Breaking Advances
Highlights from Recent Cancer Literature

Exhausted!

Significant lymphocyte infiltration is common to most solid tumors; however, these effector cells of the adaptive immune system typically fail to eradicate malignant tumors. One reason for this occurrence, as outlined in a recent article by Baitsch and colleagues, is that lymphocytes suffer from "exhaustion." During acute infections, $CD8^+$ T lymphocytes are activated and expand rapidly to attack and kill infected cells. As infectious load declines, a majority of $CD8^+$ T cells undergo aptosis, whereas memory T cells remain for many years. However, if the immune system fails to eliminate a pathogen, virus can spread and cause progressive tissue damage in the presence of a considerable number of $CD8^+$ T cells. In this scenario, T cells are functionally impaired—expressing a number of inhibitory receptors and altered molecular pathways. This impaired state has been termed exhaustion. Cancers are, of course, also chronic diseases with functional deficiencies in immune pathways, but until recently it was not clear whether T-cell exhaustion was a contributing factor. To investigate this possibility, Baitsch and colleagues chose melanoma because patients with this disease have substantial numbers of long-term persisting memory effector T cells. Using gene expression profiling of small numbers (as few as 1,000 cells) of highly purified T cells from patients vaccinated with CpG and the Melan-A/MART-1 peptide, the authors found functional tumor-specific effector T cells in peripheral blood. However, the same $CD8^+$ T cells isolated from melanoma metastases expressed a large variety of genes associated with T-cell exhaustion with molecular alterations suggesting enhanced activation and apoptosis, difficulties in maintaining DNA integrity and in sustaining cell cycling. The effector cells in the circulation had become exhausted in the tumor microenvironment, likely owing to production of ligands by malignant cells that interacted with and bound inhibitory receptors on T cells. This research opens up the possibility of targeting inhibitory receptors and other molecular pathways that characterize exhausted T cells—hence energizing the adaptive immune system to fight malignancy. *(Image courtesy of Arin Atema.)*

Baitsch L, Baumgaertner P, Devêvre E, Raghav SK, Legat A, Barba L, et al. Exhaustion of tumor-specific $CD8^+$ T cells in metastases from melanoma patients. J Clin Invest 2011;121: 2350–60.

Autophagy, Metabolism, Amino Acid, Deprivation, and Malignant Melanoma

Malignant melanoma numbers among the most lethal of cancers. In analyzing amino acids necessary for survival of melanoma, Sheen and colleagues showed that deprivation for leucine uniquely led to caspase-dependent cell death. In normal cells, leucine deprivation inhibits mTOR, which in turn activates autophagy as a survival pathway. In melanoma cells, activated Ras and mitogen-activated protein (MAP) kinase signaling disabled induction of autophagy in response to leucine deprivation, leading to cell death. In mice carrying melanoma xenografts, dietary leucine deprivation cooperated with the autophagy inhibitor chloroquine to induce apoptosis. But how does activated Ras signaling lead to defective autophagy? In an unrelated study, Settembre and colleagues showed that activation of MAP kinase signaling promoted cytoplasmic localization of the transcription factor TFEB. Inhibition of MAP kinase signaling in HeLa cells led to nuclear localization of TFEB and to a transcriptional program that activated both autophagy and lysosomal biogenesis. Thus, activation of MAP kinase signaling in cancer leads directly to defective autophagy, offering opportunities for synthetic lethal combination screens and therapies.

Sheen JH, Zoncu R, Kim D, Sabatini DM. Defective regulation of autophagy upon leucine deprivation reveals a targetable liability of human melanoma cells in vitro and in vivo. Cancer Cell 2011;19:613–28.

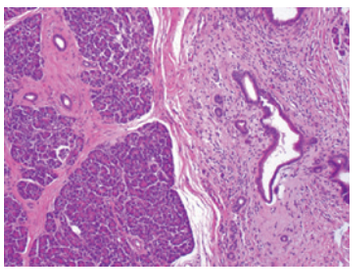
Settembre C, Di Malta C, Polito VA, Arencibia MG, Vetrini F, Erdin S, et al. TFEB links autophagy to lysosomal biogenesis. Science 2011 May 26. [Epub ahead of print].

A Manageable List of p53 Targets Critical to Tumor Suppression

The tumor suppressor p53 is among the most frequently mutated and highly studied of cancer genes. To clarify p53 targets critical to tumor suppression, Brady and colleagues generated knock-in mice carrying mutations in either or both of the 2 transactivation domains of p53. Disabling of the first transactivation domain led to mice that were compromised for transactivation of most known p53 targets and defective in both G_1 arrest and apoptosis in response to DNA damage. Surprisingly, however, mouse embryonic fibroblasts (MEF) deficient in either (but not both) transactivation domains were capable of undergoing senescence in response to activated Ras, and the corresponding mice were completely resistant to lung cancer induced by activated Kras. By comparing targets from wild-type and transactivation domain–defective MEFs and integrating gene expression studies from cancer, the investigators were able to identify 14 mostly new targets. These genes play roles in signaling, cytoskeletal studies, DNA repair, and lysosomal biology. Thus, only a small subset of p53 target genes may be important for tumor suppression, and this subset can be distinguished from those p53 targets important for DNA repair.

