona, Spain) encouraged a transition from a clinically directed drug development approach, in which responses to drugs in specific tumor types guide the next steps of experimentation, to a translational guided scenario in which the predictive biomarkers and drugs are codeveloped from the beginning of drug development until drug approval. Phase I trials provide an arena for early hypothesis testing, examining not only safety and toxicity but also target engagement, biologically effective dosages, and selection of an appropriate patient population.

Conclusions

Cancer metabolism is a promising new area of research that deserves further attention. In this sense, inhibition of the mTOR pathway, which controls metabolism and proliferation, warrants further investigation. Currently many oncology patients are receiving expensive treatments that can cause adverse side effects without getting any therapeutic benefit. In this sense, animal models of xenopatients may be useful before clinical trials because they recapitulate characteristics of the original tumor, including drug sensitivity. Since the initiation of the “-omic” era, enormous amounts of data have been generated. This information needs to be comprehensively integrated and made available to clinicians. Jonathan Knowles (EPFL, Lausanne, Switzerland), the Instituto Roche Distinguished Speaker, wrapped up the conference with an overview of personalized medicine. Knowles urged the scientific community to define ways to decide the best combination of available compounds for efficacy in particular patient cohorts. Finally, he stressed that major progress toward curing cancer is now possible through early detection from blood screens and early intervention through surgery, and a combination of radiation and chemotherapy followed by new-generation–targeted immunotherapy for all patients. The road toward personalized medicine is a challenging one and represents a major change in perspective for all involved, but it is becoming a reality.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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