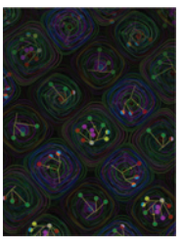
Cancer Research

Breaking Advances
Highlights from Recent Cancer Literature

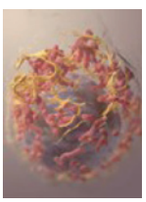
Genetic Correlates of Acquired Resistance to RAF Inhibitor Therapy in Melanoma



The *BRAF V600E* mutation is observed in half of metastatic melanoma cases and marks initial response to targeted RAF inhibitors, with most tumors eventually developing resistance. To address mechanisms of resistance, Van Allen and colleagues performed whole-exome sequencing on 45 primary metastatic melanomas and also examined matched tumors that recurred after treatment with vemurafenib or dabrafenib. Among the 45 cases, 14 showed early resistance and 31 showed delayed resistance. Resistant tumors showed mutations in *NRAS*, amplification of *BRAF*, and mutations in *MAP2K1*, with 20 of 45 cases involving the MAP kinase pathway. Four mutations were identified in *MAP2K2*, which drove resistance to both RAF and MEK inhibitors. An additional mechanism of resistance was amplification of the melanocyte master regulator *MITF*, a known driver of resistance. Interestingly, three cases showed multiple independent resistance mechanisms within the same resistant tumor biopsy. Three of the 14 cases that showed initial resistance to RAF inhibition showed mutation in *RAC1* ($RAC1^{P29S}$) and one in *HOXD8*. Although the functional significance of these mutations was not established, they may play a role in initial resistance to RAF inhibition. Overall, this work demonstrates the promise of serial tumor biopsies coupled with unbiased genetic characterization to reveal a spectrum of genetic lesions that lead to therapy resistance in melanoma. (*Image from Shi et al., Cancer Discovery; Published OnlineFirst November 21, 2013; doi:10.1158/2159-8290.CD-13-0642, courtesy of publisher.*)

Van Allen EM, Wagle N, Sucker A, Treacy DJ, Johannessen CM, Goetz EM, et al. The genetic landscape of clinical resistance to RAF inhibition in melanoma. Cancer Discovery; Published OnlineFirst November 21, 2013; doi: 10.1158/2159–8290.CD-13–0617.

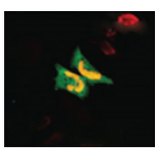
HDAC Inhibition to Overcome Resistance to MAPK Inhibition in Melanoma



While malignant melanomas harboring *BRAF V600E* mutations are exquisitely sensitive to RAF–MEK–ERK signaling inhibition, acquired resistance remains a challenge. To identify mechanisms of resistance, Johannessen and colleagues analyzed 15,906 human open reading frames in a *BRAF*-mutant melanoma cell line treated with RAF, MEK, and ERK inhibitors to identify genes whose overexpression conferred resistance to MAPK pathway inhibitors. The majority of genes conferred resistance to both RAF–MEK and ERK inhibitors, suggesting that underlying resistance mechanisms circumvented the entire RAF–MEK–ERK pathway. G-protein-coupled receptors (GPCR), the top-ranked protein class, conferred resistance to all MAPK inhibitors tested. GPCRs activate adenyl cyclase, which converts ATP to cAMP, the primary target of which is protein kinase A (PKA), and both cAMP and PKA activation were found to confer resistance to MAPK pathway inhibition in melanoma cells. Furthermore, the investigators show that cAMP-mediated resistance operated in large part through CREB, a transcription factor substrate of cAMP. Upon treatment of melanoma cells with cAMP followed by exposure to MAPK inhibitors, phosphorylation of CREB and ATF1 was blunted by exogenous cAMP, suggesting that cAMP-dependent activity of these transcription factors may be reduced by pharmacologic MAPK inhibition. The investigators identified *MITF*, a gene with an essential role in melanocyte development, as regulated by MAPK and cAMP–CREB. Expression of resistance-associated GPCRs enabled sustained MITF expression, even in the setting of MEK inhibitors, and knockdown of MITF protein levels blunted resistance to MAPK pathway inhibitors. Interestingly, MITF expression can be impaired following treatment with histone deacetylase (HDAC) inhibitors, and each of the HDAC inhibitors tested reversed cAMP-mediated resistance to MAPK pathway inhibition *in vitro*. These results suggest that the addition of HDAC inhibitors to MAPK inhibition may offer a clinical advantage in preventing or delaying resistance of some *BRAF V600E* melanomas. (*Image courtesy of NIH Image Bank.)*

Johannessen CM, Johnson LA, Piccioni F, Townes A, Frederick DT, Donahue MK, et al. A melanocyte lineage program confers resistance to MAP kinase pathway inhibition. Nature 2013 Nov 3 [Epub ahead of print].

