

## Meeting Report

## Twenty-First Annual Pezcoller Symposium: Unconventional Therapeutic Targets in Cancer

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## Introduction

The 21st Pezcoller Symposium, entitled “Unconventional Therapeutic Targets in Cancer,” was held in Trento, Italy, on June 11–13, 2009. The focus of the Symposium was on identifying nontraditional targets for anticancer drug action and using new concepts for drug development.

## Report

Sean Morrison (University of Michigan Center for Stem Cell Biology, Ann Arbor, MI) indicated that tumorigenic potential is a common characteristic of melanoma cells rather than a property of a rare population of stem cells. A fundamental question is whether cells with tumorigenic potential are common as would be expected under a clonal evolution model or rare as suggested under a stem cell model. Studies on cancers, including melanoma, have indicated that only rare human cancer cells (0.1–0.0001%) have tumorigenic potential when transplanted into nonobese diabetic/severe combined immunodeficient (NOD/SCID) mice. Using more immunocompromised NOD/SCID IL2R $\gamma$  null mice increased the detection of tumorigenic melanoma cells by several orders of magnitude. In limiting dilution assays, approximately 25% of unselected melanoma cells from 12 different patients formed tumors. In single-cell transplants, an average of 27% of unselected melanoma cells from four different patients formed tumors. High frequencies of tumorigenic cells can be detected in some cancers even in NOD/SCID or in fully immunocompetent mice. Morrison has been unable to detect any evidence for hierarchical organization or intrinsic differences in tumorigenicity among melanoma cells. Despite examining more than 50 cell surface markers (including markers previously suggested to mark “melanoma stem cells”), he was unable to distinguish tumorigenic and nontumorigenic subpopulations of melanoma cells; for example, CD133-positive and CD133-negative cells both readily formed tumors. In samples from three patients with glioblastoma, tumorigenic cells were also common. Overall, the results suggest that some cancers follow a stem cell model, whereas many others do not.

Bruce Spiegelman (Dana-Farber Cancer Institute, Boston, MA) discussed the mechanisms underlying the defects in ox-

idative metabolism in tumors. His laboratory has investigated how brown fat is generated. A large zinc-finger protein, PRDM16, is both necessary and sufficient to induce brown fat from white fat precursor cells or myoblasts. PRDM16 was isolated and found to exist in complex with several transcription factors, including CCAAT/enhancer binding protein- $\beta$  (C/EBP- $\beta$ ) and p53. The PRDM16/C/EBP- $\beta$  complex is responsible for the muscle/brown fat switch. They also found that PRDM16 binds to p53 and suppresses its actions. This is particularly interesting given that PRDM16 seems to be overexpressed via chromosomal translocation in certain human leukemias.

William Kaelin (Dana-Farber Cancer Institute, Boston, MA) identified 2-oxoglutarate-dependent dioxygenases (EglN1) as potential therapeutic targets in cancer. In the presence of oxygen, the  $\alpha$  subunit of the transcription factor hypoxia-inducible factor (HIF) is hydroxylated on one (or both) of two prolyl residues by the EglN1. This creates a binding site for VHL, which then targets HIF $\alpha$  for polyubiquitination and proteasomal degradation. When oxygen levels are low, or the VHL protein is crippled, HIF $\alpha$  accumulates, binds to HIF $\beta$ , and transcriptionally activates genes that promote survival in a low-oxygen environment. Compounds that activate EglN1 are being explored as anticancer agents. Inhibition of EglN2 leads to loss of cyclin D1 in a HIF-independent manner, decreased proliferation, and impaired tumorigenesis *in vivo*; impaired proliferation can be rescued by exogenous cyclin D1 or by loss of pRB. RBP2, a pRB-binding protein, is a 2-oxoglutarate-dependent dioxygenase that serves as a histone demethylase. Inhibition of RBP2 seems to lead to impaired proliferation, promotion of differentiation, and loss of tumorigenesis.

Vishva Dixit (Genentech, San Francisco, CA) discussed deubiquitinases as possible targets for cancer chemotherapy. The ubiquitin ligase COP1, together with its binding partner, DET1, ubiquitinates proto-oncogene *ETV1*, resulting in *ETV1* proteasomal degradation. Truncation of *ETV1* in the TMRSS2:*ETV1* translocation product, found commonly in prostate cancer, eliminates its COP1 binding motif and enhances its stability. COP1 deficiency causes prostate intraepithelial neoplasia *in vivo*. In human prostate cancer, loss of COP1 expression correlated with elevated *ETV1* protein. Thus, COP1 is potentially a tumor suppressor. In a separate unscheduled brief presentation, Dr. Dixit discussed IAP inhibitors as cancer therapeutic agents. These molecules operate by suppressing the antiapoptotic actions of the relevant IAP and also trigger cell death by triggering tumor necrosis factor signal transduction and suppressing NF- $\kappa$ B function.

