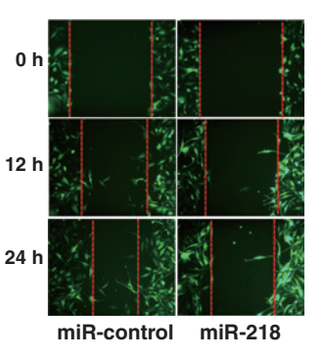
Breaking Advances
Highlights from Recent Cancer Literature

Phospho-S6 as a Biomarker of Sensitivity in BRAF-Mutant Melanoma

To identify biomarkers of sensitivity in *BRAF*-mutant melanoma, Corcoran and colleagues evaluated 16 *BRAF* V600E-mutant melanoma cell lines for sensitivity to selective RAF or MEK inhibitors. Although inhibition of pERK was necessary, it was not sufficient to predict sensitivity to MAPK inhibition; suppression of pS6 (pRPS6), downstream of TORC1, correlated robustly with sensitivity. Furthermore, maintenance of ribosomal protein S6 phosphorylation after pharmacologic inhibition of RAF or MEK was observed in therapy-refractory *BRAF*-mutant cell lines irrespective of their ability to suppress pERK, suggesting that pS6 levels may be a broader biomarker of resistance. Subsequently, the investigators knocked down TSC2 in a *BRAF*-mutant sensitive cell line as a means to activate TORC1, observing a significant decrease in the apoptotic response to each inhibitor. Additionally, inhibition of TORC1 signaling in resistant cells restored the apoptotic response to RAF inhibition. Similar downregulation of mTORC1 and increased apoptosis were seen using an inhibitor of PI3K (a regulator of mTORC1) in combination with the BRAF inhibitor vemurafenib in cell lines exhibiting ERK-independent resistance to MAPK pathway inhibitors. Expression of the BH3-only protein PUMA (BBC3) was specifically induced in response to BRAF inhibition (using vemurafenib), and PUMA knockdown significantly reduced the apoptotic response to vemurafenib, suggesting that TORC activity regulates PUMA as a major effector of apoptosis in BRAF-mutant melanoma cells. To validate this observation, the investigators showed that combining vemurafenib and the BH3 mimetic ABT263 promoted apoptosis in vemurafenib-insensitive melanoma cells. *In vivo* studies showed that pS6 suppression, unlike suppression of pERK, predicted drug sensitivity to MAPK inhibition and distinguished between sensitive and resistant melanoma xenografts. Real-time pS6 and pERK assessment was prospectively evaluated in 2 patients treated with RAF inhibitor therapy, followed by evaluation of changes in pS6 levels in paired biopsies. Reduction in pS6 levels after treatment correlated strongly with patient outcome. These data suggest that levels of pS6 may predict response to therapy for *BRAF*-mutant melanoma, a correlation that requires validation in a larger cohort of patients.

Corcoran RB, Rothenberg SM, Hata AN, Piris A, Nazarian RM, Brown RD, et al. TORC1 suppression predicts responsiveness to RAF and MEK inhibition in BRAF-mutant melanoma. Sci Transl Med 2013;5:196ra98.

MicroRNA-218: A Tumor Suppressor in Glioma



Malignant glioma, the most common primary tumor of the central nervous system, is a highly aggressive cancer, with current treatment options leading to a median patient survival of 1 to 2 years. MicroRNAs are small noncoding RNAs known to regulate gene expression. MicroRNAs are currently being shown to have several diverse roles in biologic processes such as cancer. One such microRNA is miR-218, which is downregulated in glioma. Ectopic expression of miR-218 in glioma cells caused a reduction in migration, invasion, and proliferation of the cells both *in vitro* and *in vivo*. Overexpression of miR-218 in glioma cells also caused a decrease in the stem cell–promoting oncogene *Bmi1*. Further investigations revealed that *Bmi1* is a direct downstream target of miR-218 and that miR-218 mediates at least some of its tumor-inhibitory properties via inhibition of *Bmi1*. Additionally, miR-218 blocked self-renewal of glioma stem-like cells. Because BMI1 potentially regulates stem cell growth, this further supports the finding that miR-218 regulates *Bmi1*. Tu and colleagues have thus identified miR-218 as a tumor suppressor in glioma and established that miR-218 functions by regulating genes involved in glioma development. *(Image from cited article courtesy of publisher.)*

