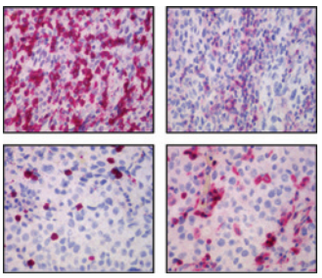
Breaking Advances
Highlights from Recent Cancer Literature

Potential Beneficial Side Effects of BRAF Inhibitors



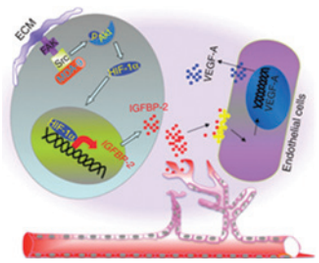
Despite advances in therapeutic targeting, most drugs still have unwanted side effects. A new study by Knight and colleagues illustrates a potential beneficial side effect of BRAF inhibitors, an increase in the $CD8^+$ T/$FOXP3^+CD4^+$ T-cell ratio. The authors suggest that augmenting this effect may lead to an increased antitumor efficacy of BRAF inhibitors. The initial observation that BRAF inhibitors may alter host immunity came from Wilmott and colleagues, who analyzed patient biopsies, demonstrating increased $CD8^+$ T-cell infiltrates in tumors treated with a BRAF inhibitor. Following up on this observation, Knight and colleagues used a syngeneic model of $Braf^{V600E}$-driven melanoma that has an intermediate sensitivity to BRAF inhibition. Through a series of experiments, they show that in their model the antitumor efficacy of BRAF inhibition is partially dependent on a drug-induced decrease in expression of tumor-derived CCL2. Importantly, it is the expression of CCR2, the receptor for CCL2, on nonneoplastic, host-derived cells that is critical for this antitumor efficacy. These authors go on to show that CCR2 is expressed on $Foxp3^+CD4^+$ tumor-infiltrating T cells and upon treatment they see a relative decrease in $Foxp3^+CD4^+$ tumor-infiltrating lymphocytes and a modest increase in $NK1.1^+$ (KLRB1C) NK cells and $CD8^+$ T cells. Using monoclonal antibodies to modulate the immune response and mice with knockout of specific immune cell subsets, Knight and colleagues show that the antitumor effect of the BRAF inhibitor is dependent on functional $CD8^+$ T cells. They suggest that future clinical trials should test the antitumor efficacy of combining BRAF inhibitors with immunotherapy, and in support of this approach, they demonstrate a synergistic antitumor activity in both mouse transplant and *de novo* tumorigenesis models treated with a combination of BRAF inhibition and anti-CD137 (TNFRSF9) antibody. (*Image from Clinical Cancer Research courtesy of publisher.*)

Knight DA, Ngiow SF, Li M, Parmenter T, Mok S, Cass A, et al. Host immunity contributes to the anti-melanoma activity of BRAF inhibitors. J Clin Invest 2013;123:1371–81.

Wilmott JS, Long GV, Howle JR, Haydu LE, Sharma RN, Thompson JF, et al. Selective BRAF inhibitors induce marked T-cell infiltration into human metastatic melanoma. Clin Cancer Res 2012;18:1386–94.

The Prometastatic Gene, MDA-9/syntenin, and IGFBP-2 Stimulate Angiogenesis

Metastasis is a complex process that requires vascularization to support survival and expansion of tumor cells at distant sites. In these contexts, understanding and ultimately inhibiting tumor angiogenesis represents, at least in principle, a means of obstructing cancer growth and metastasis. Previous studies confirm that the adapter scaffold protein melanoma differentiation associated gene 9/syntenin (*SDCBP*)



