

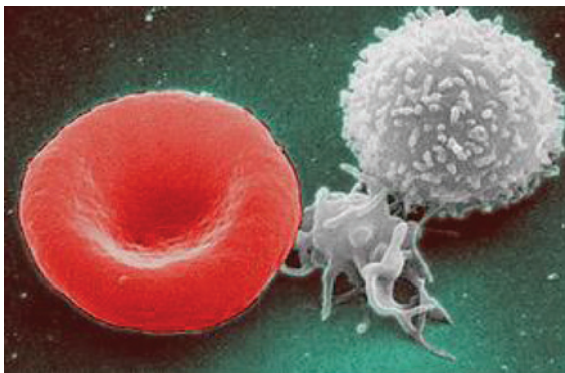
Breaking Advances Highlights from Recent Cancer Literature

A New Role for EZH2 in Glioblastoma

Polycomb proteins represent epigenetic regulators and exert transcriptional repression via polycomb repressive complexes (PRC). EZH2 is a component of PRC2, where it functions as a lysine methyl transferase, particularly for histones. In cancer, EZH2 is found to be overexpressed, and in glioblastoma multiforme, EZH2 has been shown to be important for maintenance of glioma stem cells (GSC). Although EZH2 is best understood as a histone methyltransferase, it has recently been shown to interact with other transcription factors via direct methylation. Accordingly, Kim and colleagues investigated possible partners of EZH2 in glioblastoma. Coimmunoprecipitation experiments showed an interaction with STAT3. This interaction was lost upon withdrawal of growth factors in the medium of cultured glioma cells. Importantly, shRNA depletion of EZH2 resulted in loss of STAT3 activation, as measured by loss of phosphorylation. This finding was confirmed with pharmacologic EZH2 inhibitors. EZH2 was shown to methylate STAT3, and STAT3 methylation by EZH2 at lysine residue 180 was correlated with STAT3 activation. Site-directed mutagenesis of lysine 180 resulted in loss of neurosphere formation by GSCs. The authors then examined upstream events and showed that AKT activity was required for the EZH2-STAT3 interaction, which was mediated by phosphorylation at serine 21 of EZH2. Mutation of serine 21 to alanine resulted in loss of neurosphere formation, loss of STAT3 methylation and loss of tumor aggressiveness in SCID mice. Expression of serine 21 phosphorylated-EZH2 in patient samples was correlated with poor outcome. These findings identify a new role for EZH2, in addition to its role in transcriptional silencing, with a direct link to STAT3 activation via methylation and identify an AKT-EZH2-STAT3 axis as a positive regulator of GSC self-renewal. These findings also suggest EZH2 as a possible therapeutic target in glioblastoma.

Kim E, Kim M, Woo DH, Shin Y, Shin J, Chang N, et al. Phosphorylation of EZH2 activates STAT3 signaling via STAT3 methylation and promotes tumorigenicity of glioblastoma stem-like cells. Cancer Cell 2013;23:1-14.

Reviving T Cells to Fight Cancer



Although CD8⁺ T cells can have powerful antitumor activity, chronic antigen stimulation that occurs in both malignant disease and chronic virus infections usually "exhausts" them. Exhausted

CD8⁺ T cells have decreased proliferative capacity, loss of cytokine secretion, reduced cell killing activity, low expression of canonical memory markers, and increased expression of inhibitory receptors. The inhibitory receptor programmed cell death 1, PD-1 (PDCD1), is a major player in this process. In recent clinical trials, anti-PD-1 antibody treatment has led to durable responses in patients with several types of advanced metastatic cancer, but only a minority of these patients (10%–20%) derives long-term benefit from this treatment. Using a model of chronic virus infection, West and colleagues found that low doses of interleukin (IL)-2 were able to "revive" CD8⁺ T cells in mice chronically infected with lymphocytic choriomeningitis virus. Daily low-dose IL-2 therapy enhanced CD8⁺ T-cell responses, decreased inhibitory receptor levels on virus-specific CD8⁺ T cells, and increased expression of CD127 (IL-7R) and CD44, resulting in a phenotype resembling that of memory T cells. When IL-2 treatment was combined with PD-1 blockade, striking synergistic effects were observed in enhancing virus-specific CD8⁺ T-cell responses and decreasing viral load. A major concern in the use of IL-2 as a cancer therapy is its potential to increase immune-suppressive T-regulatory cells (Treg), and low-dose IL-2 therapy did indeed increase Treg numbers in this study. However, this treatment still led to highly augmented and functional antiviral CD8⁺ T-cell responses and decreased viral burden when it was combined with PD-1 blockade. Although this work needs to be extended to experimental cancer models, the fact that both IL-2 and anti-PD-1 antibodies have been used clinically in cancer patients makes such experiments well worth pursuing and suggests one way in which the impressive results seen in some patients may be extended to more patients with currently untreatable disease. (Image courtesy of Wikimedia Commons.)

