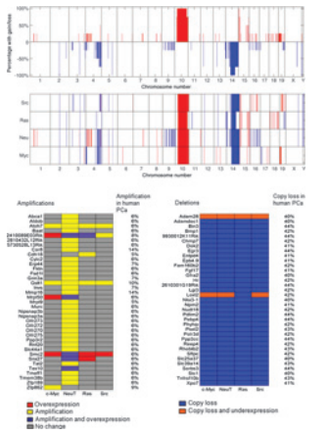
Cancer Research

Breaking Advances
Highlights from Recent Cancer Literature

Prostate Cancer Progression Signatures Identified



Metastatic prostate cancer (PCa) still remains an enigma, as the underlying molecular signature pathways are poorly defined. In the present study, Ju and colleagues attempted to identify the gene signature pathways of PCa using a murine model. To achieve this goal, a primary prostate epithelial cell line was established from FVB mice, followed by retroviral transduction with a single oncogene, c-Myc, Ha-Ras, v-Src, or NeuT (activating Erbb2 mutant). Each oncogene-transduced line displayed contact-independent growth and had a tumorigenic phenotype. Distinct genomic gain/loss and alterations of approximately 2,400 genes observed in these lines were similar to changes observed in human PCa when compared with a cohort of 205 human primary and metastatic PCa cases. Markedly increased lung metastasis was achieved with subcutaneous injection of the Ha-Ras, v-Src, and c-Myc expressing PCa cells into immunocompetent FVB mice. The authors further characterized the transformed cell lines for altered gene expression by microarray analysis. A total of 2,635 genes out of 22,115 genes were significantly altered in the oncogene overexpressing PCa cell lines. A considerable number of up and downregulated genes were shared among all 4 of the transformed cell lines. Notably, altered gene signatures observed in the Ha-Ras overexpressing cells were the most prevalent among all the lines. Remarkably, when the outcome of the gene signature between these murine PCa lines and a previously determined cohort (61 cases) of human high grade PCa was compared, an appreciable level of similarity on the alteration pattern was observed (c-Myc, 47%; NeuT, 53%; Ha-Ras, 71%; v-Src, 62%). Interestingly, the c-Myc-specific prostate gene signature of the murine PCa cells strongly resembled the gene signature of c-Myc-driven mouse mammary tumor or transformed fibroblasts. Kaplan Meier analysis was done to determine the predictability of c-Myc driven gene signatures of the murine PCa cells on recurrence-free survival compared with several defined human data sets. A panel of 14 most upregulated genes observed in the c-Myc signature bearing murine PCa cells was considered. Of note, a similar predictive outcome was seen in both the murine cell lines and human subjects. This study identifies novel oncogene-induced genomic signature pathways in PCa, which could be useful for biomarker and therapeutic design. Additionally, this genetic modification approach could prove useful for defining gene signatures for other types of cancer.

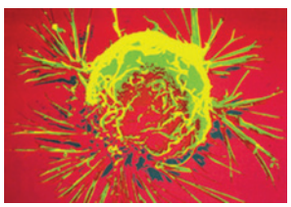
Ju X, Ertel A, Casimiro M, Yu Z, Meng H, McCue PA, et al. Novel oncogene induced metastatic prostate cancer cell lines define human prostate cancer progression signatures. Cancer Res; Published Online First November 30, 2012; doi:10.1158/0008-5472.CAN-12-2133.

