Breaking Advances
Highlights from Recent Cancer Literature

Combination Immunotherapy for Melanoma

Most cancer immunotherapy trials involve adoptive transfer of activated effector cells, immunization against relevant antigens, or administration of nonspecific immune-stimulatory agents. In the past decade, however, an approach using antibodies that block inhibitory T-cell checkpoints has resulted in substantial clinical antitumor activity, especially in melanoma, and renal and lung cancers. In patients with metastatic melanoma, ipilimumab [an antibody against cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4)] prolongs overall survival, and nivolumab [an antibody against the programmed death 1 (PD-1) receptor (PDCD1)] induced durable tumor regression in some patients. Research in mouse models revealed that immunologic checkpoints are nonredundant and can inhibit T-cell activation, proliferation, and effector function within lymph nodes or the tumor microenvironment. Moreover, CTLA-4 and PD-1 play complementary roles in regulating adaptive immunity. Whereas PD-1 contributes to T-cell exhaustion in peripheral tissues, CTLA-4 inhibits earlier points in T-cell activation. Using the transplantable B16 melanoma model, the Allison laboratory previously showed that combined blockade of PD-1 and CTLA-4 achieved more pronounced antitumor activity than blockade of either pathway alone. These observations led Wolchok and colleagues to investigate whether blockade of CTLA-4 and PD-1, given concurrently or sequentially, could cooperate in patients with advanced melanoma. In the concurrent treatment group, overall evidence of clinical activity was observed in 65% of 53 patients, as compared to 43% of 33 patients in the sequential group. Some patients who did not respond to previous treatment with ipilimumab did respond to subsequent nivolumab. The results from this small phase I trial suggest that nivolumab and ipilimumab can be administered concurrently with a manageable safety profile. More rapid and deeper clinical tumor responses were observed in patients treated with the combination therapy, as compared with previous experience with either agent alone. Responses were durable, although longer follow-up is needed, and effects on overall survival remain to be defined. The authors concluded that their data support a randomized trial to compare nivolumab alone, ipilimumab alone, and concurrent treatment in patients with advanced melanoma.

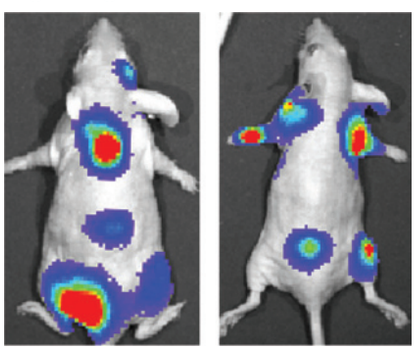
Wolchok JD, Kluger H, Callahan MK, Postow MA, Rizvi NA, Lesokhin AM, et al. Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med 2013;369:122–33.

Putting a Stop to Tumor Cell Migration by Inhibiting an Extracellular Disulfide Catalyst

Tumor cell invasion is a major obstacle to effective tumor therapy for many cancers. The substrate for cell migration, the extracellular matrix (ECM), plays a critical role in regulating cell movements. In the present article, Ilani and colleagues identify the function of a novel enzyme that catalyzes *de novo* disulfide formation in the extracellular environment. Furthermore, they demonstrate that this modification is essential to permit laminin incorporation into the ECM, integrin-mediated cell adhesion, and migration of tumor cells. Disulfide bond formation is fundamental to protein folding and assembly and it typically occurs in the endoplasmic reticulum (ER). However, quiescin sulfhydryl oxidase 1 (QSOX1) is a disulfide catalyst present in the secretory pathway and in cell secretions. Although QSOX1 was identified over 30 years ago and it is upregulated in many human cancers, its physiological function was previously unexplored. By examining several cell lines the authors determine that QSOX1 is localized to the Golgi, is present and enzymatically active in the conditioned media of confluent fibroblast cultures, and, in response to knockdown of QSOX1, cells lose their adherence to a monolayer. Based on the profound but reversible decrease in cell adherence, and an increase in unpaired thiols in the ECM, the authors examined the dependence of ECM proteins on QSOX1. Interestingly, whereas laminin trimer formation was not affected by QSOX1, lack of enzymatically active QSOX1 resulted in a severe defect in laminin incorporation into the ECM. Blocking QSOX1 activity led to a profound decrease in migration of lung and pancreatic cancer cells across a preformed fibroblast monolayer, suggesting that modulation of QSOX1 enzymatic activity could potentially alter ECM assembly and ultimately tumor cell invasion. As a test of this concept, the authors developed a function-blocking monoclonal antibody against QSOX1 and demonstrate that the antibody resulted in decreased fibroblast and epithelial cell adhesion, and decreased epithelial cell migration across a fibroblast monolayer. Future studies modulating QSOX1 enzymatic activity will determine whether this might be a promising therapeutic target in cancer.