can directly promote melanoma, breast and gastric cancer progression, and metastasis through modulation of important signal transduction pathways, including src, FAK (PTK2), p38, mitogen-activated protein kinase, and NF-κB activated processes. Through genetic gain-of-function, loss-of-function, and pharmacologic approaches, Das and colleagues now define a new proangiogenic function of MDA-9/syntenin that is mediated by secretion of insulin-like growth factor binding protein-2 (IGFBP-2). Evidence supports a pathway by which MDA-9/syntenin elicits angiogenesis through secretion of IGFBP-2. MDA-9/syntenin interacts with the extracellular matrix, activating src and FAK, resulting in activation of Akt, which induces hypoxia-inducible factor 1α (HIF-1α). The HIF-1α then induces IGFBP-2, which is secreted from melanoma cells and promotes angiogenesis by further inducing endothelial cells to produce and secrete VEGFA, thereby augmenting tumor angiogenesis. Although further studies are required, this unexpected cell-nonautonomous function of MDA-9/syntenin resulting in augmented angiogenesis may directly contribute to metastasis of melanoma and potentially to other cancers that express elevated levels of this gene product. In these contexts, targeting MDA-9/syntenin or the downstream molecules induced by this gene may provide a novel approach for inhibiting metastasis by directly inhibiting the tumorigenic/metastatic phenotype of transformed cells (autonomous function) and through indirect pathways inhibiting angiogenesis (nonautonomous function). (*Image from cited article courtesy of publisher.*)

Das SK, Bhutia SK, Azab B, Kegelman TP, Peachy L, Santhekadur PK, et al. MDA-9/syntenin and IGFBP-2 promote angiogenesis in human melanoma. Cancer Res 2013;73:844–54.

RNA Helicase Regulation of WNT Signaling

The DEAD box RNA helicase *DDX3X* shows recurrent mutation in medulloblastoma, typically associated with WNT-driven tumors. Yet, how does an RNA helicase affect WNT signaling? Cruciat and colleagues show that DDX3X binds to casein kinase 1ε (CK1ε), a serine–threonine kinase thought to be constitutively active, directly stimulating its kinase activity. Activated CK1ε (CSNK1E) in turn phosphorylates DISHEVELLED (DVL1), which is known to activate WNT signaling. The ability of DDX3X to promote WNT signaling was conserved among mammalian cells, frogs, and worms. These data offer new insights into regulation of WNT signaling and suggest that inhibitors of DDX3X might be useful to target WNT-driven tumors.

Cruciat CM, Dolde C, de Groot RE, Ohkawara B, Reinhard C, Korswagen HC, et al. RNA helicase DDX3 is a regulatory subunit of casein kinase 1 in Wnt/β-catenin signaling. Science 2013 Feb 14. [Epub ahead of print].

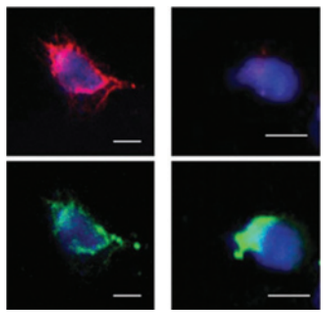
MicroRNA Regulation of a Mesenchymal Subtype in Ovarian Carcinoma

Ovarian carcinoma is highly lethal, in part due to first detection at late stage. Ovarian cancers tend to spread through direct invasion throughout the peritoneal cavity, rather than via blood-borne metastases. Invasiveness of epithelial tumors may be related to an epithelial–mesenchymal transition (EMT), the driving events for which, in ovarian carcinoma, are largely unknown. Yang and colleagues took advantage of the multidimensional dataset available on serous ovarian carcinoma from The Cancer Genome Atlas by examining the role of microRNAs (miRNA) in EMT. Consensus clustering of mRNA transcriptome data revealed mesenchymal and epithelial subtypes. They found that overexpression of the mesenchymal markers *FN1* and *SNAI2* (a master regulator of EMT) tracked with the mesenchymal subtype. Focusing on ∼200 EMT genes predicted to be regulated by miRNAs, the authors used network analysis to identify 8 key miRNAs predicted to regulate nearly 90% of these EMT signature genes. MIR506 was selected for further study based on a high degree of downregulation in the mesenchymal-subtype tumors. Ovarian carcinoma cells transfected with MIR506 displayed an epithelial phenotype (rearrangement of F-actin) and downregulated *SNAI2*. In addition, MIR506 overexpression resulted in upregulation of E-cadherin, a key epithelial marker. TGF-β, a regulator of EMT, upregulated *SNAI2* and was blocked by overexpression of MIR506. Survival analysis showed that mesenchymal tumors were more clinically aggressive, whereas patients whose tumors had high MIR506 expression had a relatively favorable outcome. These findings were validated in additional independent datasets. To explore the differential regulation of MIR506 in ovarian cancer, the authors treated ovarian cancer cells with the demethylating agent 5-azaC, which resulted in upregulation of MIR506, suggesting an epigenetic mechanism. Finally, MIR506 was delivered via nanoparticles in an orthotopic mouse model of ovarian carcinoma, resulting in fewer tumor nodules and reduced overall tumor weight. After treatment with MIR506, tumors showed decreased expression of mesenchymal markers *SNAI2* and vimentin and increased expression of the epithelial marker E-cadherin. This integrated analysis identifies a clinically aggressive mesenchymal subtype of ovarian carcinoma mediated in part by loss of MIR506. The results suggest that MIR506 is a potential therapeutic agent in patients with ovarian carcinoma.

