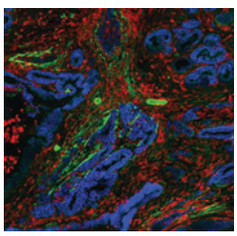
Breaking Advances
Highlights from Recent Cancer Literature

Fibroblast-Derived Exosomes Help Drive Oncogenic Signaling



Components of the tumor microenvironment, including cancer-associated fibroblasts (CAF), play a number of critical roles during tumorigenesis. In the present study, Luga and colleagues address the role of CAFs in promoting breast cancer metastasis and uncover a pathway in which exosomes derived from fibroblasts mobilize autocrine Wnt–planar cell polarity (PCP) signaling in tumor cells to promote tumor cell motility and metastasis. The authors make the observation that conditioned media from mouse fibroblast cells, termed active-conditioned media (ACM), stimulate the protrusive activity and motility of breast cancer cell lines in 2-dimensional and 3-dimensional Matrigel cultures. Moreover, coinjection of fibroblasts with breast cancer cells into the mouse mammary fat pad generated more metastatic lung lesions than tumor cells alone. Because core components of the PCP signaling pathway are important in breast cancer cell protrusive activity and motility, the authors used gene knockdown to show that ACM-induced motility is dependent on PCP components, including Smurf1, Smurf2, Dvl1, Fzd6, Vangl1, and Pk1 (Pklr) *in vitro* and Prk1 (Pkn1) *in vivo*. Importantly, they show that ACM-stimulation results in asymmetric distribution of the core PCP components with respect to cellular protrusions in breast cancer cells. During development, Wnt ligands are known to regulate PCP signaling. Surprisingly, Wnt secretion in breast cancer cells but not in fibroblasts was required for the ACM phenotype, as shown by gene knockdown of Porcupine (*Porcn*), an acetyltransferase essential for Wnt secretion. Thus, the ACM phenotype was dependent on autocrine Wnt signaling.

Using a combination of techniques, including size exclusion chromatography, ion-exchange chromatography, electron microscopy, differential centrifugation, and gene knockdown, the authors show that exosomes secreted by fibroblasts and the tetraspanin CD81 are essential for the ACM-mediated phenotype. Exosomes are membrane-bound vesicles important in intercellular communication but not previously implicated in CAF-breast cancer cell communication. By fluorescently tagging CD81in fibroblasts, the authors show that fibroblast-derived CD81 colocalizes with breast cancer cell–derived Wnt11 within the endocytic pathway in breast cancer cells. To demonstrate the potential relevance of these observations to human breast cancer, the authors isolated exosomes from human CAFs and showed that these exosomes also can stimulate breast cancer cell protrusions and asymmetric accumulation of PCP components. Based on these data, the authors suggest a model whereby autocrine Wnt-PCP signaling is mobilized by fibroblast-derived exosomes to help drive tumor metastasis. (*Image from Clinical Cancer Research 2012;18:4266–76, Fig. 3, courtesy of the publisher.)*

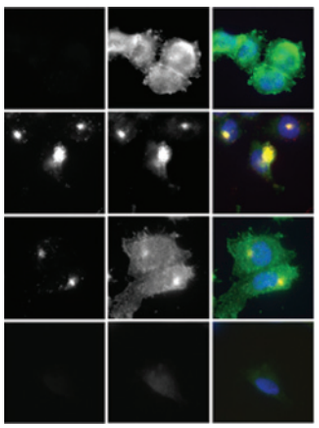
Luga V, Zhang L, Viloria-Petit AM, Ogunjimi AA, Inanlou MR, Chiu E, et al. Exosomes mediate stromal mobilization of autocrine Wnt-PCP signaling in breast cancer cell migration. Cell 2012;151:1542–56.

Ephrins and Glioma Stem Cells

Recent findings have shown the existence of a subpopulation of cancer stem cells in glioblastoma multiforme (GBM), with properties of self-renewal, tumorigenesis, and treatment resistance. Ephrin receptor tyrosine kinases and their ligands play a role in central nervous system development and cancer cells. Specifically, the EphA2 receptor has been shown to be overexpressed in a variety of human cancers, including GBM. In this study, the authors investigated the role of the EphA2 receptor in GBM cancer stem cells. The authors' first show that *EPHA2* mRNA was overexpressed in GBM relative to non-neoplastic brain and lower-grade gliomas. In GBM tumor samples, EphA2 was coexpressed with markers of neural precursors, including Nestin, Sox2, and Olig2. Primary cultures showed coexpression of EphA2 with previously described cell surface markers of cancer stem cells, including CD15 (FUT4) and CD44. Furthermore, upon differentiation/loss of stemness, EphA2 levels also decreased. Cell sorting of $EphA2^{high}$ and $EphA2^{low}$ subpopulations showed that the $EphA2^{high}$ fraction was more clonogenic and showed increased tumorigenicity in nude mice. For example, tumor formation reliably occurred after implantation of as few as 100 $EphA2^{high}$ cells, whereas injection of as many as 5,000 $EphA2^{low}$ cells failed to result in consistent tumor formation. Treatment with soluble ephrinA1-Fc caused EphA2 downregulation in stem-like cells and suppressed self-renewal and tumorigenicity. This result was confirmed with the use of siRNA-mediated knockdown of EphA2, which showed similar results. Loss of EphA2 resulted in a coordinate loss of the neural stem cell markers CD15, CD44 and CD133 (PROM1) and upregulation of GFAP, an astroglial differentiation marker. Interestingly, EphA2 downregulation resulted in an increase in ERK (MAPK) phosphorylation, and self-renewal was rescued upon treatment with an ERK inhibitor, implicating ERK activation in the loss of stemness. Finally, examination of data from The Cancer Genome Atlas showed poor survival in patients with EphA2 overexpression within the proneural and mesenchymal transcriptomal subclasses. Interestingly, additional data suggest that preexisting orthotopic human GBM xenografts were successfully treated with intraparenchymal infusion of ephrinA1-Fc, providing a model for clinical use. Overall, the authors call attention to a novel marker of stemness in GBM and point to a possible therapeutic strategy for patients with EphA2-overexpressing tumors.

