Breaking Advances
Highlights from Recent Cancer Literature

**ALPPL-2 for Management of Pancreatic Adenocarcinoma**

Pancreatic ductal adenocarcinoma (PDAC) is a lethal disease with a dismal survival rate following current treatment approaches, mandating the rapid development of novel disease management strategies. Dua and colleagues conducted a high-throughput analysis called systemic evolution of ligands by exponential enrichment (SELEX) on specific PDAC cells, Panc-1 and Capan-1. Through this Cell-SELEX approach, they identified an RNA aptamer, SQ-2, that specifically binds to PDAC cells. This unique approach led to the discovery of an oncofetal antigen, alkaline phosphatase placental-like 2 (ALPPL-2, chr. 2q37.1), which is expressed in the cell membrane of the PDAC cells via the GPI anchor. The specific binding of the SQ-2 with ALPPL-2 was confirmed by subsequent knockdown studies. Of note, ALPPL-2 expression was detectable in both the membrane and the secretome. Knockdown of ALPPL-2 from Panc-1 cells markedly reduced cellular growth and invasion. Moreover, genomic microarray analysis identified reduced mRNA expression levels of several growth-regulatory molecules, including interleukin-1 and -6 receptors, S-phase kinase-associated protein-2 (SKP2), and CyclinD2 in the ALPPL-2 knockdown Panc-1 cells. Identification of ALPPL-2 as a novel PDAC-associated molecule expressed in the membrane and in the circulation could be an attractive target for therapy as well as for diagnosis and monitoring, warranting a comprehensive analysis in human PDAC samples. *(Image from cited article courtesy of publisher.)*


**BRAF Mutations and the Warburg Effect in Melanoma**

**BRAF** mutations are common in melanoma and are most often represented by the V600E mutation, which results in constitutive activation of its serine/threonine kinase activity, with downstream effects on the mitogen-activated protein kinase (MAPK) signal transduction pathway. The BRAF inhibitor vemurafenib (PLX4032) is effective in patients with the V600E mutation, and the presence of the V600E allele is used clinically as a predictive marker to select patients for this therapy. Although tumor regression after vemurafenib is often seen with **BRAF**-mutant melanomas, relapse and tumor resistance are common within months of treatment. How **BRAF**-mutant melanomas grow even under conditions of nutrient scarcity is not well understood. The Warburg effect (preferential glycolysis even under conditions of high oxygen) has been linked to cancer-activating genes but is poorly understood in melanoma. Haq and colleagues have linked **BRAF** inhibition to activation of oxidative phosphorylation through a pathway involving microphthalmia-associated transcription factor (MITF) and proliferator-activated receptor gamma coactivator 1 alpha (PGC1α, PPARGC1A). They first show that **BRAF** activation induces metabolic reprogramming in melanoma. Treatment of **BRAF**-mutant melanoma cells with vemurafenib or an inhibitor of the MAPK pathway resulted in induction of the Krebs cycle and subsequent oxidative phosphorylation. Vemurafenib treatment led to an increase in the number of mitochondria per cell, associated with increased expression of BRAF(V600E), and suppressed expression of PGC1α, the latter of which is a known inducer of mitochondrial metabolism. The authors also show lineage specificity to this mechanism. They observed that MAPK/extracellular signal–regulated kinase (ERK) pathway inhibition in a variety of cancer cell lines affected PGC1α only in melanoma cells, and to elucidate this mechanism, they showed that PGC1α expression was directly regulated by MITF, a transcription factor largely restricted to the melanocyte lineage. In turn, they found that **BRAF** negatively regulates MITF activity and that MITF expression in melanomas is correlated with expression of genes associated with oxidative phosphorylation. Furthermore, they showed that MITF expression in cells resulted in decreased lactate production under conditions of similar glucose uptake. Overall, the authors show that **BRAF**-mutant melanomas become dependent on ATP generation from mitochondria. Therapeutic targeting of mitochondrial metabolism could represent an approach toward enhancing the effect of **BRAF** inhibitors and potentially delaying resistance to these agents.


**Myeloid Cells, Myeloid-Derived Suppressor Cells, and the Retinoblastoma Gene**

Myelopoiesis is significantly altered in mouse and human cancers with expansion of immature activated cells, myeloid-derived suppressor cells (MDSC) that can suppress immune responses. Youn and colleagues analyzed the fate of MDSCs in transplantable or genetic mouse models and uncovered a new way in which these cells are regulated. MDSCs can be subdivided into monocytic MDSCs (M-MDSC) and polymorphonuclear MDSCs (PMN-MDSC). Under physiologic conditions, inflammatory monocytes, which are the normal counterpart of M-MDSCs, differentiate into macrophages and dendritic cells. In cancers, PMN-MDSCs accumulate in the tumor and spleen. The investigators found that a large proportion of M-MDSCs in tumor-bearing mice were able to acquire phenotypic, morphologic, and functional features of PMN-MDSCs. Moreover, acquisition of this phenotype was mediated by transcriptional silencing of the retinoblastoma gene, **RB1**, in the myeloid cells through epigenetic modifications mediated by histone deacetylase 2 (HDAC2). MDSCs from patients with pancreatic, lung, and head and neck cancers were also predominantly PMN-MDSCs; they could be generated in vitro from M-MDSCs and had low **RB1** expression. Therefore epigenetic silencing of **RB1** expression in myeloid cell populations is important in accumulation of these tumor-promoting cells in malignant disease. HDAC inhibitors may be indicated in preclinical and clinical studies that target MDSCs.