Ilani T, Alon A, Grossman I, Horowitz B, Kartvelishvily E, Cohen SR, et al. A secreted disulfide catalyst controls extracellular matrix composition and function. Science 2013;341:74–6.

Therapeutic Targeting of the EGFR-MET Signaling Axis in NSCLC



In non–small cell lung carcinoma (NSCLC), alterations of EGFR and MET play key roles in therapeutic resistance. However, the molecular mechanism of interaction between these molecules is poorly defined. In the present study, Breindel and colleagues examined this interaction using a 32D mouse myeloid cell model system lacking EGFR, ERBB2/3/4, and MET and lacking expression of appropriate ligands EGF, TGF-α, AREG, BTC, NRG1, and HGF. The 32D cells engineered to express EGFR or MET only were activated in a ligand-specific manner, and EGFR signaling led to the upregulation of MET without the involvement of other ERBB family of receptors. EGFR-mediated activation of MET was due to stabilization of MET protein expression and predominant MAP kinase signaling. Both EGFR and MET kinases were found to be necessary for EGF-mediated activation of MET, and both wild-type and activated (gain of function mutant) EGFR triggered MET phosphorylation. Interestingly, ligand activated ERBB3 could also induce MET phosphorylation when co-expressed with EGFR. Of

note, EGFR-MET crosstalk at multiple levels was evident in many NSCLC cell lines, suggesting relevance of the 32D model system. Furthermore, a definitive contribution of EGFR-MET in inducing invasion and brain metastasis was demonstrated *in vitro* and *in vivo* using PC9/PC9-BrM3 cells. This study established a crucial role of EGFR-MET interaction through MAP kinase signaling in NSCLC progression, and thus the interaction between these receptor tyrosine kinases could be an attractive target for therapeutic development. (*Image from cited article courtesy of publisher.*)

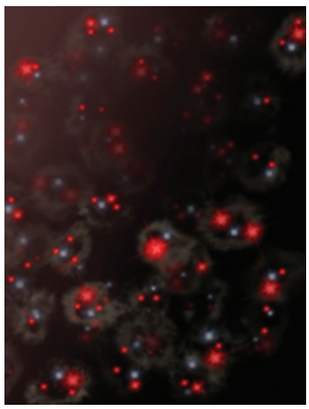
Breindel JL, Haskins JW, Cowell EP, Zhao M, Nguyen DX, Stern DF. EGF receptor activates MET through MAP kinases to enhance non–small cell lung carcinoma invasion and brain metastasis. Cancer Res; Published OnlineFirst June 21, 2013; doi:10.1158/0008-5472.CAN-12-3775.