Tu Y, Gao X, Gang Li, Fu H, Cui D, Liu H, et al. MicroRNA-218 inhibits glioma invasion, migration, proliferation and cancer stem-like cell self-renewal by targeting the polycomb group gene Bmi1. Cancer Res; Published OnlineFirst August 15, 2013; doi:10.1158/0008–5472.CAN-13–0358.

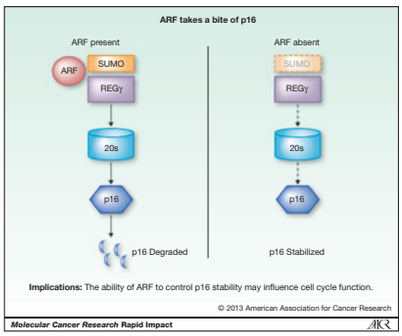
CDK6: Linking Two Hallmark Features of Cancer

Cyclin D kinases 4 and 6 both permit cell-cycle progression and are amplified and overexpressed in many cancer types, thereby representing promising targets for anticancer therapy that have already entered the clinic. These complexes have distinct kinase-dependent mechanisms that drive proliferation, but it has been postulated that CDK6 also has important kinase-independent functions as a transcriptional regulator. Kollman and colleagues unexpectedly reveal that overexpression of CDK6 inhibited cell-cycle progression and growth of BCR–ABL transformed B-cell leukemias and lymphomas. Upon injection of leukemia cells into recipient mice, they showed that overexpression of CDK6 delayed tumor formation and that CDK6 induced high levels of the tumor suppressor and cell-cycle inhibitor $p16^{INK4a}$. In a separate mouse model of T-cell NPM-ALK–driven lymphoma, in which methylation is responsible for the loss of p16 (*Cdkn2a*), CDK6 performed its well-known growth-promoting role, highlighting the importance of silencing p16. Further, the authors observed an inverse correlation between protein expression levels of CDK6 and p16 levels in human B-cell and T-cell lymphomas. Interestingly, they did not observe the same effects in the close homolog CDK4. Additional investigation into the role of CDK6 demonstrated a direct link with angiogenesis. In lymphoid tumor models, the authors showed that overexpression of CDK6, but not CDK4, had a proangiogenic effect and induced VEGFA (a well-known angiogenic factor) independent of its kinase activity. Mechanistic studies showed that CDK6 drives angiogenesis by binding to p16 and VEGFA promoters as part of a transcriptional complex that includes STAT transcription factors and D-type cyclins. Until recently, the roles of CDK4 and CDK6 were thought to be primarily kinase dependent, and this idea had been the driving factor for drug design. However,

Kollman and colleagues show compelling data taking into account the kinase-independent function of the CDK6 protein in promoting angiogenesis, supporting development of a new class of inhibitors for the clinic.

Kollman K, Heller G, Schneckenleithner C, Warsch W, Scheicher R, Ott RG, et al. A kinase-independent function of CDK6 links the cell cycle to tumor angiogenesis. Cancer Cell 2013;24:167–81.

Stabilization of INK4A Protein by ARF



CDKN2A gene is among the most frequently deleted genes in cancer. Through use of separate reading frames, this single gene encodes two nonhomologous proteins, the tumor suppressors INK4A (inhibitor of CDK4) and ARF (alternative reading frame). Kobayashi and colleagues show that ARF destabilizes INK4A in normal cells and in cancer cells by interacting with REG gamma (REG3G), an activator of ubiquitin-independent proteolysis, leading to degradation of INK4A. The authors speculate that ARF evolved to provide a fail-safe mechanism to maintain appropriate levels of INK4A. *(Image from cited article courtesy of publisher.)*

Kobayashi T, Wang J, Al-Ahmadie H, Abate-Shen C. ARF regulates the stability of p16 protein via REGγ-dependent proteasome degradation. Mol Cancer Res 2013;11:828–33.