CXCL12/CXCR4 Signaling in MPNSTs

Neurofibromatosis type 1 (NF1) is a relatively common tumor predisposition syndrome that confers increased risk of developing both benign and malignant tumors, including optic pathway gliomas (OPG) and malignant peripheral nerve sheath tumors (MPNST). Mo and colleagues used mouse models for MPNSTs to demonstrate the importance of CXCR4/CXCL12 signaling in the progression of NF1-deficient tumors. In 2 NF1-deficient murine models for MPNST, they demonstrate upregulation of Ccxl4 at the mRNA and protein levels. Using a combination of gene knockdown and overexpression, they also show that CXCR4 can drive tumor cell proliferation and tumor growth in vivo through regulation of cyclin D1 (Cnd1) expression. Multiple signaling pathways can regulate cyclin D1. By comparing pathway activity in cells with and without CXCR4 knockdown, the authors identified a dramatic reduction in nuclear β-catenin levels and decreased β-catenin transcriptional activity in cells with Ccxl4 depletion. Importantly, overexpression of β-catenin or cyclin D1 could rescue the proliferation phenotype in Ccxl4-depleted cells. Although many cell types, including cells in the tumor microenvironment, can produce the CXCR4 ligand CXCL12, the authors show that tumor cell-derived CXCL12 can drive increased cyclin D1 levels and increased proliferation in their murine model. Furthermore, they demonstrate the potential utility of the CXCR4 antagonist AMD3100 to suppress tumor growth in vivo. Through examination of a set of human NF1-deficient MPNSTs, they found robust expression of CXCR4, β-catenin, and cyclin D1, suggesting that this may be an important pathway to inhibit in human tumors. Interestingly, earlier work by Warrington and colleagues demonstrated that CXCL12 also promotes tumorigenesis in optic pathway gliomas in the setting of neurofibromin loss, further supporting the use of CXCR4 inhibitors for NF1-associated neoplasms. (Image from Cancer Research courtesy of publisher.)


Ovarian Cancer Stem Cells

Ovarian cancers are uncommon but lethal, represent the fifth cause of cancer death among U.S. women, and show frequent loss of p53 (Ptp53) and RB (Rb1). Classified histologically, over 90% of ovarian cancers arise from surface epithelium. The ovarian surface epithelium is ruptured and regenerates during ovulation, suggesting this epithelium as a source of stem cells. Flesken-Nikitin and colleagues identified the hilum region of the mouse ovary, the transitional area between the ovarian surface epithelium, mesothelium and tubal/ovudial epithelium, as a stem cell niche. Cells of the hilum ovarian surface epithelium cycled slowly and expressed stem/progenitor markers including ALDH1 (ALDH1A1), LGR5, LEP1, CD133 (PROM1), and CK6B (KR16B). Hilum ovarian epithelial cells displayed stem cell properties evidenced by sphere generation and long-term lineage-tracing assays. Importantly, transplantation of ovarian surface epithelium cells deleted for both p53 (Ptp53) and Rb1 led to efficient tumor formation in recipient mice, suggesting that stem cells in transitional zones of the ovarian surface epithelium are susceptible to malignant transformation. These data suggest hilum cells of the ovarian surface epithelium as a source of cancer stem cell niche.


Selective Inhibition of Notch1 Signaling in T-ALL

Identifying strategies to target aberrant transcription factors has been challenging and not amenable to conventional small-molecule screening approaches owing to the complexity of developing high-throughput and robust screening assays. Roti and colleagues highlight the difficulties in targeting Notch1 in cancer due to its pleiotropic roles as an oncogene in some cancers and a tumor suppressor in others and integrate a cDNA screen with a gene expression–based high-throughput screen to identify potential therapeutic targets in Notch1-driven leukemias. The authors identified SERCA (Sarco/endoplasmic reticulum calcium ATPase), a family of 3 genes—ATP2A1, ATP2A2, and ATP2A3—as a Notch1 signaling enhancer at the intersection of these 2 screens and isolated thapsigargin as a highly potent natural inhibitor of SERCA. Low nanomolar concentrations of thapsigargin induced the Notch1 “off” signature, reduced the expression of direct Notch1 target genes like MYC, HES1, and STX1A, and resulted in G0/G1 arrest in human Notch1-dependent T-cell acute lymphoblastic leukemia (T-ALL) cells. Furthermore, thapsigargin treatment reduced the level of Notch1 on the cell surface and resulted in colocalization of Notch1 with a Golgi membrane protein, leading to defective Notch1 maturation in T-ALL cells. The investigators used a Drosophila stem cell model in which Notch inhibition perturbs differentiation, followed by a T-ALL xenograft model to show that SERCA antagonism inhibits Notch function and T-ALL growth in vivo through effects that require the calcium-binding modules of the Notch1 extracellular domain and Notch1 receptors bearing leukemogenic mutations. These data suggest that selective targeting of the mutant oncoprotein versus wild-type Notch1 in T-ALLs, much like selective targeting of mutant BRAF in melanoma, provides a therapeutic window for thapsigargin as well as a mechanism that could mitigate the potential cancer-promoting effects of inhibiting wild-type Notch1. However, Pan-SERCA inhibitors like thapsigargin will need to be modified prior to clinical application, given the prevalent role of calcium signaling in normal physiology.


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