West EE, Jin HT, Rasheed AU, Penaloza-Macmaster P, Ha SJ, Tan WG, et al. PD-1 blockade synergizes with IL-2 therapy in reinvigorating exhausted T cells. J Clin Invest 2013 May 15. [Epub ahead of print].

Quantifying What It Takes to Invade

Tumor cell invasion is a fundamental feature of malignant solid tumors and contributes greatly to their associated morbidity and mortality. The ability of a tumor cell to deform its shape and travel through existing extracellular matrix or across cell-cell junctions requires specific biomechanical properties, including deformability and surface friction. Quantification of these properties and their comparison across different tumors and between tumor cells with specific genetic alterations would provide important insight into the determinants of tumor cell invasion. Byun and colleagues describe the design and use of a device that can quantitatively measure the size and velocity of a single cell as it traverses a constricted lumen using a suspended microchannel resonator. Importantly, the technique is both high throughput and precise and allows for the determination of cell size, the velocity of the cell as it enters a constricted multichannel, and the velocity of the cell as it transits through the constricted channel. These studies suggest that perturbations of the cytoskeleton primarily alter the entry velocity of a cell. In contrast, increased surface friction, generated by a positive surface charge at the constriction, primarily altered transit velocity. Thus, the differential contributions of viscoelastic and frictional properties to cell

invasion could be broken down into entry velocity and transit velocity, respectively. Interestingly, when the authors examined cell lines with different metastatic abilities, those lines with greater metastatic ability tended to exhibit a greater entry velocity. In a subset of highly metastatic cell lines, however, the transit velocity was much shorter than would be expected. In these cells, the surface friction, possibly conferred by alterations in the glycocalyx, is a potential determinant of overall metastatic ability. The potential utility of this high-throughput technique is broad and includes the identification of novel determinants of deformability and surface friction and the detection of metastatic carcinoma cells circulating in body fluids based upon their unique viscoelastic and frictional properties.

Byun S, Son S, Amodei D, Cermak N, Shaw J, Kang JH, et al. Characterizing deformability and surface friction of cancer cells. *Proc Natl Acad Sci U S A* 2013;110:7580–5.

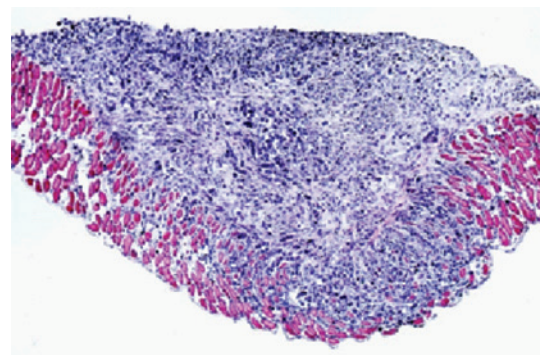
Upregulation of ERBB3 as a Mechanism of Primary Resistance to RAF Inhibition in Melanoma