Binda E, Visioli A, Giani F, Lamorte G, Copetti M, Pitter KL, et al. The EphA2 receptor drives self-renewal and tumorigenicity in stem-like tumor-propagating cells from human glioblastomas. Cancer Cell 2012;22:765–80.

Cancer Cell–Specific Receptor Targeting Enhances Delivery and Efficacy of Chemotherapeutics



Tumor-specific delivery of anticancer agents, including chemotherapy, represents the "holy grail" of therapy. However, achieving this objective to effectively treat cancer patients has been difficult. In this interesting study, Wang and colleagues used a cell surface receptor, EphA2, which is specifically and abundantly expressed on various cancers and represents a key promoter of tumor angiogenesis, to effectively deliver the chemotherapeutic agent paclitaxel (PTX). The authors have designed and synthesized a natural EphA2 ligand peptide named YSA (amino acid sequence YSAYPDSVPMMS), which can selectively target EphA2 receptors on cancer cells. The YSA molecule was subsequently linked to PTX. In the next step, modified YSA-tagged PTX molecules, YNH-PTX and dYNH-PTX, were synthesized, characterized, and validated stringently for specificity and activity. The efficacy of both YNH-PTX and dYNH-PTX as therapeutic molecules was examined further in PC-3 prostate cancer cells expressing EphA2 and LNCaP cells lacking EphA2. PC-3 cells expressing EphA2 receptors were efficient in internalizing both of the PTX conjugates, indicating their potential applications in tumors expressing EphA2. Of the 2 molecules, dYNH-PTX, which was superior in activity, showed a remarkable effect on PC-3 xenografts by inhibiting tumor growth, resulting in further tumor regression. Moreover, dYNH-PTX treatment significantly reduced tumor angiogenesis in a murine model of renal cancer. Notably, no adverse effects were detected following administration of these molecules in animals. Targeting of uniquely expressed cancer surface receptors, which are internalized, using the novel approaches for efficient drug delivery described in this article could provide a sensitive and selective approach for effectively treating cancer patients. (*Image from cited article courtesy of the publisher.)*

Wang S, Noberini R, Stebbins JL, Das S, Zhang Z, Wu B, et al. Targeted delivery of paclitaxel to EphA2-expressing cancer cells. Clin Cancer Res 2013;19:128–37.

Blocking Resistance in SHH-Driven Medulloblastoma

The Sonic Hedgehog (SHH) pathway is mutated in basal cell carcinoma and in some subtypes of medulloblastoma. Inhibitors of SMO, a key intermediate in SHH signaling, show short-lived activity in patients due to development of resistance mutations in SMO. Kim and colleagues show that arsenic trioxide and itraconazole (which act through a mechanism distinct from that of current clinical SMO inhibitors) in combination show activity against drug-resistant SMO alleles *in vitro* and *in vivo*.

Kim J, Aftab BT, Tang JY, Kim D, Lee AH, Rezaee M, et al. Itraconazole and arsenic trioxide inhibit hedgehog pathway activation and tumor growth associated with acquired resistance to smoothened antagonists. Cancer Cell 2013;23:23–34.

Vaccinating against Colorectal Cancer

Vaccination against tumor-associated antigens is playing an increasing role in treatment of cancer patients, although the immune system is usually compromised in advanced disease. Such vaccines would be expected to work best in a preventative setting. However, their immunogenicity has rarely, if ever, been tested in this way–until now. Kimura and colleagues describe immunization of patients at high risk for colorectal cancer with a vaccine against a tumor-associated antigen called MUC1. The trial showed that the vaccine was highly immunogenic, with immunogenicity limited to a subset of recipients. Of 39 vaccinated patients with advanced premalignant colonic adenomas, 17 developed high levels of specific anti-MUC1 IgG and long-lasting immune memory, and 22 failed to respond. This lack of response was not associated with age, body mass index, size of adenoma at surgery, or time after removal. There was also no association between response and HLA-DR/DQ. However, the nonresponders had a higher proportion of myeloid-derived suppressor cells (MDSC) in their blood compared with vaccine responders or normal controls. Although MDSCs are found in mice and humans with advanced cancers, they have not been reported in a premalignant setting.

This study has several important implications. First, it provides a rationale for vaccination against a tumor-associated antigen in patients with premalignant disease. There were no significant adverse effects of the vaccine, and no signs that autoimmune reactions were stimulated. Second, if confirmed in further studies, these results show that response to vaccine is compromised in the presence of MDSCs, which has broad implications for the use of cancer vaccines. When vaccines are given alongside other treatments and in advanced disease, it is difficult to tease out reasons for their success or failure. Finally, the presence of MDSCs in patients with premalignant disease certainly warrants further study. Because the vaccine was well tolerated and response was not restricted to a limited number of HLA types, future studies should be able to determine whether this relatively simple intervention could delay or prevent further adenomas and colon cancers.

Kimura T, McKolanis JR, Dzubinski LA, Islam K, Potter DM, Salazar AM, et al. MUC1 vaccine for individuals with advanced adenoma of the colon: a cancer immunoprevention feasibility study. Cancer Prev Res 2013;6:18–26.

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.