Meeting Report

Targeting PI3K/mTOR Signaling in Cancer

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Abstract

The American Association for Cancer Research (AACR) Special Conference on Targeting PI3K/mTOR Signaling in Cancer was held in San Francisco, California from February 24 to 27, 2011. The meeting was cochaired by Drs. Lewis C. Cantley, David M. Sabatini, and Funda Meric-Bernstam. The main focus of this event was the therapeutic potential of drugs targeting the PI3K/mTOR signaling pathway for the treatment of cancer. This article summarizes the recent discoveries in the field, with particular emphasis on the major themes of the conference. Cancer Res; 71(24); 7351–9. ©2011 AACR.

Introduction

The phosphoinositide 3-kinase (PI3K) family of lipid kinases phosphorylate the 3’-hydroxyl group of phosphoinositides (1). There are 3 classes of PI3K, each with distinct lipid products and substrate specificity (1). To date, the class IA PI3Ks are the most widely implicated class in human cancers (2). PI3K activation initiates a signal transduction pathway that promotes cancer cell growth, metabolism, and survival (Fig. 1). Akt, a serine-threonine kinase that is directly activated in response to PI3K, is a crucial effector of PI3K in tumorigenesis. Activation of Akt leads to increased cellular growth and survival. One of the key downstream effectors of Akt is mTOR complex 1 (mTORC1). The regulation of mTORC1 is complex in that the PI3K/Akt signaling is not the only means of regulating mTORC1. mTORC1 is under the control of many pathways, including growth factor signaling, nutrient and oxygen availability, and the energy state of the cell. Therefore, from a therapeutic standpoint, the regulation of mTORC1 is crucial as some PI3K inhibitors will block both PI3K and mTOR signaling, whereas others will only target PI3K itself. The importance of targeting the PI3K/mTOR for cancer therapy was highlighted at the recent American Association for Cancer Research (AACR) Special Conference on Targeting PI3K/mTOR Signaling in Cancer in San Francisco from February 24 to 27, 2011. This meeting underscored the challenge that those in the PI3K/mTOR field face in developing dual PI3K/mTOR inhibitors and emphasized this exciting era of targeted therapies for cancer. In this short article, we review some of the key presentations from this meeting.

PI3K/mTOR Signaling

It is well recognized that the PI3K/mTOR signaling pathway is vital for the growth and survival of cancer cells. With the rapid pace that PI3K/mTOR inhibitors are entering the clinic, there was no shortage of scientific research and clinical data to go through at this meeting. To start off the meeting, the keynote address was given by José Baselga (Massachusetts General Hospital, Boston, MA), who presented PI3K mutations in cancer and their contribution to resistance to hormonal therapy. The first agents targeting the PI3K pathway were rapamycin analogues, and breast cancer patients who received RAD001 in combination with endocrine therapy showed a clinical benefit. Among the new inhibitors now under development are BEZ235 (PI3K, mTORC1/2 inhibitor), BKM120 (PI3K inhibitor), XL147 (selective class I PI3K inhibitor), and BYL719 (alpha isoform–specific PI3K inhibitor). Dr. Baselga emphasized the need to change the way clinical trials are done. Trials need to be smaller and smarter, and combinational approaches need to be taken. In addition, screening for mechanisms of resistance to PI3K inhibition via short hairpin RNA screens and other types of screens are needed. Moreover, novel therapies need to be incorporated earlier into disease treatment. Dr. Baselga gave an informative introduction to targeting the PI3K pathway and elucidated the future of targeted therapies to PI3K/mTOR, particularly combination treatments.

Lewis C. Cantley (Beth Israel Deaconess Medical Center, Boston, MA) started the morning addressing PI3K and cancer cell metabolism. Dr. Cantley underscored the need for coclinical trials with mouse models. Even when agents are already in clinical trials, mouse models give invaluable insights. For example, in animal models, antitumor effects of PI3K inhibitors correlate very well with positron emission tomography (PET) response, suggesting 2[18F]fluoro-2-deoxy-D-glucose (FDG) PET may be an important early marker of response in the clinic. However, when PI3K is shut off with drug but glucose uptake is not completely shut off as measured by PET, these tumors will rebound aggressively. Dr. Cantley showed convincing PET images of mice to highlight this point. Also, he showed that PTEN mutant prostate mice tumors respond to
the GlaxoSmithKline PI3K/mTOR inhibitor, but the tumor does not go away completely. Combination therapy with a MAP/extracellular signal–regulated kinase (MEK) inhibitor is needed to achieve complete tumor regression and complete inhibition of FDG uptake. Furthermore, one of the best predictors of relapse was reemergence of PET signal. Of importance also is to use mice with known mutational backgrounds and see what therapy will be successful and to uncover resistance mechanisms. Furthermore, Dr. Cantley discussed that the heterogeneity in PI3K activity is beneficial to normal tissues by restricting PI3K activation to only a subset of cells. This work was reported recently by Yuan and colleagues, who elegantly showed that this heterogeneity might serve to protect the population as a whole from overactivation of the PI3K pathway, which ultimately can lead to cellular senescence or cancer (3).

