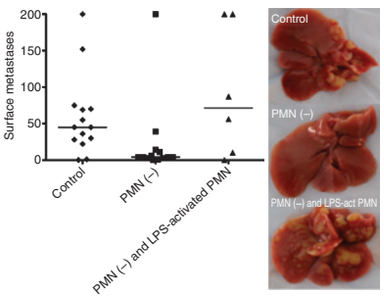
Breaking Advances
Highlights from Recent Cancer Literature

Neutrophil Homing Facilitates Metastasis through Interaction with Circulating Tumor Cells



It is well established that circulating neutrophils are frequently associated with distant metastases and poor patient outcome in several epithelial malignancies. However, whether this association is causative in initiating and establishing a metastatic niche in concert with circulating tumor cells (CTC) is largely unknown. Using an intrasplenic model of liver metastasis, Spicer and colleagues show that selective depletion of neutrophils markedly reduces metastatic lung tumor foci in the liver. The decreased number of metastatic foci was largely due to the reduced adherence capacities of the lung cancer cells in the liver. This phenotype was reversible by the reinfusion of activated neutrophils into the circulation. The neutrophils directly interacted with the circulating lung cancer cells and promoted their establishment in the liver via attachment with endothelial cells. Tumor cell adhesion was partially reversed by blocking Mac-1 (Itgam/Cd11b) and intercellular adhesion molecule (ICAM), the key molecules involved in neutrophil-mediated metastatic induction. In addition, decreased tumor cell adhesion was also observed in Mac-1 (*Itgam*) knockout mice. Monitoring neutrophil homing in the tumor microenvironment and deciphering the cross-talk(s) of neutrophils with CTCs could be useful for managing metastatic tumor spread.

Spicer JD, McDonald B, Cools-Lartigue JJ, Chow SC, Giannias B, Kubes P, et al. Neutrophils promote liver metastasis via Mac-1 mediated interactions with circulating tumor cells. Cancer Res 2012;72:3919–27.

mRNA Stability Plays a Critical Role in Myc-Induced Lymphomagenesis

Alterations in posttranscriptional control of mRNAs are becoming recognized as a critical node of gene expression underlying tumor formation. Indeed, a number of oncogenic pathways have been shown to regulate the levels and/or activity of several components involved in this specific level of gene expression. In this context, many short-lived transcripts harbor adenylate-uridylate (AU)-rich mRNA-destabilizing elements (ARE) in their 3′UTRs, which promote mRNA decay. A cohort of RNA-binding proteins known as AU-binding proteins (AUBP) regulates the metabolism of ARE-containing mRNAs. Importantly, a causal role has been suggested for AUBP activity toward mRNA stability in cancer through poorly understood mechanisms. Rounbehler and colleagues use a convergence of *in vitro*, *ex vivo*, and *in vivo* approaches to show that the protein tristetraprolin (TTP/ZFP36) acts as a tumor suppressor by destabilizing ARE-containing mRNAs. The authors performed expression profiling analyses of B cells harvested from a mouse model of cancer in which Myc is overexpressed in the B-cell compartment (Eμ-Myc) and determined that a large percentage (∼20%) of genes that have been identified in an ARE database (ARED) were aberrantly expressed in precancerous and transformed Eμ-*Myc* B cells. These findings were extended to a dataset of primary human Burkitt lymphoma samples, which harbor the *MYC/Ig* translocation, as well as other tumor types such as neuroblastoma. In addition, the authors showed that premalignant and cancerous Eμ-*Myc* B cells and lymphomas displayed elevations in the expression of mRNA-stabilizing AUBPs, including HuR (Elavl1), Auf1 (Hnrnpd), Auf2 (Hnrnpab), and Ncl, whereas AUBPs such as Ttp and Tis11b (Zfp36l1), which bind to mRNAs for degradation, were decreased at both the transcript and protein level. Strikingly, downregulation in the expression of TTP and/or the closely related TIS11D (ZFP36L2) was also found in *MYC*-expressing breast, colorectal, and metastatic prostate cancer as well as in neuroblastomas harboring Myc amplifications and *MYC*-overexpressing colorectal cancers. As such, *TTP* and *MYC* activity may be coordinately regulated during cancer development. This hypothesis is in part supported by the authors' findings that *TTP* and *TIS11B* mRNA levels were elevated in B cells deprived of the growth factor interleukin (IL)-7, which is known to upregulate Myc activity, and introduction of this Myc-associated mitogenic signal led to reductions in the transcript levels of TTP and TIS11b. The authors showed that the mRNA levels of *TTP* and *TIS11B* decrease upon Myc activation in P493-6 lymphoblastoid B cells harboring a tetracycline-inducible *Myc* gene, and chromatin immunoprecipitation experiments revealed that Myc binds to the initiator (Inr) elements of *TTP* and *TIS11B* genes to repress their transcription.