Brady CA, Jiang D, Mello SS, Johnson TM, Jarvis LA, Kozak MM, et al. Distinct p53 transcriptional programs dictate acute DNA-damage responses and tumor suppression. Cell 2011;145: 571–83.

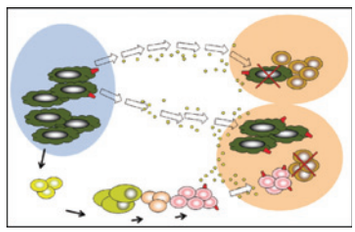
Antimalarial Drug Slows Pancreatic Cancer Growth in Mouse Model



Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest forms of cancer and a disease in which patients have extremely limited therapeutic options. Recent work by Yang and colleagues indicates that the antimalarial compound hydroxychloroquine leads to robust tumor regression and prolongs survival in preclinical models of pancreatic cancer. Hydroxychloroquine and its derivatives have gained attention in cancer treatment due to their ability to inhibit the late stages of autophagy, a tightly regulated lysosomal degradation and recycling process used by cancer cells to survive starvation and stress. In contrast to normal cells, PDAC cells exhibit an uncharacteristically high level of basal autophagy even in the absence of an overt stress or stimulus. *In vitro* functional studies indicate that PDAC cells are highly dependent on this increased level of autophagy to proliferate as well as sustain oxidative metabolism. Accordingly, upon autophagy inhibition using hydroxychloroquine in xenograft models, all untreated mice died of their cancer within 140 days, whereas only 1 of 8 treated mice died by 180 days. Importantly, similar results are obtained in a genetic PDAC model driven by the *K-Ras* oncogene; upon monotherapy with hydroxychloroquine, median survival increased by 27 days compared with survival in untreated controls. The relative decrease in efficacy observed in the genetic model may be partly due to reduced penetration of the drug into a highly fibrous stroma (a situation that closely resembles human tumors). In light of the limited success of numerous other agents in this genetic model, these results with hydroxychloroquine are highly encouraging. Furthermore, because hydroxychloroquine and its derivatives are potent inhibitors of autophagy and have a long history of use in humans against malaria and in diseases such as rheumatoid arthritis, these results are immediately translatable for use in the treatment of patients with pancreatic cancer. *(Image courtesy of L. Coussens, University of California, San Francisco.)*

Yang S, Wang X, Contino G, Liesa M, Sahin E, Ying H, et al. Pancreatic cancers require autophagy for tumor growth. Genes Dev 2011;25:717–29.

Regulatory B Cells Promote Mammary Metastasis



Solid tumors are commonly infiltrated by diverse assemblages of immune cells that regulate discrete parameters of multistage neoplastic progression. In murine models of breast cancer, T-helper 2–effector $CD4^+$ T cells, $CD4^+$ T regulatory cells, and macrophages have previously been implicated as critical effectors of the penultimate stages of carcinogenesis, in that each cell type contributes to cell-intrinsic programs fostering metastasis. Olkhanud and colleagues now provide evidence that a distinct population of CD25-expressing $B220^+CD19^+$ B cells in peripheral blood and secondary lymphoid organs also contributes to metastasis in mammary carcinoma. Tumor-evoked B-regulatory cells (tBreg) resemble mature B2 cells ($CD19^+CD25^{hi}CD69^{hi}$) that express constitutively active Stat3 and B7-$HI^{hi}CD81^{hi}CD86^{hi}CD62L^{lo}IgM^{int}$. Using the 4T1 murine model of mammary carcinogenesis that readily metastasizes to lung, these investigators found that a major role for tBregs was to induce TGF-β–dependent conversion of resting $CD4^+$ T cells into $FoxP3^+$ T-regulatory cells, which in turn inactivate natural killer cells and subsequent antitumor defenses, thereby supporting metastatic dissemination. The significance of this program was also evaluated in melanoma; B16 melanoma was unable to progress in the absence of mature B cells, a phenotype that was reinstated by adoptive transfer of congenic tBregs. Importantly, tBregs do not appear limited in significance to murine tumors, because $CD19^+$ B cells isolated from the peripheral blood of cancer-free patients treated with conditioned medium of ovarian or colon carcinoma cells resulted in upregulation of CD25, and suppressed proliferation of $CD3^+$ T cells stimulated by anti-CD3/CD29 monoclonal antibodies plus interleukin-2. These data extend observations made previously in mouse models of squamous and prostatic carcinogenesis revealing protumor bioactivity of B cells and thus implying that therapeutics, such as rituximab, that deplete B cells may have efficacy in combating some solid tumors. *(Image from cited article courtesy of publisher.)*

Olkhanud PB, Damdinsuren B, Bodogai M, Gress RE, Sen R, Wejksza K, et al. Tumor-evoked regulatory B cells promote breast cancer metastasis by converting resting $CD4^+$ T cells to T-regulatory cells. Cancer Res 2011;71:3505–15.

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.