Breaking Advances
Highlights from Recent Cancer Literature

Colitis and Colorectal Cancer

Most, if not all, cancers are characterized by tumor-elicited inflammation even though there is no evidence of preexisting inflammatory disease at the tumor site. One explanation is that inflammatory cytokines and chemokines are induced downstream of oncogenic mutations in the malignant cell. Grivennikov and colleagues offer an alternative explanation, at least for cancers that develop in epithelia exposed to commensal or pathogenic bacteria. Using data from human colorectal cancer biopsies and a relevant mouse model of the disease in which tumors develop in the distal colon (CPC-APC mice, as shown in image above), these authors discovered that early genetic lesions lead to defective barrier protein expression in the premalignant epithelial cells of adenomas. Microbial products are then able to infiltrate these adenomas, stimulating interleukin (IL)-23 production by tumor-associated macrophages (TAM), tumor-promoting Th17 responses, IL-6 production, and STAT3 activation in the cancer cells. Short-term depletion of intestinal microflora with a combination of broad-spectrum antibiotics, which reduced microbial counts by over 99.9%, inhibited IL-23 expression by TAMs, reduced IL-17A in the tumors, and decreased STAT3 activation in cancer cells. Prolonged antibiotic depletion of commensal microflora starting at weaning reduced tumor size and load in control mice but not in mice in which the IL-23 receptor (*Il23*$^{-}$/*Il23*$^{-}$) was deleted. Tumor development also coincided with elevated endotoxin in portal blood, and occasional bacteria were detected within colorectal tumors and proximal to tumor epithelial cells in early mouse lesions that resembled aberrant crypt foci and in early human adenomas. Mucus from goblet cells usually prevents bacteria from penetrating the colonic epithelial barrier, and there was an absence of mucus-producing cells in mouse and human colorectal tumors. Epithelial barriers also depend on tight and adherent junctions between the cells. A notable loss of polarized expression of the junctional proteins JAM-A (F11R) and JAM-B (JAM2) was reported in human colorectal tumors and claudins no longer localized to tight junctions. Importantly, early human adenomas showed defective mucin production and tight junction organization that coincided with elevated IL-23 and IL-17A. Collectively, these data led the authors to propose that early barrier loss and activation of IL-23/IL-17–driven tumor-elicited inflammation act additively and sequentially to genetically controlled events that govern development and progression of colorectal cancer. (*Image of mouse model of colonic adenoma–carcinoma progression from Hinoi et al., Cancer Res 2007;67:9721–30; courtesy of publisher.*)

Grivennikov SI, Wang K, Mucida D, Stewart CA, Schnabl B, Jauch D, et al. Adenoma-linked barrier defects and microbial products drive IL-23/IL-17-mediated tumour growth. Nature 2012 Oct 3. [ePub ahead of print].

Clinically Relevant Genomic Heterogeneity in Diffuse Large B-cell Lymphoma

Diffuse large B-cell lymphoma (DLBCL) shows a range of biologic alterations and clinical outcomes. Monti and colleagues used an integrated analysis to characterize this heterogeneity. To analyze DNA copy number alterations (CNA), the authors first used the Genomic Identification of Significant Targets in Cancer (GISTIC) algorithm as applied to copy number profiles on 180 samples of DLBCL. They then compared the CNAs in DLBCL with recurring CNAs in over 2,000 samples of nonhematologic cancers. Nine of 21 (43%) regions of copy gain and 10 of 26 (38%) regions of copy loss were identified as specific to DLBCL, including gains of 2p16.1 and 19q13.42. Furthermore, these DLBCL-selective CNAs were largely absent in non–germinal-center lymphoid malignancies. Using transcriptomic profiling data on the same samples, Monti and colleagues identified genes that were concordantly regulated according to DNA copy number alteration (i.e., higher expression when copy number was elevated). They called these "*cis*-acting genes." Pathway analysis showed that the most significantly enriched *cis*-acting gene sets were associated with p53 signaling, apoptosis, and cell-cycle regulation. CNAs of p53 pathway components all pointed in a similar direction—decreased p53 activity and reduced levels of p53 targets. Additional pathways that were perturbed were activation of antiapoptotic function, as well as cell-cycle regulation, including activation of E2F function. To further evaluate E2F function, they examined so-called "*trans*-acting genes" (defined as those genes outside the CNA with the most significant association between transcript abundance and the CNA). This analysis showed enrichment of genes with E2F promoter binding sites. The authors then assessed the patterns and combinations of alterations that occurred in individual tumors. When the 180 primary DLBCLs were clustered in the space of the CNAs that affect the p53 pathway as well as the cell cycle, 66% of the tumors had multiple alterations (which they called "complex"), whereas the remaining 34% of tumors lacked these lesions (they called these "clean"). Consistent with the overall findings, DLBCLs with complex CNA patterns also had significantly higher proliferation indices as determined by the Ki-67 labeling index. Furthermore within the subset of patients who received rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (the R-CHOP regimen), patients with a clean CNA profile had a 5-year survival rate of 100%, with only 62% for patients with complex tumors. Finally, the authors examined targeted therapeutics in cell lines and showed that CDK inhibitors could be effective in tumors with genetically driven cell-cycle alterations, a finding that is especially relevant because the tumors with the most aggressive clinical outcome had perturbations in these pathways. Overall, these findings provide clinically relevant genetic and genomic insights into the biology and potential therapeutics for these tumors.

