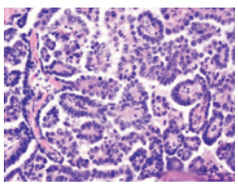
Breaking Advances
Highlights from Recent Cancer Literature

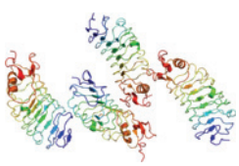
Predicting Time to Ovarian Cancer Recurrence



High-grade serous ovarian cancer has a poor prognosis for two main reasons: Most cases are detected at a late stage when there are disseminated peritoneal metastases, and after initial response to chemotherapy, 30% to 40% of patients experience a relapse within 12 months and are resistant to standard therapy. Were it possible to predict the outcome of therapy, oncologists might be able to give more targeted, personalized drugs or intensive chemotherapy to subsets of patients in this genetically heterogeneous disease. Yang and colleagues used reverse-phase protein arrays of 412 patient biopsies to construct a PROtein-driven index of OVARian cancer (PROVAR). A predictive set of nine protein markers was associated significantly with progression-free and overall survival. These nine proteins represent three major intracellular signaling pathways (STAT, PKC, and MEK/ERK) involved in cell proliferation, survival, and invasion. PROVAR was validated using a second cohort of 224 high-grade serous ovarian cancer patient biopsies, discriminating between high and low risk of recurrence after chemotherapy, as well as between short-term and long-term survivors. The protein array data were superior to gene expression-based classification of prognosis. Previous gene expression studies have shown that both mesenchymal and immunoreactive gene expression signatures were associated with a poor prognosis; the PROVAR protein signature was correlated with both these subgroups. Should this nine-protein signature be validated further, these outcomes could be measured by immunohistochemistry at the time of diagnosis. *(Image courtesy of Wikimedia Commons.)*

Yang JY, Yoshhara K, Tanaka K, Hatae M, Masuzaki H, Itamochi H, et al. Predicting time to ovarian carcinoma recurrence using protein markers. J Clin Invest 2013;123:3740–5.

Rescuing Intestinal Stem Cells Improves Therapeutic Index of Chemotherapy



The treatment of patients harboring metastatic cancer remains an important clinical problem, often complicated by tissue injury induced by the chemotherapy or radiation used as part of their treatment. Shou and colleagues tested whether induction of adult intestinal stem cells (ISC) overcome injury to the gastrointestinal tract as a potential means to improve survival of patients with colorectal cancer receiving chemotherapy or radiation. They showed that the cell surface receptor Robo1 and its ligand (Slit2) colocalized to ISC, enriching in the Lgr5 positive cells that mark this stem cell compartment. Using mice lacking Robo function or overexpressing Slit2, they also showed the functional requirement of this signaling axis to maintain ISC viability. They further demonstrated that the Slit2-Robo1 axis acts synergistically, *in vitro* and *in vivo*, with the Wnt agonist R-Spondin1 (Rspo1). Although therapeutic doses of either the chemotherapeutic agent 5-fluorouracil (5-FU) or radiation dramatically reduced ISC markers, increased expression of Slit2 or R-Spondin1 maintained ISC markers during such challenges *in vitro* or *in vivo*. Significantly, the combination of Slit2 and R-Spondin1 acted synergistically to maintain ISC markers during 5-FU treatment or radiation. In mice treated with 5-FU or radiation, these treatments significantly increased survival, with the combination of Slit2 and R-Spondin1 providing the greatest protection. Most importantly, given the role Wnt signaling plays in colorectal cancer, these agents did not accelerate cancer in their colorectal cancer mouse model. The combined treatment also did not reduce the sensitivity of the tumors to 5-FU, as they provided increased survival to an otherwise lethal dose of 5-FU while significantly eliminating the intestinal tumors. These results suggest that a combined Slit2/R-Spondin1 treatment might be used in an adjuvant fashion to maintain ISC function during chemotherapy or radiation, increasing the survival of these colorectal cancer patients by expanding the therapeutic index of such treatments. (*Image courtesy of Wikimedia Commons.*)

Shou WJ, Geng ZH, Spence JR, Geng JG. Induction of intestinal stem cells by R-spondin 1 and Slit2 augments chemoradioprotection. Nature 2013;501:107–11.