Role of Inflammatory Cells in Modulating the Response to Chemotherapy

Chemotherapy was thought traditionally to simply target malignant cells. Over the past few years, experiments in murine cancer models showed that cells in the tumor microenvironment were also influenced by chemotherapy. 'Danger' signals generated by some chemotherapeutic drugs can stimulate the immune system to generate adaptive antitumor immune responses to novel tumor-associated antigens. This can be tempered by a 'wound-healing' damage response to chemotherapy with an influx of macrophages, endothelial progenitors, and fibroblasts that stimulates growth of remaining malignant cells. In a paper published in *Nature Medicine*, Bruchard and colleagues described another way in which the immune system may limit the activity of two common chemotherapy drugs, gemcitabine and 5-fluorouracil (5FU). In experiments in murine cancer models and on blood leukocytes derived from patients undergoing chemotherapy, Bruchard and colleagues found that gemcitabine and 5FU activated the NOD-like receptor family, pyrin domain containing-3 protein (NLRP3)-dependent caspase-1 activation complex (termed the inflammasome) in myeloid-derived suppressor cells, increasing their tumor-promoting activity. During chemotherapy, the myeloid-derived suppressor cells produced interleukin (IL)-1β that in turn induced secretion of IL-17 by CD4+ T cells, blunting the anticancer efficacy of the chemotherapy. Gemcitabine and 5FU had greater antitumor activity when a variety of solid tumor xenografts tumors were established in *Nlrp3*−/− mice or when established in wild-type mice treated subsequently with an IL-1 receptor antagonist. Inhibitors of the NLRP3 inflammasome or use of an IL-1 receptor antagonist may thus enhance the efficacy of 5FU or gemcitabine in patients.

Bruchard M, Mignot G, Derangère V, Chalmin F, Chevriaux A, Végran F, et al. Chemotherapy-triggered cathepsin B release in myeloid-derived suppressor cells activates the Nlrp3 inflammasome and promotes tumor growth. Nature Med AOP Dec 2, 2012.

Cell Organization Matters in Cancer



http://www.standup2cancer.org/article_archive/view/what_is_cancer
Solid cancers are very commonly derived from epithelial cells that display impaired cell polarity, which results in disruption of epithelial organization. The PAR complex, which comprises the adaptor protein PAR6 (*PARD6*) bound to atypical protein kinase C (aPKC) and the polarity protein PAR3 (*PARD3*), is a critical regulator of apical-basal polarity. However, the mechanisms by which this key determinant of epithelial polarity may contribute to tumor progression have yet to be unraveled. In this manuscript, McCaffrey and colleagues determined that loss of PAR3 activity triggers increased MMP9 expression through aPKC-mediated activation of the JAK/STAT signaling pathway, resulting in breast cancer invasion and metastasis. The authors transplanted PAR3-depleted primary mammary epithelial cells into syngeneic mice

and found that loss of PAR3 alone was insufficient for tumor formation. However, knockdown of PAR3 when combined with either overexpressing NOTCH or RAS (*NICD/shPAR3* and *RAS^{61L}/shPAR3*, respectively) led to striking reductions in latency and epithelial organization, as well as increases in tumor size and metastatic potential compared with NOTCH or RAS overexpression alone. Interestingly, despite their decreased tissue organization, NICD/shPAR3 and RAS^{61L}/shPAR3 tumors retained epithelial characteristics, including expression of the luminal epithelial marker cytokeratin 8 and the tight junction marker ZO1 (TJP1). Contrary to the conventional notion that cancer cells must lose all epithelial markers and gain mesenchymal characteristics (epithelial-mesenchymal transition) before migration to distal sites, these findings suggest that invasive cancer cells may exhibit both mesenchymal and epithelial properties. To complement their *in vivo* approach, the authors used three-dimensional Matrigel experiments and showed that NICD/shPAR3 and RAS^{61L}/shPAR3 mammary epithelial cells migrated through Matrigel much more efficiently than cells exhibiting a single oncogenic lesion. As migration and invasion requires the activity of adhesion-related genes such as metalloproteinases (MMP) that degrade the extracellular matrix (ECM), McCaffrey and colleagues sought to unravel the contribution of PAR3 expression towards MMP activity. Quantitative RT-PCR analysis revealed that loss of PAR3 cooperated with RAS or NOTCH oncogenic activity to alter the expression of MMPs and other adhesion-related genes. Furthermore, staining experiments conducted on cells grown on fibronectin-coated dishes show that NICD/shPAR3 cells detached from their plates due to degradation of the ECM. Intriguingly, these cells detached as spheroid colonies as opposed to single cells, which may be due to the presence of epithelial markers that promote intercellular adhesion (e.g., E-cadherin). Rescue experiments using shRNA-resistant PAR3 show that these adhesion defects can be attributed to loss of PAR3 function. Interestingly, a PAR3 mutant that was unable to bind and be phosphorylated by aPKC maintained the adhesion deficiency, and pharamacologic inhibition of aPKC activity restored cell adhesion. The authors shed further mechanistic insight into the role of PAR3 in tumor progression and metastasis by showing that silencing of PAR3 in NOTCH-overexpressing tumors increased the abundance of activated STAT3, activating MMP during tumorigenesis. NICD/shPAR3 cells treated with aPKC inhibitors reduced STAT3 to normal levels, indicating that STAT3 was downstream of aPKC activity. Furthermore, treatment of NICD/shPAR3 and RAS^{61L}/shPAR3 mammary epithelial cells with the STAT3 inhibitor Cucurbitacin-I decreased their invasive potential. JAK activity was also increased upon loss of PAR3 and a similar pharmacologic approach was used with the JAK inhibitor Pyridone 6 to show that STAT3 activation was downstream of JAK. Importantly, the authors extended their findings to human cancer samples. They found that levels of PAR3 and its associated mRNA encoded by *PARD3* were severely reduced in human breast invasive ductal and lobular carcinomas. Decreased expression of PAR3 also correlated with increased abundance of MMP9. Additionally, costaining experiments in a panel of human breast carcinomas revealed that samples with low PAR3 expression also displayed high levels of activated aPKC and STAT3. Overall, the authors' findings establish a novel signaling axis that is co-opted by cancer cells to promote invasion. They propose a model in which loss of PAR3 upon acquisition of an oncogenic lesion such as RAS leads to the mislocalization and aberrant activation of aPKC, which in turn activates JAK/STAT3 signaling, resulting in increased MMP expression and ECM degradation.