Bart Vanhaesebroeck (Queen Mary University of London Institute of Cancer, London, UK) introduced the 8 isoforms of PI3K in mammals and showed that by using genetic and pharmacologic approaches, his laboratory has uncovered isoform-selective roles of the PI3K isoforms. Interestingly, Foukas and colleagues derived primary cell lines from their PI3K-dead-knock-in mice, which carry a mutation within the ATP binding site leading to inactivation of the kinase, and showed that in reality, cells can proliferate effectively with greater than 90% of p85-associated PI3K activity gone (4). This implies that inhibiting PI3K alone may not be effective in cancer cells. Therefore, there may be a role for inhibitors that target the tumor stroma. p110δ-null knock-in mice have reduced growth and metastasis of solid tumors in syngeneic B16 and 4T1 xenograft models. Moreover, the main effect of the δ inhibitor PI-3065 in the 4T1 model in the knock-in mice is on adaptive immunity (B/T cells), and less so on innate regression (macrophages, neutrophils). The real take-home message of Dr. Vanhaesebroeck’s presentation was that p110δ inhibition is not immunosuppressive and is a potential target in cancer inflammation and immunity. Langdon Miller (Calistoga Pharmaceuticals Inc., Seattle, WA) underscored the importance of constitutive PI3Kδ pathway in driving the overgrowth of malignant B cells and showed striking evidence that selective inhibition of this pathway in the clinic can safely abrogate aberrant PI3Kδ signaling with the CAL-101 drug, which is an oral, small-molecule PI3Kδ inhibitor with a low nanomolar EC50 and greater than 200-fold selectivity for PI3Kδ relative to the other PI3K isoforms. Phase I trials showed high levels of durable antitumor activity in mantle cell lymphoma, indolent non-Hodgkin lymphoma, and chronic lymphocytic lymphoma patients who have received extensive prior chemoimmunotherapy. Reversible lymphoid depletion was observed as a side effect. These findings show that CAL-101 holds significant promise as a therapeutic option for patients with lymphoid malignancies. Phase II/III trials of CAL-101 are planned for indolent non-Hodgkin lymphoma and chronic lymphocytic lymphoma.
The first talk of the meeting specifically addressing mTOR was by David M. Sabatini (Whitehead Institute for Biomedical Research, Cambridge, MA). mTOR is the target of the immunnosuppressive drug rapamycin and is a vital player in nutrient and growth factor signaling. Recently, members of the Rag family of GTPases were shown to be amino acid–specific regulators of the mTORC1 pathway (5, 6). Sancak and colleagues from the laboratory of Dr. Sabatini identified a new trimeric protein complex, which they termed Ragulator (7). The Ragulator is encoded by the MAPKSP1, ROBLD3, and c11orf59 genes and interacts with the Rag GTPases. They further showed that the Ragulator recruits the Rag GTPases to the lysosomes, which also induces translocation of mTORC1 to lysosomes and is crucial for the activation of mTORC1 by amino acids through Rheb (7). Interestingly, individuals with partial loss of function of a component of the Ragulator are small, immunosuppressed, and very young-looking. Ragulator null cells thus have very low mTOR signaling and are absolutely sensitive to rapamycin. Sancak and colleagues showed that one could rescue this effect by forcing mTOR to the lysosome, where proteins are degraded and amino acids are made. It thus makes sense that mTOR needs to be around the storage centers of amino acids. Moreover, RagA(12)GTP/GTP mice die perinatally with slightly decreased size, which very nicely phenocopies the Atg5−/− autophagy–deficient mice, who die of starvation (8).

Thus, it seems like there is a loss of amino acid sensing in these mice. Dr. Sabatini further introduced the identification of the mTOR-dependent phosphoproteome. They defined the mTOR phosphoproteome by looking at cells under starvation, insulin stimulated, rapamycin– and Torin-treated (mTORC1/2 inhibitor) conditions. On the basis of global phospho profile, mTOR inhibition mimics serum starvation. Rapamycin-resistant Torin sensitive sites seem to be downstream of mTOR signaling. In addition, they identified Grb10 as a novel mTORC1 substrate. Grb10 is a known negative regulator of insulin-like growth factor (IGF) signaling. They propose an intriguing model in which mTOR activates Grb10, which negatively regulates the pathway by acting on receptor tyrosine kinases (RTK) and insulin receptor substrate 1 (IRS1), possibly embellishing the classical negative feedback through S6K loop.