The Nuclear Factor One Family in Neural Stem Cell Quiescence

Adult stem cells can remain for long periods of time in the quiescent state. Active maintenance of quiescence is critical, and its dysregulation can drive diseases, including cancer. Although cell–cell and cell–extracellular matrix (ECM) interactions are known determinants of quiescence, the cell-intrinsic factors regulating these interactions are largely unknown. Martynoga and colleagues used a cell culture model of neural stem cell (NSC) quiescence and epigenomic profiling to identify nuclear factor one X-type (NFIX) as a transcription factor critical to the gene regulatory network controlling NSC quiescence. Because few experimentally tractable models of NSC quiescence exist, the authors developed a cell culture model of quiescence in which exposure of NSCs to BMP4 resulted in the reversible acquisition of cellular and transcriptional characteristics of quiescent cells, including cell-cycle arrest. By comparing the epigenomic profile of arrested and proliferating NSCs, they identified quiescent-specific enhancer element activity, and they confirmed that these enhancer regions were enriched near genes with quiescent-specific alterations in gene expression. Using these data, they screened for transcription factors potentially regulating these quiescent-specific patterns of gene expression. They made the unique observation that the nuclear factor one (NFI) motif is strongly enriched in enhancers with quiescent-specific activity. Moreover, using luciferase reporter assays, gene knockdown, and gene overexpression they demonstrated that NFIX, one member of the NFI family of transcription factors, is both required and sufficient for specific aspects of NSC quiescence. Interestingly, enhancer studies and gene expression studies confirmed an upregulation of genes associated with the ECM and cell–cell adhesion in cell cycle–arrested versus proliferating NSCs. The authors suggest that NFIX may regulate NSC quiescence, in part, via alterations in cell–cell and cell–ECM interactions. Future studies examining the importance of NFI transcription factors in

quiescence and exploring their potential dysregulation in disease will be of interest.

Martynoga B, Mateo JL, Zhou B, Andersen J, Achimastou A, Urbán N, et al. Epigenomic enhancer annotation reveals a key role for NF1X in neural stem cell quiescence. Genes Dev 2013;27:1769–86.

Tumor and Microenvironment Innervation Drives Prostate Cancer Progression

A connection between the nervous system and tumor progression has long been recognized. For example, perineural invasion in several epithelial cancers is linked to poor prognosis. However, the causal relationships have remained unclear. Magnon and colleagues breaks new ground by identifying the functional relationship between the sympathetic nervous system (SNS) and parasympathetic nervous system (PNS) in contributing to different stages of prostate cancer progression. Using both prostate cancer xenografts (i.e., PC3) or a transgenic prostate cancer model (Hi-Myc) and surgical, genetic, or chemical ablation, they were able to outline two main functions for the SNS and PNS. First they show that similar to angiogenesis, tumors recruit newly formed nerves in the stroma. They showed that the SNS, characterized by fibers signaling through β2- and β3-adrenergic receptors and innervating primarily the stroma surrounding the prostate gland, foster the development of prostatic intraepithelial neoplasia. Surgical or chemical (using 6-hydroxydopamine, which destroys tyrosine hydroxylase^{+} sympathetic fibers) ablation of the SNS nerves or genetic ablation of the β2- and β3-adrenergic receptors in the microenvironment almost completely prevented tumor formation by xenografted or spontaneous tumors. The PNS nerves appear not to play a role during early stages of prostate cancer progression and in contrast facilitate migration, invasion, and distant metastasis development; unlike the SNS, these nerves clearly innervate the tumor tissue. In fact, agonists of the cholinergic receptor, muscarinic 3 (CHRM3), favored invasive properties and also proliferation of xenografted tumors while antagonists blocked these events. Interestingly, these effects were dependent on the microenvironment alone. It will be interesting to determine whether the metastatic lesions in bone or visceral organs follow a PNS or SNS innervation. The authors also show that nerve density assessment in human prostate cancer tissue predicted for relapse outside the prostate capsule. In addition, they highlight the fact that intake of clinically used β-blockers is associated with improved survival in prostate cancer. Much has been said about targeting the tumor microenvironment through efforts focused on immune cells or the cancer-associated fibroblasts. These findings provide a novel twist on accurately and powerfully targeting the tumor microenvironment with exceptional results. Given the potent and selective pharmacology that is available to target the SNS and PNS, our data raise the tantalizing possibility that drugs targeting both branches of the autonomic nervous system may be useful therapeutics for prostate cancer.