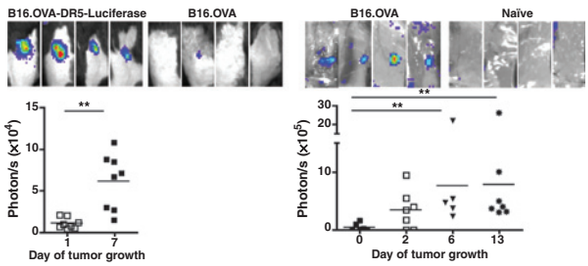
Monti S, Chapuy B, Takeyama K, Rodig SJ, Hao Y, Yeda KT, et al. Integrative analysis reveals an outcome-associated and targetable pattern of p53 and cell cycle deregulation in diffuse large B cell lymphoma. Cancer Cell 2012;22:359–72.

A "Twist" on p53 Inactivation in Cancer

TWIST1 and TWIST2 are important transcription factors in tissue specification including mesoderm development, with expression of both factors reduced and restricted to quiescent mesodermal stem cells in adults. Twist proteins are associated with cancer development, in which they contribute to metastatic progression through induction of the epithelial-to-mesenchymal transition and/or stem cell renewal. However, because Twist proteins regulate several different regulatory pathways, it has been difficult to determine the exact mechanisms by which they drive cancer development. Piccinin and colleagues investigated the role of Twist proteins in sarcomas, as these tumors are mesenchymal in origin and should provide an ideal system to study Twists in tumorigenesis. Specifically, they studied the relationship between Twist proteins in sarcomas that still retain wild-type p53 (*TP53*) but display defects in the function of this tumor suppressor. The authors showed that the *TWIST1* gene was amplified in a large panel of sarcoma tissues, which consequently led to overexpression of TWIST1 as determined by immunohistochemistry. The authors then asked whether TWIST1 overexpression interfered with p53 function in a panel of tumors that retained wild-type p53. They found that, in this subset of liposarcomas, TWIST1 overexpression negatively correlated with levels of the p53 ubiquitin ligase MDM2. Their findings were functionally validated in a mouse xenograft study, demonstrating that injection of *TWIST*-transduced human fibroblasts led to tumor development, at least in part by antagonizing p53 function. The authors used an RNA interference approach to determine that downregulation of TWIST1 stabilized p53 expression, and this effect was rescued upon treatment with the proteasome inhibitor MG132, suggesting a role for TWIST1 in proteasomal degradation of p53. To corroborate their data linking TWIST1 and the p53 response, the authors also showed that knockdown of TWIST1 in sarcoma cells led to induction of senescence-associated β-galactosidase activity and the expression of p53 target genes. Piccinin and colleagues shed mechanistic light into the role of TWIST1 in modulating p53 degradation by undertaking a mutagenesis approach, which demonstrated that TWIST1 does not require binding between its basic domain and p300/CBP to antagonize p53 expression. They show that the p53 C-terminal regulatory domain is associated with a Twist regulatory region known as the Twist box. This interaction impairs the phosphorylation of site Ser392 on p53, which is critical for the activity and stabilization of this protein. The authors further provide evidence that ultimately TWIST induces a more stable interaction between p53 and MDM2, which leads to direct degradation of the p53 protein. These findings provide a new layer of complexity in tumors retaining wild-type p53 that is inactivated not at the genetic level but through an unexpected association with transcription factors such as Twist. Overall, these studies may provide a new rationale for novel therapeutic approaches that could disrupt the Twist–p53 complex in cancer.

Picinnin S, Tonin E, Sessa S, Demontis S, Rossi S, Pecciarini L, et al. A "Twist box" code of p53 inactivation: Twist box:p53 interaction promotes p53 degradation. Cancer Cell 2012;22:404–15.

Novel Role of Retinoic Acid in T-cell–Mediated Antitumor Immunity



Various vitamin A derivatives (retinoids, all-*trans* retinoic acid) have been extensively investigated in the context of cancer therapy due to their ability to induce cellular differentiation and arrest proliferation. However, their role in potentially facilitating host defenses against cancer and specifically augmenting antitumor immunity of T cells has not been investigated in detail. In this study, Guo and colleagues examined the role of retinoic acid (RA) on various immune cell functions using C57BL/6-B16.OVA and CD4/OTI transgenic mouse model tumor systems. Using an RA-specific B16.OVA-DR5-Luciferase reporter system, they observed an increase in RA signaling induced into the tumor microenvironment (TME). Remarkably, the increased RA was produced in the TME by the tumor-infiltrating dendritic cells and macrophages and not by the tumor cells. They further discovered that the tumor-specific $CD8^+$ T cells obtained from the tumor bed, lymph nodes, or spleen were the responsive populations of cells displaying enhanced RA signaling. These $CD8^+$ T cells with increased RA signaling exhibited significant antitumor activity and notably, blockade of RA signaling in these cells significantly reduced their antitumor efficacy. Guo and colleagues also showed that sustained RA signaling was necessary for *in vivo* clonal expansion of these $CD8^+$ T cells, suggesting that RA is essential for the survival of tumor-reactive $CD8^+$ T cells within the TME. This study provides new insights into the potential role and use of retinoids in modulating tumor immunotherapy. (*Image from cited article courtesy of publisher.*)

Guo Y, Pino-Lagos K, Ahonen CA, Bennett KA, Wang J, Napoli JL, et al. A retinoic acid-rich tumor microenvironment provides clonal survival cues for tumor-specific $CD8^+$ T cells. Cancer Res;1727.2012; Published OnlineFirst August 17, 2012; doi:10.1158/0008-5472.CAN-12-1727.

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.

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Cancer Res 2012;72:5433-5434.