Next, Brendan D. Manning (Harvard School of Public Health, Boston, MA) focused on the biologic effects of mTOR signaling. Dr. Manning reported on his team’s investigation of the downstream targets and functions in cancer and in human diseases. They carried out a simple, clean genetic and metabolomics assessment of wild-type, tuberous sclerosis complex (Tsc1)1/2−/− mouse embryonic fibroblast (MEF) and Tsc1/2−/− MEF cell lines treated with rapamycin, which stimulated transcription factors controlling metabolic pathways. They found that mTOR seems to be exerting its biggest effects on metabolism through influencing the oxidative branch of the pentose phosphate pathway through sterol regulatory element-binding protein 1 (SREBP1) to upregulate lipid and sterol biosynthesis (9). SREBP traffics to the Golgi, where it is cleaved and translocates to the nucleus to function as a transcription factor, activating genes such as fatty acid synthase. Davel and colleagues found that mTORC1 acts in the processing of SREBP1 and SREBP2 through S6K1 (9). Furthermore, they proposed that mTORC1 has 2 major roles: through hypoxia-inducible factor (HIF) to induce glucose uptake and glycolysis and through SREBP to ramp up the pentose phosphate pathway. In addition to playing a role in protein synthesis, mTORC1 promotes specific bioenergetics and anabolic cellular processes. TSC1 knockout mice are protected from age- and diet-induced hepatic steatosis. In hepatocytes, mTORC1 activation is necessary but not sufficient to activate lipogenesis; SREBP1 and 2 are required for mTORC1-driven proliferation.

Akt activates SREBPs and regulates expression of enzymes involved in cholesterol and lipid biosynthesis. Caroline A. Lewis (CRUK London Research Institute, London, UK) presented that mTORC1 inhibitor rapamycin blocked induction of SREBP target genes in immortalized human retinal pigment cells, whereas activated mTORC1 increased the level of nuclear SREBP1 and also regulated the transcriptional activity of SREBP1. Glioblastoma multiforme is associated with mTOR pathway activation and hypoxia. In U87 glioblastoma cells, in response to hypoxia, expression of a subset of SREBP target genes, as well as SREBP1 protein, decreases, which results in a decrease in de novo lipid synthesis. However, another subset of genes involved in lipid uptake and transport are increased, such as stearyl-CoA desaturase (SCD). The mechanism of differential expression is not known yet.

Cheryl L. Walker [The University of Texas MD Anderson Cancer Center, Houston, TX (MDACC)] presented progress in the field of reactive oxygen species (ROS)-induced ATM signaling. ATM is the gene mutated in the genetic disease ataxia telangiectasia and is the cellular damage sensor that plays a crucial role in signaling to DNA repair machinery and the cell-cycle checkpoints. Recently, Alexander and colleagues identified a cytoplasmic pathway for oxidative stress–induced ATM activation of tumor suppressor TSC2 to regulate mTORC1 signaling and autophagy (10, 11). ROS play an important role in many physiologic and pathophysiologic processes, including cancer. Upon elevated ROS, ATM activates TSC2 through the LKB1/AMP kinase metabolic sensing pathway in the cytoplasm to repress mTORC1 and induce autophagy. She showed that TSC signaling is regulated by ROS at the peroxisome. TSC1 and 2 both contain PTS1 sequences to be transferred to the peroxisome and PTS1 mutations result in TSC disease. mTOR suppression by TSC2 requires localization to peroxisome. ATM acts at the peroxisome as a local sensor of ROS. This also explains the induction of autophagy after exposure to H2O2 or etoposide via the ATM/AMPK/TSC2/mTORC1 pathway because of mTOR suppression. Elucidation of this stress pathway offers a molecular role for ATM in the cytoplasm and shows that ATM is more than a DNA repair protein; it functions in different cellular compartments.

Alexandra Newton (University of California, San Diego, La Jolla, CA) discussed the balance that is needed between kinases and phosphatases in cancer. Lipid second messenger pathways are strictly controlled by the kinases protein kinase C (PKC) and Akt and the serine–threonine phosphatase PH domain leucine-rich repeat protein phosphatase (PHLPP). Basically, PHLPP provides “the brakes” for Akt and PKC signaling, whereas PDK1 and mTORC2 phosphorylate them. Both are central players in cancer cell proliferation. PHLPP controls...
cellular levels of PKC, and in cells PKC is almost always phosphorylated. PHLPP also suppresses epidermal growth factor receptor (EGFR) and controls amplitude of agonist-stimulated EGFR kinase activity. PHLPP knockdown increases p-Akt, p-ERK, and EGFR. Unlike the well-known PTEN, which is somatically mutated in cancer, the PHLPP phosphatases are actually functional in many cancer cell lines examined thus far. There is a delicate balance between phosphorylation and dephosphorylation states which may lead to cancer. Consequently, PHLPP phosphatase activity could be modulated for the treatment of cancer. Specific inhibitors that activate PHLPP may inhibit multiple cancer signaling pathways that are activated by PKC and Akt. The laboratory of Dr. Newton currently focuses on the function of the PHLPP phosphatases in modulating signaling via Akt and PKC, with the hope of developing small-molecule inhibitors for the treatment of cancer.