Gregory Verdine (Dana-Farber Cancer Institute, Boston, MA) used specifically stapled peptides to affect targets that would otherwise be difficult or impossible to attack directly. As many as 80% to 90% of all potential targets are undruggable.

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The Verdine laboratory is developing “synthetic biologics,” molecules that, like biologics, possess an ability to target large, flat surfaces, but, like small molecules, are fully synthetic and can be readily modified. Hydrocarbon-stapled  $\alpha$ -helical peptides and dipeptides have been developed. Proteins involved in cell survival were among the first that were targeted (e.g., Bcl2 and Bax), and therapeutic effects were observed in animals. Transcription factors can be targeted in this way.

Nika Danial (Dana-Farber Cancer Institute, Boston, MA) discussed the role of the apoptosis-regulating protein BAD in glucose sensing. Glycolysis and mitochondrial oxidative phosphorylation suppression is associated with metabolic aberrations in both cancer cells and in diabetes. When phosphorylated, the BAD minimal death domain, BH3, engages proteins involved in glucose phosphorylation, mitochondrial respiration, and ATP production. When dephosphorylated, the BH3 domain inactivates Bcl-2 and Bcl-x<sub>L</sub>. Hydrocarbon-stapled phospho-BAD BH3 helices simulated the metabolic function of BAD.

Steven Elmore (Abbott Laboratories, Abbott Park, IL) reported on the use of specific compounds to target Bcl-2 as cancer treatment. Cancer cells gain a survival advantage by changing the balance between proapoptotic and antiapoptotic factors. Antagonists of antiapoptotic Bcl-2 proteins have been developed. ABT-263 is a small molecule that disrupts Bcl-2 or Bcl-x<sub>L</sub> interactions with proapoptotic proteins (e.g., Bim), leading to rapid initiation of Bax mitochondrial membrane translocation, cytochrome *c* release, and Bax/Bak-dependent apoptotic cell death. ABT-263 can kill cell lines dependent on Bcl-2 and/or Bcl-x<sub>L</sub> for survival but is incompletely potent as a single agent in most solid tumors. However, by lowering the apoptotic threshold, ABT-263 synergistically enhanced the killing effect of chemotherapeutic agents in several tumors clinically.

Wilhelm Krek (Institute of Cell Biology, Zurich, Switzerland) discussed microtubule-based VHL tumor suppressor mechanisms. Regulation of microtubule cytoskeletal stability by VHL binding is critical for its tumor-suppressing activity. VHL suppresses spindle misorientation and promotes chromosomal stability by positively regulating Mad2 mitotic checkpoint protein expression. Loss of Mad2 leads to aneuploidy, and aneuploidy arising after VHL inactivation can be rescued by Mad2 reexpression. This newly identified function of VHL is rendered defective in VHL mutants, linked, at least in part, to microtubule-dependent activities. An association between VHL inactivation, reduced Mad2 levels, and increased aneuploidy was also found in human renal cancer. Quantitative live-cell imaging of microtubule growth was important when the plus-end marker end-binding protein 3-green fluorescent protein was imaged in conjunction with the spatiotemporal clustering of growth tracks to reveal the effect of VHL function loss on microtubule dynamics.

Emilio Hirsch (University of Torino, Torino, Italy) studied the inhibition of phosphoinositide 3-kinases (PI3K) in mice. PI3Ks consist of heterodimers of a 110-kDa catalytic (p110) subunit and a regulatory/adaptor subunit. p110 $\alpha$ , p110 $\beta$ , p110 $\gamma$ , and p110 $\delta$  share homology, assume certain nonredundant

biological roles, and work both as kinases and as scaffolds supporting certain protein-protein interactions. Thus, p110 $\gamma$  plays a crucial role in mounting inflammatory reactions but is also part of a complex controlling cardiac contractility. Deletion of the *p110 $\beta$*  gene causes embryonic lethality, but the expression of catalytically inactive p110 $\beta$  is compatible with life. Although the absence of p110 $\gamma$  blocks proliferation of fibroblasts, inhibition of its kinase activity does not affect their growth. The p110 $\gamma$  protein is required for epidermal growth factor receptor endocytosis, independently of its kinase activity. Clathrin-coated vesicle production is severely impaired in the absence of p110 $\gamma$ , yet is normal when p110 $\gamma$  catalytic function is ablated. Despite its function as a scaffold protein, p110 $\gamma$  catalytic activity is involved in signal transduction triggered by tyrosine kinase and G-protein-coupled receptors. p110 $\gamma$  seems to be required for oncogenic Erbb2-mediated mammary cancer development because mice expressing catalytically inactive p110 $\gamma$  revealed delayed mammary gland cancer development when crossed with transgenic mice that overexpressed Erbb2 in the mammary gland.