McCaffrey LM, Montalbano J, Mihai C, Macara IG. Loss of the Par3 polarity protein promotes breast tumorigenesis and metastasis. Cancer Cell 2012;22:601-14.

Reversible EMT and Cancer Metastasis

The epithelial-mesenchymal transition (EMT) has been postulated to play an important role in aggressiveness of epithelial malignancies. Acquisition of mesenchymal features, including cell motility and invasive capacity is an important feature of advanced carcinoma, particularly cancer metastasis. The exact role and relevance of EMT in the metastatic process nevertheless remains controversial, due in part to the fact that metastatic cancer tissues do not always display mesenchymal characteristics. In this manuscript, the authors clarify the role of EMT in cancer metastasis using a mouse model of spontaneous squamous cell carcinoma. They generate a mouse with skin-specific TWIST1 expression through application of the Tet-on system driven by the keratin 5 promoter. TWIST1 is known to induce EMT in a variety of systems, and they cleverly use this mouse to compare and contrast local induction of TWIST1 (via topical administration) with systemic induction of TWIST1 during the metastatic process via Tet administration in the drinking water. Local administration was considered "reversible EMT," because cells lost TWIST1 induction during metastasis. In contrast, systematic administration was termed "irreversible EMT," because TWIST1 induction would be operative even after tumor cells leave the primary site. The authors first show that TWIST1 induction was sufficient to promote invasive carcinoma in their mouse model. Interestingly, loss of TWIST1 expression proved important for metastasis, as the majority of mice with topical TWIST1 induction developed metastases, while only a minority of the mice with systemic TWIST1 induction developed metastases. Consistent with the importance of reversible EMT in metastasis, they showed that TWIST1 induction resulted in EMT (loss of E-cadherin; induction of vimentin) in primary tumors, with re-establishment of epithelial characteristics (MET) in the metastatic site. Realizing that carcinoma cells need to invade, intravasate the bloodstream, extravasate, and then colonize, the authors dissected where in the process reversibility of EMT was required for successful metastasis. They show that EMT promoted both intravasation and extravasation. However, loss of EMT was required for colonization of tumor cells in distant sites and was associated with increased tumor cell proliferation. Taken together, this elegant set of experiments shows that reversible EMT, caused by reversible induction of TWIST1in this model, is required to complete the metastatic process and that a level of plasticity is important in this process.

Tsai JH, Donaher JL, Murphy DA, Chau S, Yang J. Spatiotemporal regulation of epithelial-mesenchymal transition is essential for squamous cell carcinoma metastasis. Cancer Cell 2012;22: 725-36.

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.