Jean J. Zhao (Dana-Farber Cancer Institute, Boston, MA) presented elegant work using genetic mouse models in targeting PI3K in cancer. The 2 commonly expressed class Ia catalytic isomers of PI3K are p110α and p110β. To date, in most cell types studied, p110α is the major player in the PI3K signaling through RTKs, although p110β is necessary for signaling via G protein–coupled receptors. In the mouse, p110α and p110β have been shown to be more essential in some processes and dispensable for others. Unexpectedly, Dr. Zhao’s laboratory discovered that p110α and p110β actually play opposing roles in the normal development and pathology of mammary tissue. The absence of p110α during breast development impaired mammary duct outgrowth, whereas mice deficient in p110β in mammary tissue triggered developmental hypertrophy. Thus, p110 isoform–specific inhibition may potentially be a therapeutic strategy in the future. Dr. Zhao also presented a mouse model of breast cancer that conditionally expressed an H1047R oncogenic allele of PIK3CA to address resistance mechanisms to PI3K-targeted therapy. This model initiates hyperplasia, followed by palpable tumors and metastasis. If the mutation is removed, tumors sometimes show partial regression but may keep on growing. If recurrent tumor relies on high p-Akt levels, then it responds to PI3K inhibitor treatment. Interestingly, genomic analysis revealed Met, c-Myc, and Mdm2 amplification in recurrence; thus, tumors recur via both PI3K pathway–dependent and PI3K pathway–independent mechanisms, highlighting the challenges for the development of therapeutic agents that target the PI3K pathway in cancer.

To wrap up the PI3K/mTOR signaling part of the meeting, Davide Ruggiero (UCSF Helen Diller Family Comprehensive Cancer Center, San Francisco, CA) discussed the pharmacogenetic targeting of the translational machinery downstream of oncogenic mTOR signaling. Dr. Ruggiero provided insight into the postgenomic mechanisms of prostate cancer development regulated by mTOR signaling and showed data indicating that ATP active site inhibitors of mTOR perturb the aberrant translation program in prostate cancer. For example, the potent and selective TORC1/2 inhibitor, INK128 (Intellikine, Inc.) decreased prostate tumors in mice and was more effective than various rapalogs, which only interfere with TORC1 activity. With the use of a new technology, ribosome profiling, the translational state of prostate cancer was assessed. Inhibition of mTOR regulated 148 target genes, and YB-1 was identified as a major posttranslational regulon, which was regulated both by rapamycin and PP242 (mTORC1/2 inhibitor). YB-1 is involved in cancer cell invasion and PI3K/mTOR/elf4E/YB1 axis regulates epithelial–mesenchymal transition genes. Interestingly, YB-1 expression is reduced at the translational level by mTORC1/2 in vivo.

PI3K Pathway Aberrations in Human Cancer

When cancer cell lines are addicted to RTKs, usually PI3K or MEK pathway inhibitors inhibit growth. However, it is not clear if PI3K inhibitors alone will be successful in the clinic. Jeffrey A. Engelman (Massachusetts General Hospital) reported on strategies for targeting PI3K when using combination therapy. Dr. Engelman stressed that the need to inhibit both the PI3K and MEK signaling hinges on the key concept of inducing apoptosis, not just inducing cytostasis. MEK regulates BIM and PI3K regulates MCL-1, a key factor in inducing tumor regression. KRAS tumors are inherently difficult because they activate both PI3K and MEK. In the KRAS mutant lung cancer model, the PI3K inhibitor BEZ235 failed to shrink the tumor, but the combination with MEK1/2 inhibitor AZD6244 (ARRY-142886) inhibited both pathways, leading to an apoptotic response. Another approach would be to inhibit both pathways indirectly, so as to avoid toxicities to patients. For example, for EGFR mutations, hitting the RTK is an indirect method to inhibit both signaling events. The combination IGF-IR and MEK inhibition may be a good strategy for colorectal KRAS mutant tumors. Dr. Engelman showed data that in a large panel of patient samples, p85 is immunoprecipitated consistently with IRS1, indicating that this is a commonly engaged network in colorectal cancers to activate PI3K signaling. The data support that in KRAS mutated tumors, PI3K signaling is critical for KRAS-driven tumor growth in the early stages, but as the tumor progresses, its reliance on signaling comes more from the RTKs. Dr. Engelman further emphasized the dynamics of resistance mechanisms and showed very intriguing and impressive patient data (12). In non–small cell lung cancer (NSCLC), patients who were originally responsive to the EGFR inhibitors gefitinib or erlotinib but whose disease starts to progress, through multiple biopsies, it can be shown that there is acquisition of other mutations such as EGFR T790M or PIK3CA mutations. These patients are taken off the targeted therapy, put on chemotherapy alone for 1 year, and as mutations disappear, they can become sensitive to EGFR-targeted therapy again. It remains unclear whether resistant mechanisms are acquired or whether they preexist, affecting a small fraction of cells. Resistance is probably due to both and is a major challenge in designing treatment strategies for targeting the PI3K pathway.