David Sabatini (Whitehead Institute for Biomedical Research, Cambridge, MA) discussed the therapeutic potential of targeting the mammalian target of rapamycin (mTOR) pathway. One mTOR complex regulates growth through S6K, and another regulates cell survival through Akt. mTORC1 and mTORC2 define both the rapamycin-sensitive and the rapamycin-insensitive branches of the mTOR pathway. Amino acids promote mTORC1 movement to a part of the endomembrane system containing its activator Rheb. Targeting Rheb-mTORC1 interactions may represent a new therapeutic approach in tumors. Amino acids signal through the conserved Rag family of small GTPases, which interact directly with the Raptor component of mTORC1 depending on the GTP-loaded status of the Rags. mTORC1 inhibition by the mTOR-interacting protein, Deptor, hyperactivates PI3K signaling in multiple myeloma. This interaction may represent another potential target of therapeutic intervention.

Tom Roberts (Dana-Farber Cancer Institute, Boston, MA) discussed targeting of PI3K isoforms in cancer. The only class of cancer-associated PI3Ks is 1A, consisting of three enzymes (p110 $\alpha$ , p110 $\beta$ , and p110 $\delta$ ) signaling downstream from receptor tyrosine kinases (RTK), G-protein-coupled receptors (GPCR), and certain oncoproteins. Pathway activation in tumors is usually achieved by activating mutations in p110 $\alpha$  via inactivation or mutations of PTEN. p110 $\alpha$  carries the majority of the PI3K signal in RTK signal transduction, while p110 $\beta$  responds to GPCRs. p110 $\alpha$  is essential for the growth of tumors driven by mutations and/or oncogenic RTKs/Ras, whereas p110 $\beta$  is the major isoform in mediating PTEN-deficient tumorigenesis. In a prostate tumor model with deletion of PTEN, BEZ235, which inhibits both PI3K $\alpha$  and PI3K $\beta$ , blocked prostate tumor progression. In an ovarian model with lost PTEN and activated Kras, ablation of p110 $\alpha$ , but not of p110 $\beta$ , markedly inhibited tumor formation. p110 isoform-specific inhibition holds promise as a therapeutic strategy.

Giulio Superti-Furga (Center for Molecular Medicine, Vienna, Austria) uses molecular networks to discern future drug

targets. Five drugs for chronic myeloid leukemia were investigated. Imatinib resistance necessitated the development of second-generation (nilotinib and dasatinib) and third-generation (bosutinib and INNO-406) inhibitors. The disease-relevant protein target profiles of these drugs were identified in patients' cells by chemical proteomics, which combines drug-affinity chromatography with mass spectrometry. One nonenzymatic target identified, NQO2, was inhibited by imatinib at nanomolar concentrations. The interaction profiles of the five drugs display strong differences, overlapping only at the ABL kinases, with dasatinib and bosutinib being particularly promiscuous. In conclusion, (a) "modern" targeted drugs are quite promiscuous; (b) generally, drug targets are likely part of larger protein complexes; (c) binding partners may influence drug action on the complex and be affected; (d) the complex components that become available after drug treatment may redistribute and affect other signaling pathways; and (e) drugs are system perturbators and not "erasers" of protein activity, with some gain-of-function effects matching loss-of-function.

Peter Finan (Novartis, Cambridge, MA) discussed target identification by chemical genetics. Wnt pathway inhibitor development has been hampered by the paucity of pathway components amenable to small-molecule inhibition. A chemical genetic screen was used to identify modulators selectively inhibiting  $\beta$ -catenin-activated transcription. By a solid-phase chemical and proteomic approach, it was found that the most potent and specific of these modulators inhibits a member of the phosphoribosyl pyrophosphate family, tankyrase, which had not been previously associated with the Wnt pathway. These studies highlighted novel approaches to identifying new therapeutic targets.

William Hahn (Dana-Farber Cancer Institute, Boston, MA) described systematic functional approaches to targeting K-Ras. A strategy for targeting *KRAS* is to identify gene products that, when suppressed or inhibited, result in synthetic lethality (i.e., cell death only in the presence of a relevant oncogenic allele). By systematic RNA interference screens, two kinases, *TBKI* and *STK33*, were identified that synthesize activated mutant *KRAS* and selectively kill cancer cell lines. Thus, synthetic lethality screens visualize the existence of heretofore unpredicted cancer target proteins.