Yang D, Sun Y, Hu L, Zheng H, Ji P, Pecot CV, et al. Integrated analyses identify a master MicroRNA regulatory network for the mesenchymal subtype in serous ovarian cancer. Cancer Cell 2013;23:186–99.

Plastin-3: Novel Marker for Colorectal CTCs Undergoing EMT

Circulating tumor cells (CTC) are attractive targets to develop novel cancer management strategies. The present study sought



to identify novel CTC biomarkers for colorectal cancer. Initially, through cDNA microarray analysis on 132 colorectal cancer patients from Japan, 2,969 overexpressed genes were identified in tumors compared with normal tissue. Further scrutiny of 22 colorectal cancer distant-metastasis–associated genes from this list identified Plastin-3 (*PLS3*) as a potential marker of CTC, which was exclusively expressed at very high levels in metastatic colorectal cancer. Gene set enrichment analysis showed significant association of *PLS3* with TGFB-specific, metastasis, stemness, and mesenchymal gene expression signatures. Tumor-specific overexpression of *PLS3* at both the mRNA and protein level was further validated in independent sets of colorectal cancer patients. Induction of *PLS3* expression due to epithelial–mesenchymal transition (EMT) and stemness was also shown with specific colorectal cancer cell lines. The strong potential of *PLS3* as a marker for EMT-induced CTCs was further shown in cytokeratin-expressing CTCs that were positive or negative for EPCAM. Moreover, *PLS3* expression with concomitant high expression of *vimentin* was detected in cells with reduced cytokeratin expression. At the same time, *PLS3* expression was negative in peripheral blood mononuclear cells obtained from healthy individuals without cancer. Clinicopathologic analysis in peripheral blood samples from 711 colorectal cancer patients showed significant association of *PLS3* expression with greater depth of invasion, lymph node metastasis, liver metastasis, peritoneal dissemination, recurrence rate, and Dukes staging progression. A multivariate analysis also showed an association between *PLS3*-positive CTCs in peripheral blood samples from CRC patients with a poor prognosis. Remarkably, a significantly elevated level of *PLS3* was detected in a cohort of patients with recurrent colorectal cancer compared with patients without recurrence. Of note, high levels of *PLS3* were detected in several solid tumors, including esophageal, gastric, liver, pancreatic, breast, lung, and prostate cancer and melanoma. Thus, *PLS3* appears to be a novel marker for CTCs in colorectal cancer with EMT as well as defining a stemness phenotype and could be further explored in colorectal cancer and possibly in other malignancies. (*Image from cited article courtesy of publisher.*)

Yokobori T, Iinuma H, Shimamura T, Imoto S, Sugimachi K, Ishii H, et al. Plastin-3 is a novel marker for circulating tumor cells undergoing the epithelial-mesenchymal transition and is associated with colorectal cancer prognosis. Cancer Res.0326.2012; Published OnlineFirst February 1, 2013; doi:10.1158/0008–5472.CAN-12-0326.

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.