Magnon C, Hall SJ, Lin J, Xue X, Gerber L, Freedland SJ, Frenette PS. Autonomic nerve development contributes to prostate cancer progression. Science 2013;34:1236361.

Hypoxia Regulates Collagen Formation and Metastasis in Sarcoma

Metastases are responsible for the vast majority of cancer-associated deaths, but a paucity of therapeutic approaches target metastasis. Malignant tumors of soft tissue (soft-tissue sarcomas) include a variety of histologic subtypes and frequently show lethal metastatic behavior, often to the lungs. Poorly differentiated undifferentiated pleomorphic sarcoma (UPS) is among the most aggressive clinically, with frequent early metastases. Eisinger-Mathason and colleagues explore the involvement of tumor hypoxia as a driver of sarcoma metastasis. Hypoxia is known to induce HIF-1α, which in turn promotes the release of enzymes that remodel collagen in the extracellular matrix, including procollagen-lysine, 2-oxoglutarate 5-dioxygenase (PLOD2). The authors first show that high levels of *HIF-1α* and *PLOD2* correlate with sarcoma metastasis, rather than primary tumor formation. Using a genetically engineered conditional murine model of UPS, they found that injection of Adeno-Cre into skeletal muscle resulted in $Kras^{G12D}$ expression and *Trp53* deletion and produced sarcomas within 8 weeks. Deletion of *HIF-1α* in this model decreased metastases and led to increased collagen maturity without affecting latency or primary tumor size. To establish a role for *HIF-1α* and *PLOD2*, they knocked down these genes in a human xenograft sarcoma model. Loss of either gene resulted in decreased lung colonization following tail vein injection. Using Boyden chamber–based migration assays, they then showed that knockdown of HIF-1α and PLOD2 significantly decreased sarcoma cell motility. Using differential labeling, they performed cell mixing experiments. The addition of control cells rescued migratory deficiency in HIF-1α and PLOD2 knockdown cells, suggesting that that HIF-1α drives sarcoma cell migration in a cell-extrinsic manner. In addition, PLOD2 expression rescued cell migration in some HIF-1α-deficient cells under hypoxic conditions, stimulated migration in normoxic cells, and rescued *in vivo* metastatic ability in HIF-1α-deficient sarcoma cells. Treatment with minoxidil, a pharmacologic PLOD2 inhibitor, resulted in decreased cell migration and decreased pulmonary metastases in the mouse model. These findings characterize molecular mechanisms that link hypoxia to metastatic behavior in soft-tissue sarcoma via HIF-1α, PLOD2, and collagen maturation and identify PLOD2 as a potential therapeutic target to control metastases in these tumors. *(Image from cited article courtesy of publisher.)*

Eisinger-Mathason TSK, Zhang M, Zhang M, Qiu Q, Skuli N, Nakazawa MS, et al. Hypoxia-dependent modification of collagen networks promotes sarcoma metastases. Cancer Discov; Published OnlineFirst August 1, 2013; doi:10.1158/2159-8290.CD-13-0118.

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.