Todd Golub (Dana-Farber Cancer Institute, Boston, MA) discussed how gene expression signatures reflecting a biological state can be used to identify small molecules modulating the signature of interest and that biological state. This process, termed gene expression-based high-throughput screening, was successfully applied to the identification of compounds, inducing myeloid differentiation of acute myeloid leukemia cells, inhibiting the activity of Ewing Sarcoma oncogene *EWS/FLI*, and abrogating androgen receptor signaling in prostate cancer. To systematically connect signatures of diseases to signatures of gene product function or signatures of drug action constitutes the Connectivity Map project in which new drug targets operating in pathways of interest are discovered. The Connectivity Map approach identified modulators of glucocorticoid resistance in acute leukemia and candidate mechanisms of action of small molecules dis-

covered in cell-based screens such as a natural product inhibitor of transcription. Thus, gene expression-based approaches are an important new adjunct to conventional approaches to drug discovery.

Charles Sawyers (Memorial Sloan Kettering Cancer Center, New York, NY) discussed novel approaches to prostate cancer therapy. Androgen receptors (AR) can promote prostate cancer growth in human tumor xenografts growing in animals treated with androgen deprivation therapy. AR can be overexpressed in this setting. AR antagonists function as weak agonists in this context. Novel AR antagonists are sought using a cell-based screen that might retain AR inhibitory function in the context of increased AR expression. Using the high-affinity AR agonist RU59063 as a starting point, more than 200 derivatives were synthesized and screened. The novel compound RD162 retained potent antiandrogen activity in cells expressing increased levels of AR, blocked AR function in mice with 10-fold greater potency/affinity than bicalutamide, and impaired the growth of LNCaP and LAPC-4 xenografts engineered to express high levels of AR. An RD162 derivative, MDV3100, has shown clinical activity in a phase I-II clinical trial. MDV3100 impaired the growth of the TMPRSS2-ERG-positive cell line VCAP in culture and induced apoptosis. Some results suggested that PI3K pathway activation may blunt the efficacy of antiandrogen therapy.

Pier Paolo Pandolfi (Harvard Medical School, Boston, MA) stressed the need to target cancer-initiating cells (CIC) for effective therapy. The elucidation of a novel PTEN/PI3K/mTOR signaling regulatory network for the control of "stem cell-ness" has allowed for the testing of concepts for CIC eradication of immediate therapeutic applicability. Excessive signaling can also trigger fail-safe mechanisms leading to stem cell pool exhaustion, depending on the genetic milieu. Thus, therapeutic intervention could aim at transiently enhancing, rather than blocking, PTEN/PI3K/mTOR signaling. Cancer cell types that benefit from aberrant activation of this pathway (e.g., prostate cancer) would be particularly sensitive to a transient superactivation of the pathway. Transient inactivation of the tumor suppressor promyelocytic leukemia (PML) could be used toward the eradication of the leukemia-initiating cells (LIC) in chronic myelogenous leukemia (CML). PML represses mTOR signaling, and its inactivation leads to LIC exhaustion in CML. Arsenites (e.g.,  $A_2O_3$ ) transiently inhibit PML by triggering its proteasome-dependent degradation and causing the exhaustion of the CML LICs. Initial treatment with  $A_2O_3$ , followed 3 to 5 days later by Gleevec, could eradicate CML residual disease, a concept currently tested clinically. Thus, Pandolfi underscored how transient pharmacologic inhibition of tumor suppressors could represent a new therapeutic avenue.

Zaver Bhujwala (Johns Hopkins University, Baltimore, MD) discussed imaging to guide therapies based on unconventional targets. In prodrug enzyme activation systems, imaging the enzyme delivery so that the prodrug is administered when enzyme levels are highest in tumor and lowest in systemic circulation and normal tissues would optimize this therapeutic strategy. Cytosine deaminase converts

5-fluorocytosine (5-FC) to 5-fluorouracil (5-FU). 5-FC and 5-FU can be detected noninvasively by  $^{19}\text{F}$  magnetic resonance spectroscopy. Imaging was used to time prodrug administration optimally. Image-guided targeting of choline kinase, the first step in choline phospholipid biosynthesis, is also done. Target specific pathways, microenvironments, and cell types within tumors can be targeted under image guidance.

Robert Kerbel (University of Toronto, Toronto, Canada) outlined unexpected outcomes of anti-vascular endothelial growth factor treatments. After an initial antitumor effect, unexpectedly enhanced tumor cell growth and metastasis occur. Through increases in the hypoxia-induced transcription factor HIF-1, drug-exacerbated tumor hypoxia likely results in the adaptive upregulation of angiogenesis, cell invasion, and metastasis. Circulating growth factors, cytokines, and chemokines are induced following the administration of antiangiogenic drugs, such as sunitinib, and can promote tumor growth and angiogenesis. In preclinical models of neoadjuvant or adjuvant-like therapy of micrometastatic breast cancer, evidence of accelerated metastatic growth was obtained. To improve the effect of antiangiogenic

drugs, their combination with metronomic chemotherapy is being investigated.

### Prospects

The novel and unconventional molecular targets and systems discussed at this Symposium should provide the basis for the development of more effective and specific treatments of cancer.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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