Mitochondrial Resident Pyruvate Dehydrogenase Governs Oncogene-Induced Senescence

Oncogene-induced senescence (OIS) plays a pivotal role in tumorigenesis. However, the metabolic programming events and the altered molecular niche of the senescent cells are not well defined. In this study, Kaplon and colleagues identified a novel role of mitochondrial gatekeeper pyruvate dehydrogenase (PDH, PDP1) in regulating OIS. The authors utilized the $BRAF^{V600E}$ mutation as an OIS inducer in human diploid fibroblast (HDF) cells, and observed increased rates of oxygen consumption, pyruvate oxidation, and redox stress—all indicative of an altered metabolic program compared to the normally cycling cells. Mechanistically, PDH was identified as the key component of metabolic reprogramming in the OIS cells, with a deregulated PDK1-PDP2-PDH signaling axis. In the OIS cells, two opposite events, including downregulation of the PDH-inhibitory enzyme PDK1, and induction of the phosphatase PDP2 (which activates PDH), were evident, which both normalized after abrogation of OIS in response to depletion of the transcription factor C/EBP-β (CEBPB). Notably, other PDK and PDP isozymes were not affected in the manner PDK1/PDP2 were altered. Further, depletion of PDP2 remarkably resulted in the suppression of PDH activity and diminished $BRAF^{V600E}$-induced arrest. On the other hand, overexpression of PDK1 rescued the decrease in PDH phosphorylation and suppressed the increase in PDH activity in OIS cells. Concomitantly, PDK1 expression also reversed the increase in TCA cycle activity and prevented the rise of PDH function in the OIS cells. In parallel studies, depletion of PDK1 negatively affected melanoma growth initiation and progression both *in vitro* and *in vivo*. Under an *in vivo* condition and DOX-inducible setting, cells depleted for PDK1 not only failed to develop viable tumors but also showed almost complete regression of pre-established tumors. This study identified a crucial signaling axis of PDK1-PDP2-PDH in regulating OIS, and identified PDK1 as a potential and clinically relevant therapeutic target for treating melanoma patients bearing BRAF mutations.

Kaplon J, Zheng L, Meissl K, Chaneton B, Selivanov VA, Mackay G, et al. A key role for mitochondrial gatekeeper pyruvate dehydrogenase in oncogene-induced senescence. Nature 2013;498:109–12.

MET Amplification: A Novel Mechanism of Both Primary and Acquired Resistance



Patients with metastatic colorectal cancer whose tumors initially respond to EGFR-targeted antibodies develop acquired resistance early on in therapy. Resistance in this setting is primarily driven by secondary mutations in the *KRAS* oncogene, a downstream component of the EGFR signaling pathway. Interestingly, *KRAS* mutational status is also a key predictor of primary resistance to anti-EGFR therapy in patients with metastatic colorectal cancer. To identify novel mechanisms of resistance, Bardelli and colleagues performed exome sequencing from three cases obtained before and after anti-EGFR treatment and identified high-level amplification of the *MET* oncogene mutually exclusive from *KRAS* mutations in the posttreatment specimens. The *MET* gene is constitutively activated in other human cancers, and along with its ligand HGF, it is implicated in both acquired and primary resistance to targeted therapies. Using a digital PCR approach to detect the presence of the *MET* amplicon in circulating cell-free DNA, the investigators sought to assess the possibility that this genetic event could be detected in the circulation of patients during therapy and prior to development of resistance by conventional imaging. This testing was applied to serial blood samples from one of the three exome-sequencing cases and detected the presence of *MET* amplification as early as 3 months from start of therapy, and also detected low levels of *MET* amplification in the pretreatment tissue, supporting the hypothesis that anti-EGFR therapy selects preexisting clones that become the leading population during treatment. Additionally, the investigators go on to show that the same genetic alteration is responsible for *de novo* resistance to EGFR-targeted antibodies in colorectal cancer patient–derived xenografts, potentially identifying a new biologically distinct (albeit very rare, ∼1%) subpopulation. As MET is an actionable target, *in vivo* clinical trials were performed with two clinically available inhibitors and provide the preclinical rationale for designing MET inhibition strategies for colorectal cancer patients harboring somatic *MET* amplification, either *de novo* or acquired. (*Image from cited article courtesy of publisher.*)

Bardelli A, Corso S, Bertotti A, Hobor S, Valtorta E, Siravegna G, et al. Amplification of the MET receptor drives resistance to anti-EGFR therapies in colorectal cancer. Cancer Discov 2013;3:658–73.

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.