Ramon Parsons (Columbia University, New York, NY) outlined the history of PTEN, the most commonly mutated tumor suppressor to date, which was discovered in 1997. Dr. Parsons and his colleagues found that adenosival expression of PTEN could lead to apoptosis in cancer cells. Furthermore, they
screened for PTEN-regulated genes (that were FOXO-regulated in a PI3K-dependent manner) and identified NFIL3 (nuclear factor, interleukin 3 regulated)/E4BP4. NFIL3 binds to the endogenous \textit{TRAIL} promoter and represses transcription. It also has effects on numerous other FOXO target genes such as \textit{FasL}, and \textit{GADD45}. Increased expression of NFIL3 correlates with poor prognosis in breast cancer, glioblastoma multiforme, and ovarian cancer, and high NFIL3 mRNA correlates with PTEN-negative status in breast cancer. Dr. Parsons underscored the following model: PI3K activates Akt, which in turn activates NFIL3, a transcriptional repressor. NFIL3, in association with histone deacetylase 2, can inhibit FOXO transcription of its target genes by the remaining pool of nuclear FOXO. Thus, correlating with the data from Dr. Engelman, the goal of cancer therapy should be to induce cell death.

Eric C. Holland (Memorial Sloan-Kettering Cancer Center, New York, NY) further emphasized that the loss of PTEN in gliomas induces transcriptional changes (13). In normal brain, ABCG2 transporter activity generates "side population" phenotype, and this phenotype marks stem-like cells. Loss of PTEN increases PI3K/Akt (but not mTOR), which induces ABCG2 activity, which in turn increases tumorigenicity and chemoresistance (14). Dr. Holland presented a new technology, translating ribosome affinity purification (TRAP), which allows isolation of ribosome-bound mRNA and measures translational efficiency under different conditions or treatments. Six hours of radiation exposure of glioma enriched for p53 and E2F1 transcriptional targets. At 2 hours, p53 targets are activated and at 6 hours E2F1 targets are lost. In a following experiment with PTEN loss, 255 genes were downregulated and 98 genes were upregulated; however, differential gene expression profiles of radiation and PTEN loss did not show overlapping genes. Radiation and mTOR blockage showed overlaps but at the transcriptional level only.

Carlos L. Arteaga (Vanderbilt-Ingram Cancer Center, Nashville, TN) discussed the importance of signaling feedback upon inhibition of PI3K and the implications this has for clinical trials. Dr. Arteaga presented data on XL147 (an ATP-competitive reversible PI3K inhibitor from Exelixis, Inc.) against a panel of breast cancer cell lines harboring alterations, such as \textit{HER2} gene amplification. XL147 decreases Akt activity but leads to the upregulation of the \textit{HER3} transcript. In \textit{HER2}+ cells, PI3K inhibition is followed by an increase in expression and phosphorylation of RTKs, including \textit{HER3}. This increase is suppressed by knocking down FoxO1 and FoxO3a. In \textit{HER2}+ cells, \textit{HER3} siRNA knockdown or \textit{HER2} inhibition by trastuzumab or lapatinib combined with XL147 shows a synergistic effect \textit{in vitro} and \textit{in vivo}, p-HER3 and p-Akt are lower compared with tumors treated with single agents. It seems that in \textit{HER2}+ breast tumors, PI3K inhibitors used in combination with \textit{HER2}/3 inhibitors can stop compensatory feedback mechanisms, which may limit therapeutic efficacy of PI3K inhibitors alone. Overall, in \textit{HER2}+ cancers, PI3K inhibitors will have limited clinical activity if used as single agents, and in \textit{HER2}+ cancers, activated pathways in response to PI3K inhibitor therapy are not clear yet.

Lloyd C. Trotman (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY) noted that \textit{Pten}−/− \textit{Phlp}−/− compound mutants cause a worse outcome than signal deficiencies in mouse prostate models. Moreover, \textit{PHLPP} gene loss (approximately 40%) is about as frequent as PTEN and p53 in patient prostate samples. Interestingly, the compound PTEN/PHLPP mutant acquires massive Akt activation late in tumor development, correlating to loss of p53, PTEN and PHLPP combined loss triggers p53 activation and senescence arrest. Consistent with this, human metastatic prostate tumors are the only samples that show the PTEN + PHLPP + p53 triple deletion, which rarely occurs in primary tumors. Both PHLPP proteins (1 and 2) compensate for PTEN loss initially, and this feedback response depends on \textit{PI3K} pathway activation.