HK2 as a Target for Cancer Therapy

Increased aerobic glucose metabolism (the Warburg effect) is a hallmark of cancer. Hexokinases (HK) catalyze the first committed step in glucose metabolism, converting glucose into glucose-6-phosphate. Cancer cells express high levels of HK2, leading to increased glucose metabolism. Using knockout fibroblasts from floxed *Hk2* conditional mice, Patra and colleagues show that deletion of *Hk2* blocked oncogenic transformation by activated RAS and was not compensated by HK1. They found elevated HK2 levels in *Kras*-induced murine lung tumors. Conditional loss of Hk2 reduced tumor burden and extended survival time in *Kras*-driven lung cancer and in *Erbb2*-driven mouse breast cancer. Similar results were seen in human tumor xenografts following *HK2* RNA interference. Germline *Hk2* deletion in mice was embryonically lethal. However, deletion of *Hk2* was well tolerated in adult mice and reduced lung tumor burden in the *Kras* model of lung cancer. To explore the mechanism underlying *Hk2* deletion, they conducted a metabolomic screen and identified the most significant decreases in the levels of fructose 1,6 bisphosphate and phosphoserine. Examining nucleotide biosynthesis via the pentose phosphate pathway, they found that *Hk2* deletion suppressed glucose-derived ribonucleotide formation in *Kras*-driven lung cancer. Finally, they show that HK2 expression is required for efficient flow of carbon from glycolysis to the Krebs cycle, as loss of HK2 resulted in attenuated Krebs cycle activity generally. Overall, the study provides insight into the role of HK2 in cancer by showing that this enzyme plays a critical role in anabolic cancer metabolism. Although HK1 is sufficient for normal cell metabolism, the increased demands of cancer cells on glycolysis could account for a requirement that cancer cells overexpress HK2. These results suggest HK2 as a possible therapeutic target and provide a rationale for the development of HK2 inhibitors for cancer therapy.

Patra KC, Wang Q, Bhaskar PT, Miller L, Wang Z, Wheaton W, et al. Hexokinase 2 is required for tumor initiation and maintenance and its systemic deletion is therapeutic in mouse models of cancer. Cancer Cell 2013;24:213–28.

Receptor Transactivation and Resistance to ErbB Inhibition

Receptor tyrosine kinases (RTK) are dysregulated in many cancers and are important drivers of disease. Therapies targeting specific RTKs, however, have had limited long-term clinical efficacy, a failure attributed to both the unmasking and the development of resistance mechanisms. Meyer and colleagues used data from the Cancer Cell Line Encyclopedia to predict mechanisms of drug resistance. Testing these predictions experimentally in human breast cancer cell lines, they identified the receptor AXL as a strong predictor of resistance to ERBB inhibitors. Vital to their study was the use of multivariate signaling network analysis, which allowed them to determine the association of drug resistance with combinatorial expression of multiple RTKs including the target RTK. AXL can be transactivated in a ligand-independent manner by EGFR, and knockdown and inhibition of AXL demonstrated its role in both EGFR and downstream signaling pathway activation. In the absence of AXL, there was decreased phosphorylation of multiple phosphosites. Thus, AXL activation can diversify and amplify the signaling response. Interestingly, this AXL-mediated signaling was required for EGF-induced migration of triple-negative breast cancer cells, and inhibition of AXL decreased EGFR-mediated proliferation and migration. In addition to identifying a clinically relevant mechanism of resistance to ERBB inhibitors, Meyer and colleagues impart an important cautionary note with respect to the use of univariate analysis, as univariate analysis failed to identify this very strong predictor of response to ERBB inhibitors.

Meyer AS, Miller MA, Gertler FB, Lauffenburger DA. The receptor AXL diversifies EGFR signaling and limits the response to EGFR-targeted inhibitors in triple-negative breast cancer cells. Sci Signal 2013;6:ra66.

Note: Breaking Advances are written by *Cancer Research* Editors. Readers are encouraged to consult the articles referred to in each item for full details on the findings described.