The authors used an *in vivo* approach to assess whether Myc-mediated transcriptional repression of AUBPs contributes to lymphomagenesis. They generated novel mouse models displaying elevated expression of specific mRNA-destabilizing AUBPs, including Ttp1, Ttp2, or Tis11b, which were subsequently bred to Eμ-*Myc* mice to produce single- and double-transgenic offspring. Strikingly, mice overexpressing both *Myc* and *Ttp1*/*Ttp2*(Eμ-*Myc*;Eμ-*Ttp1* and Eμ-*Myc*;Eμ-*Ttp2*, respectively) exhibited extended life spans compared with Eμ-*Myc* mice, whereas *Tis11b* transgenic mice did not have any changes in survival. This finding suggests that TTP is the key AUBP that inhibits *Myc*-induced lymphomagenesis. The authors determined that the increased life span of Eμ-*Myc*;Eμ-*Ttp* mice was due to a prolonged premalignant state in this mouse model as opposed to any direct changes in *Myc* expression levels. Additionally, this extended premalignant state is caused by Ttp disabling *Myc*-mediated proliferation. To determine whether Ttp suppression is necessary for tumor maintenance, the authors carried out GFP expression analyses using *Nude* mice injected with Eμ-*Myc* lymphomas infected with *GFP*-only versus Ttp-*GFP* retroviruses. Although *Ttp*-expressing lymphoma cells made up nearly half of the lymphomas injected (40% compared with 54% *GFP*-only cells), the percentage of *Ttp*-expressing cells in lymph node and peripheral blood samples collected at tumor development was dramatically reduced, indicating a strong selection against *TTP* expression in Eμ-*Myc* lymphomas. Additionally, *Nude* mice injected with TTP-expressing lymphoma cells developed tumors later than mice injected with

GFP-only lymphoma cells, supporting the idea that suppressing TTP activity increases tumorigenic potential. The authors used expression profiling to provide insight into the mechanisms by which Myc represses *TTP* expression during tumorigenesis. A large subset of genes differentially expressed between Eμ-*Myc* and Eμ-*Myc*;Eμ-*Ttp* premalignant B cells harbor AU-rich elements and may represent direct targets of TTP. Importantly, many of these genes have roles in cancer: *Cyclin D*1 (cell cycle and proliferation) and *Fstl1* (proinflammatory cytokine) were downregulated in Eμ-*Myc*;Eμ-*Ttp* B cells, whereas *Gabarapl1* (autophagy) and *Uaca* (apoptosis) were increased. Additionally, the expression of genes downregulated in Eμ-*Myc* cells, including *Tes* (putative tumor suppressor) and *Tns3* (metastasis repressor) was restored to normal levels in B cells overexpressing both Myc and Ttp. Significantly, the TTP and ARE-containing mRNA regulatory axis is altered in malignant lymphomas. This new circuitry between MYC and TTP activity underscores the exquisite specificity of mRNA stability in cancer etiology. These novel studies are unraveling important new mechanisms by which perturbations in gene expression at the posttranscriptional level underlie distinct cellular processes during tumorigenesis. This work by Rounbehler and colleagues lays the foundation for the design of new therapeutic compounds. For example, drugs that specifically reactivate TTP expression may be considered as novel therapies for *MYC*-mediated cancers.

Rounbehler RJ, Fallahi M, Yang C, Steeves MA, Li W, Doherty JR, Tristetraprolin impairs MYC-induced lymphoma and abolishes the malignant state. Cell 2012;150:563–74.