Gordon Mills (MDACC) introduced the endometrial cancer mutation program and showed that \textit{PIK3CA} mutations and PTEN loss are the driving mutations in endometrial cancer. Unlike most other cancers, loss of PTEN and \textit{PIK3CA} mutation may coexist or at least one of them is detected in 80% of cancers. The \textit{PI3K} pathway is frequently hyperactivated in this disease, and Dr. Mills focused on somatic mutations of \textit{PI3K} regulatory subunit 1 (p85\(\alpha\); \textit{PIK3R1}), p85 homodimerizes when there is an excess of p110 and bounds to PTEN via the RhoGAP domain of p85 and the C2 domain of PTEN. p85 gain of function mutations in endometrial cancers are unable to bind PTEN, thereby leading to PTEN degradation and pathway activation.

Development of Novel PI3K/mTOR Pathway Inhibitors

Kevan Shokat (University of California, San Francisco, CA) began the session on the development of novel PI3K/mTOR pathway inhibitors by introducing chemical genetic investigations of protein and lipid kinase signaling. They tested PP242 (ATP site mTORC1/2 inhibitor) in a xenotransplantation model of human liver tumors serially passaged in mice. This model maintains the integrity of the tumor model quite nicely. They found that cell lines that had wild-type KRAS/PI3K responded to the drug, yet tumor lines with mutant \textit{KRAS} and wild-type or mutant \textit{PI3K} responded poorly. Even more specifically, wild-type \textit{PI3K} was less responsive than lines with \textit{H1047K} mutant \textit{PI3K}. From this, Dr. Shokat proposed the model that mutant \textit{KRAS} drives some mTOR activity, yet when there is a \textit{PI3K} mutation as well, mTOR activity is rewired to a strong PI3K-driven route, thereby making it more acutely sensitive to PP242. Dr. Shokat also presented the kinase suppressor of Ras (KSR), which is a scaffolding protein and assembles Raf–MEK–ERK complexes, facilitating MEK phosphorylation (15). Furthermore, mass spectrometry identified MEK1 Ser24 and Ser72 as KSR-dependent phosphorylation sites.

After a summary of \textit{PI3K} pathway regulation, William R. Sellers (Novartis Institutes for BioMedical Research) presented data on new inhibitors: BEZ235, BKM120, and BYL719. Dr. Sellers described an exciting research initiative, The Cell Line Encyclopedia. Novartis screened 1,000 cancer cell lines (characterized their expression profiles, genetics, and single-nucleotide polymorphisms), and their sensitivity/resistance to various compounds. Amazingly, they can profile approximately 500 cell lines in 3 months and 1,300 compounds in more than 500 cell lines. This will reveal biomarkers to sensitivity of
Targeting mTOR

Funda Meric-Bernstam (MDACC) presented some of the ongoing studies to determine the mechanism of rapamycin action and markers for rapamycin response/resistance. Preclinical studies have suggested that activation of Akt/mTOR signaling may be a predictor of response. However, there have been challenges in validating these markers clinically as predictors of response. By immunohistochemistry, comparison of p-Akt and p-4E-BP1 expression in primary and matched distant metastases of breast tumors showed poor concordance, which might reflect tumor heterogeneity or challenges in doing immunohistochemistry on archived tissue (18). Dr. Meric-Bernstam presented modulation of downstream signaling by rapamycin and novel pharmacodynamic markers of response. Rapamycin-regulated transcriptome predicts survival in breast cancer patients, showing the importance of the pathway in human cancer biology (19). Rapamycin also was shown to regulate the expression of SCD1, a critical enzyme in fatty acid metabolism, SCD1 knockdown decreases, and SCD1 overexpression increases breast cancer cell growth; thus, rapamycin-mediated downregulation of SCD1 may play an important role in its antitumor effect (20). Studies are ongoing to determine the role SCD1 in cancer biology and as a pharmacodynamic marker. Dr. Meric-Bernstam also presented work in functional proteomics approaches to identify predictors and pharmacodynamic markers of response to these agents.

Robert J. Schneider (New York University Cancer Institute, New York, NY) has shown that inflammatory breast cancer is resistant to radiation, in part, because of overexpression of eIF4GI and constitutively active mTOR pathway. In response to ionizing radiation, expression of DNA damage response (DDR) proteins increase and protect inflammatory breast cancer cells. In vitro, reducing high levels of eIF4GI sensitizes non-transformed breast epithelial cells to ionizing radiation, but surprisingly increases the cancer stem cell population. Overall, there is no enhancement in radiation sensitivity. Next, using an inflammatory breast cancer cell line, SUM149, these investigators compared everolimus (allosteric mTOR inhibitor), PP242 (mTORC1/2 inhibitor), and eIF4GI silencing. PP242 and eIF4GI siRNA knockdown, but not everolimus, prevented growth of inflammatory breast cancer xenografts, enhanced radiosensitivity, decreased clonogenic survival, inhibited Akt activation, and more efficiently inhibited downstream targets and translation of mRNAs involved in DDR. In vivo, treatment of SUM149 xenografts with PP242 and ionizing radiation led to better tumor control and longer survival. Dr. Schneider concluded that eIF4G silencing or mTORC1/2 inhibition might also sensitize cancer stem cells to ionizing radiation.
limited effect on tumor vasculature, and inhibits tumor growth more than rapamycin. As Akt is inhibited, glucose uptake by tumor cells decreases. Uptake of glucose analogue 2[18F]fluoro-2-deoxy-o-glucose, observed by PET scanning, is correlated with tumor growth and used as a biomarker. Dr. Guichard justified combination treatment by 2 examples: in HER2-amplified breast cancer cell lines, AZD8055 increases p-HER3, and combination of AZD8055 and HER2 inhibitor lapatinib is synergistic. Second, AZD8055 results in a temporary increase in p-MEK. Combination of AZD8055 and the MEK inhibitor selumetinib (AZD6244) produces cell death and tumor regression in NSCLC xenografts.