Although the BRAF inhibitor vemurafenib has gained approval by the U.S. Food and Drug Administration for the treatment of *BRAF*^{V600} mutant melanoma, a subset of patients progress on this therapy or lose responsiveness early on in treatment. This situation provides the impetus for elucidating mechanisms of *de novo* resistance that protect melanoma cells with activated BRAF from responding to inhibitors of *BRAF*^{V600}. Abel and colleagues speculated that FOXD3, a transcription factor selectively induced upon BRAF/MEK pathway inhibition and capable of promoting cell survival, was an adaptive mediator of early treatment resistance. To identify FOXD3-regulated druggable targets, they used ChIP-seq to probe the FOXD3 transcriptome. These experiments revealed that FOXD3 strongly enriched the intronic enhancer region of *ERBB3*. A series of experiments identified *ERBB3* as a direct transcriptional target of FOXD3, possibly linking upregulation of ERBB3 signaling as a means of bypassing effective inhibition of the mutant BRAF/MEK/ERK pathway. To strengthen this link, they demonstrated that ERBB3 signaling was significantly enhanced in *BRAF*^{V600} melanoma cells treated with RAF and MEK inhibitors, indicating that BRAF/MEK inhibition, like FOXD3 overexpression, positively regulates ERBB3 expression levels. Further experiments demonstrated that high levels of ERBB3 were responsible for diminishing the BRAF inhibitor effects on cell viability and tumor growth in xenografts. Although ERBB3 is deficient in intrinsic kinase activity, the investigators showed that ERBB3 partnered with ERBB2, and that the enhanced signaling from this complex could be overcome by dual targeting with inhibitors like lapatinib (ERBB2/EGFR inhibitor) to enhance efficacy in *BRAF*^{V600} vemurafenib-resistant melanoma xenografts. Depletion of ERBB3 *in vivo* showed a marked reduction in tumor growth in the vemurafenib-treated group, further supporting the importance of ERBB3 as a driver of

resistance to RAF inhibitors and providing a rationale for developing ways to directly target ERBB3 in combination with vemurafenib.

Abel EV, Basile KJ, Kugel CH III, Witkiewicz AK, Le K, Amaravadi RK, et al. Melanoma adapts to RAF/MEK inhibitors through FOXD3-mediated upregulation of ERBB3. *J Clin Invest* 2013;123:2155–68.

Novel Insights into Malignant Pleural Mesothelioma



Tissue factor (TF), also known as thromboplastin CD142 and coagulation factor III (F3), is a cell surface glycoprotein abundantly expressed in various cancers that might provide an attractive therapeutic target. However, its mechanism of action is not well defined. Keshava and colleagues examined the role of TF and its possible association with endothelial cell protein C receptor (EPCR, PROCR) and protease activated receptor-1 (PAR1, F2R) in malignant pleural mesothelioma (MPM). A number of human MPM cell lines (e.g., REN, MS-1, and M9K) were first characterized for the expression level of TF and various other relevant molecules, including EPCR, PAR1, PAR2 (F2RL1), TM (THBD), and TFPI. The REN cell line was chosen for further investigation because it displayed high expression of TF and PAR1, with barely detectable levels of EPCR and undetectable expression of TFPI. In an orthotopic model, the REN cells formed highly invasive tumors, with depletion of TF from these cells dramatically restricting tumor growth into the pleural cavity. In contrast, depletion of PAR1 from the REN cells not only reduced *in vivo* tumor growth but also decreased invasion potential. However, overexpression of TF in less aggressive MS-1 and M9K cells did not increase their tumorigenicity. When EPCR was introduced into the REN cells, a very low expresser of EPCR, *in vivo* tumor growth was drastically reduced, and moreover depletion of EPCR from the nonaggressive MS-1 and MK9 markedly increased their *in vivo* tumorigenicity, confirming a key role of EPCR in regulating TF-driven tumor progression. Identification of these key factors could be beneficial in developing effective management strategies for MPM. (Image from cited article courtesy of publisher.)

Keshava S, Sahoo S, Tucker TA, Idell S, Vijaya L, Rao M, et al. Endothelial cell protein C receptor opposes mesothelioma growth driven by tissue factor. *Cancer Res*; Published OnlineFirst March 28, 2013; doi:10.1158/0008-5472.CAN-12-1690.

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.