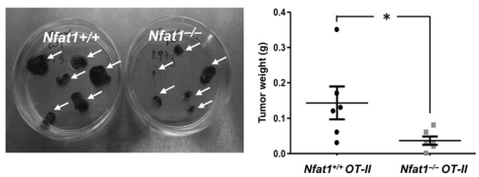
Stromal WNT Signaling Drives Chemoresistance

Many cancer drugs are initially successful, but relapsed disease is typically resistant to treatment. Although some aspects of this resistance are caused by genetic or epigenetic changes in the malignant cells themselves, it is increasingly clear that cancer therapy also has an impact on other cells in the tumor microenvironment. Sun and colleagues examined prostate cancer biopsies collected before and after neoadjuvant chemotherapy. Using genome-wide analyses of transcriptional responses, they identified a spectrum of secreted proteins derived from the tumor microenvironment that included the Wnt family member WNT16B. Having confirmed these findings in biopsies from breast cancer and ovarian cancer patients, they performed further experiments that showed that chemotherapy induced DNA damage in fibroblasts and smooth muscle cells of the tumor microenvironment, which induced *WNT16B* expression via the inflammatory transcription factor NF-κB. In a paracrine manner, WNT16B activated the canonical Wnt program in tumor cells, attenuating the effects of cytotoxic chemotherapy *in vivo* and promoting tumor cell survival and disease progression.

Several important conclusions can be drawn from this work. First, the outcomes of chemotherapy depend on the innate damage response capabilities of the tumor microenvironment; second, although some malignant cells become resistant to chemotherapy, resistance can come from the tumor microenvironment; third, the composition of the microenvironment damage response indicates the potential to promote resistance to pathway-targeted agents such as epidermal growth factor inhibitors; and fourth, specific microenvironment proteins that promote therapy resistance are attractive targets for augmenting responses to genotoxic drugs. This study focused on fibroblasts in the tumor microenvironment. Recently published articles have shown that chemotherapy also stimulates an influx of macrophages that repair tissue damage, protect tumor cells from further destruction, and aid relapse. However, the response of the tumor microenvironment to treatment is not always "bad." On the good side, some cytotoxic therapies may stimulate a long-lasting immune response to the malignant cells, uncovering new tumor antigens that stimulate the immune system as the malignant cells are destroyed.

Sun Y, Campisi J, Higano C, Beer TM, Porter P0, Coleman I, et al. Treatment-induced damage to the tumor microenvironment promotes prostate cancer therapy resistance through WNT16B. Nat Med 2012 Aug 5 [Epub ahead of print].

T-cell Anergy Inducer Identified



Immune evasion by tumor cells represents one of the critical mechanisms associated with tumor progression. Clonal anergy and adaptive tolerance are major causes of T-cell hyporesponsiveness; however, the ways in which they promote their pathophysiologic effects in the tumor microenvironment require further clarification. In this context, identification of the key molecules and pathways associated with immune evasion and clarification of their precise roles in mediating cancer development and progression are of paramount importance. In the present study, Abe and colleagues have examined the molecular mechanisms governing helper $CD4^+$ T-cell anergy with the use of a soluble peptide-induced model of B16 melanoma expressing OVA antigen (B16-OVA). In this model system, tumor-specific $CD4^+$ T cells were found to be hyporesponsive, producing less IL-2 and triggering upregulation of several key anergy-associated genes, including E3 ubiquitin ligases *Grail (Rnf12)*, *Cblb*, and *Itch* and transcription factors *Egr2*, *Grg4 (Tle4)*, and *Casp3*. The investigators also found that $CD4^+$ T cells obtained from *Nfat1*$^{-/-}$ (*Nfatc2*$^{-/-}$) mice were resistant to soluble peptide-induced anergy *in vivo*. Further studies documented that only *Nfat1*$^{-/-}$ mouse-derived $CD4^+$ T cells are resistant to anergic induction and produced Th1 cytokines. Moreover, this population of T cells markedly delayed and reduced tumor growth *in vivo* by eliciting an effective antitumor response. NFAT1 appears to be a key factor in mediating T-cell anergy and thus could be an attractive therapeutic target for enhancing antitumor response. (*Image from cited article courtesy of publisher*).

Abe BT, Shin DS, Mocholi E, Macian F. NFAT1 supports tumor-induced anergy of $CD4^+$ T cells. Cancer Res 2012;72:4642–51.

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;72:4609-4610.