Neil Rosen (Memorial Sloan-Kettering Cancer Center) classified PI3K signaling pathway as "pathway activation without inflammation-associated pathologies, such as cancer."

Late-Breaking Research/Hot Topics

This session of the meeting was filled with short talks that were proffered from abstract submissions. To start, William C. Cobb (University of North Carolina, Chapel Hill, NC) presented his research on IKK-dependent phosphorylation of PI3K, which induces nutrient deprivation–induced autophagy. IKK phosphorylates p58It in response to starvation in vivo, which leads to feedback inhibition of the PI3K/Akt/mTOR pathway, promoting autophagy. This cross-talk between IKK/NF-kB and PI3K/Akt probably will have significant implications for inflammation-associated pathologies, such as cancer.

Next, Jonathan Ross Hart (The Scripps Research Institute, La Jolla, CA) described an elegant SILAC screen (stable-isotope labeling by amino acids in cell culture) in which he identified the SIAT3 targets as a major group of proteins that are over-expressed during PI3K-mediated oncogenic transformation. PI3K activates the Tec family of tyrosine kinases, which in turn phosphorylates and activates STAT3. Inhibition of Tec kinases/STAT3 interferes with oncogenic transformation induced by p110a or p110z.

Nathan T. Ihle (MDACC) showed that specific amino acid substitutions in mutant KRAS G12C signaling and may predict patient survival and their response to targeted therapeutics. NSCLC KRAS G12C and colon or pancreas cancer G12D amino acid substitutions are common. Analysis of NSCLC cell lines with G12D showed activation of the PI3K/Akt and MEK pathways, whereas G12C mutants have a weak activation of PI3K but strong activation of RAL signaling. In NSCLC patients, G12C mutations reveal a worse progression-free survival compared with other mutations, including G12D mutation, which shows that different mutations may require different inhibitors.

Devin T. Worster (Harvard Medical School, Boston, MA) described research showing that the loss of p57Kip2 increases the PI3K/AKT oncogenic signaling pathway. p57Kip2 is a cyclin-dependent kinase inhibitor that is induced under conditions such as IGF-I or insulin stimulation. Interestingly, p57Kip2 is downregulated in response to EGF and EGF induces proliferation in the mammary epithelial cell line, MCF10a. Also, knockdown of p57Kip2 releases acini from standard growth arrest during morphogenesis of mammary epithelia cell in 3-dimensional culture. p57Kip2 is activated by Akt but inhibited by ERK, sensitive to Akt/ERK ratio, resulting in cell-cycle arrest if the ratio is high. Remarkably, the p57Kip2 locus is silenced in many breast cancers, which frequently show hyperactivation of the PI3K pathway. p57Kip2 level may help to identify targeted therapy against ERK and PI3K pathways. This interesting hypothesis needs to be prospectively validated in the clinic.

Both the PI3K and MEK pathways contribute to PHLPP-dependent proliferation. Matt J. Niederst (University of California, San Diego) showed that not only do the PHLPP family phosphatases regulate the PI3K pathway via their effect on Akt, but they also regulate growth factor receptor signaling. PHLPP suppresses Ras activation and PHLPP knockdown increases EGF protein levels and activity, as well as transcription of other growth factors such as VEGF receptor 1, MET, platelet-derived growth factor 1α, and IGF receptor II. Many cancers overexpress growth factor receptors, such as EGFR, thereby making regulation of PHLPP an attractive therapeutic strategy.

APC loss results in WNT signaling activation and WNT activation is important in intestinal regeneration, particularly crypt growth. William J. Faller (Beatson Institute for Cancer Research, Glasgow, UK) presented research in mice that raptor knockout or rapamycin treatment inhibits tumor growth induced by APC and PTEN deletion within the LGR5-positive stem cell population. This growth effect is mediated through S6K1 and indicates that the mTOR pathway mediates effects of WNT activation in intestine.

Olga K. Mirzoeva (University of California, San Francisco) showed that in pancreatic ductal adenocarcinoma, inhibiting PI3K or mTOR induces growth inhibition, whereas inhibiting both induces cell death. Use of PI103. XL765 (dual inhibitors), or combination of PIK90. XL147 (p110α inhibitors) with rapamycin or with PP242 (mTOR inhibitors) resulted in apoptosis but induced autophagy. Inhibiting autophagy by chloroquine enhanced apoptosis. Thus, combining PI3K/mTOR inhibitors with autophagic inhibitors may promote pancreatic cancer treatment.

Novel PI3K/mTOR Pathway Inhibitors in Clinical Trials

Ana Maria Gonzalez-Angulo (MDACC) discussed molecular marker–based clinical trial designs and once again emphasized a general theme of the meeting—that clinical trials need to be smaller, shorter, cheaper, and individualized. Evidence-based
Emerling and Akcakanat

medicine is generally practiced today, and this is best for the average population but not specific for individuals. Treatments for individual patients should be personalized on the basis of molecular characteristics of their tumor and their genetic map. For selection of correct markers, Dr. Gonzalez-Angulo stressed the importance of identifying pathway drivers, understanding limitations of marker research, and the fact that pathway signatures may be superior to single markers. Even if a marker is technically valid and scientifically reasonable, clinical validation may come later, even in the trial. Thereby, carefully matching patients and specific single agents or combination therapies will improve our likelihood of accomplishment. Furthermore, Dr. Gonzalez-Angulo discussed molecular marker-based clinical trials targeting the PI3K/Akt/mTOR pathway, promising results of combination therapies, and specific challenges to clinical trials, such as time from sample acquisition to processing, additional time needed for patient enrollment, high cost, and extensive regulations.

Li Yan (Merck & Co., Rahway, NJ) presented data about MK-2206, an oral allosteric AKT inhibitor. In a phase I study, it was shown to be fairly well tolerated, with widespread rash as the major toxicity, and resulted in a decrease in AKT signaling but increase in p-ERK and p-MEK. Rationale combination trials are currently ongoing with MK-2206 in combination with chemotherapies as well as targeted agents, such as MEK inhibitors, to make the most of the overall clinical outcome.

Razelle Kurzrock (MDACC) presented the PREDICT program, in which all patients are screened for common oncogenic mutations (PIK3CA, KRAS, NRAS, BRAF, EGFR, PTEN loss, etc.) with the goal of enhancing efficacy of personalized targeted agents tested in phase I trials.

Mark R. Lackner (Genentech, Inc., South San Francisco, CA) further emphasized this point and highlighted that we need to follow tumor genetics to optimize therapy for cancer patients. Genentech has several clinical trials in progress that strive to identify patients likely to benefit from targeted therapy in which they are testing putative predictive signatures.

Joanne J. Lager (Sanofi-Aventis, Cambridge, MA) described early clinical studies of XL147 (pan-PI3K inhibitor) and XL765 (pan PI3K/mTORC1/2 inhibitor). Interestingly, XL147 inhibits not only p-Akt but also p-MEK, and this is evident across many tumor types with no change in total MEK or ERK. In addition as a single agent, there are antiproliferative effects but no apoptosis. XL765 passes the blood–brain barrier and also inhibits p-Akt and p-ERK with no change in total MEK or ERK levels.

Dr. Lager postulates that the p-ERK effect is not unique to these PI3K/mTOR pathway inhibitors. Furthermore, p-ERK inhibition likely lags behind Akt inhibition, indicating that combination with a MEK inhibitor may be more effective than these compounds as monotherapies.

Ryan J. O. Dowling (Ontario Cancer Institute, Toronto, ON, Canada) presented the biologic effects of metformin in early-stage breast cancer. Metformin is a first-line therapy for type 2 diabetes that reduces circulating insulin and glucose levels. Metformin has emerged as a potential anticancer agent because it can activate AMPK and decrease mTOR signaling, leading to a decrease in proliferation in cancer cells. Dowling and colleagues conducted a neoadjuvant, single-arm, “window of opportunity” clinical trial to treat breast cancer patients with metformin prior to breast surgery. Their interim analysis showed that metformin decreased proliferation; there were no changes in p-AMPK, but there was a decrease in p-Akt; together, these findings suggest that there may be an indirect mechanism of metformin that provides its antitumor effects.

Summary

This conference highlighted the fact that although there has been a large amount of research done on the PI3K/mTOR signaling pathway over the years, there is still a great deal of work to do to better understand how to effectively use the inhibitors of this pathway in the clinic. Moreover, we need to intimately understand the oncogenic mechanisms of individual tumors, allowing us to treat patients more effectively, with the least toxicity. These are exciting times in cancer research, particularly in the PI3K/mTOR signaling field. We look forward to the next AACR Special Conference on Targeting PI3K/mTOR Signaling in Cancer.

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