SIRT1 and AMPK Play a Role in Mediating Hypoxia-Induced Drug Resistance



SIRT1 (Sirtuin 1) belongs to the family of mammalian class III histone deacetylases implicated in diverse cellular processes, including cell division, differentiation, senescence, and tumorigenesis. However, a potential role of SIRT1 in tumor progression, and in particular its involvement in tumor response to chemotherapy, remains controversial. The present study reveals a role of SIRT1 in hypoxia-mediated resistance against cisplatin or doxorubicin in the context of non–small cell lung cancer (NSCLC). Shin and colleagues observed that both SIRT1 and AMPK (AMP-activated protein kinase) serve as metabolic sensors based on the nutritional status of cells. They observed that SIRT1 along with AMPK was significantly repressed during hypoxic conditions in tumor cells. The inactivation of the SIRT1–AMPK pathway was one of the causative factors underlying hypoxia-induced drug resistance, which was reversed upon treatment of NSCLC cells with SIRT1–AMPK modulators. Upon further investigation, it was observed that during hypoxic conditions, SIRT1–AMPK suppression prevents tumor cells from undergoing caspase-mediated apoptosis in the presence of cisplatin or doxorubicin. The tumor resistance to cytotoxic agents was reversed upon combined treatment of tumor xenografts with SIRT1 activator and cisplatin. The findings of this study suggest that rational combinations of agents that would activate the SIRT1–AMPK pathway along with conventional chemotherapeutic drugs could be used as an alternative strategy to overcome hypoxia-induced chemoresistance, thereby enhancing the overall therapeutic index of this combination therapy in NSCLC. (*Image from Lee et al., Cancer Res 2012;72:4394–404, courtesy of publisher.*)

Shin DH, Choi YJ, Park J-W. SIRT1 and AMPK mediate hypoxia-induced resistance of non-small cell lung cancers to cisplatin and doxorubicin. Cancer Res; Published OnlineFirst November 15, 2013; doi: 10.1158/0008-5472.CAN-13-2541.

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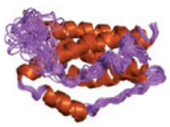
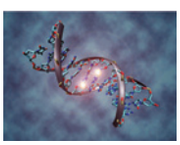
Precision Medicine for Minimal Residual Disease



After initial successful treatment, tumors can remain dormant for many years before local or distant recurrence. Progression from a state of minimal residual disease (MRD) is unpredictable, and underlying mechanisms are poorly understood. A novel insight into this important clinical problem comes from Kottke and colleagues, who studied mice given a range of experimental treatments, apparently cured of their syngeneic transplanted tumors. After many weeks, some of these tumors recurred at the original site of tumor cell injection. Transition from MRD to recurrent tumor led to a host innate acute phase protein response triggered by proliferation of malignant cells and their release of inflammatory signals. This infection-like response could be detected locally, and also by a transient elevation in serum IL-6 and VEGF, a few days before recurrent tumors were palpable. The authors postulated that successful tumors would need to evolve to evade this potent host response. This hypothesis also suggested that premature induction of recurrence (by local treatment of tumors with VEGF) would resensitize the tumors to the original therapy—and this was indeed the case. Furthermore, screening for this inflammatory response, when combined with second-line treatments that targeted innate insensitivity, prevented relapse. If these findings are confirmed in other mouse models and validated clinically, these data could lead to a paradigm shift in treatment of dormant disease in patients—active attempts to uncover MRD before the tumor evolves to escape the host immune response. (*Image courtesy of Wikimedia Commons.*)

Kottke T, Boisgerault N, Diaz RM, Donnelly O, Rommelfanger-Konkol D, Pulido J, et al. Detecting and targeting tumor relapse by its resistance to innate effectors at early recurrence. Nat Med 2013 Nov 17 [Epub ahead of print].

Detection of Circulating DNA with Genome-Wide Hypomethylation

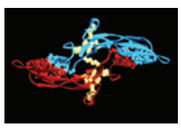


Next-generation sequencing, which enables rapid whole-genome analyses of genetic and epigenetic alterations, is sure to have a dramatic impact on assessment of risk, diagnosis, treatment, and response to therapy. Earlier this year, Dawson and colleagues demonstrated its use to detect circulating tumor DNA in patients with metastatic breast cancer. More recently, Chan and colleagues report on their examination of bisulfite conversion followed by next-generation sequencing to detect hypomethylated DNA in the circulation to provide insight into both initial diagnosis and recurrence monitoring. A reference set of 16 healthy controls was used to define the threshold for hypomethylation. Dividing the genome up into 1-MB regions, the authors determined the mean methylation density in each bin across all 16 controls in the reference set. Using these criteria, for each case they determined the percentage of bins across the genome that was hypomethylated (mean methylation density >2 SD below the reference mean). Receiver operating characteristic curve analysis demonstrated that the area under the curve was 0.93%. Using a cutoff of 1.1% of bins hypomethylated, the sensitivity was 81% and specificity was 94% for the detection of patients with hepatocellular carcinoma. Based on two patients who were followed over time, the authors provide tantalizing data regarding use of this assay for detecting recurrence. In one patient, circulating hypomethylated DNA remained high even after tumor resection and subsequent studies showed widely metastatic disease. In contrast, the other patient with stable disease had a precipitous and long-lasting drop in circulating hypomethylated DNA following tumor resection. While the potential utility of this assay is intriguing, larger studies in age-matched, high-risk patients, including those with cirrhosis, are clearly needed. (*Image courtesy of Wikimedia Commons.*)

Dawson SJ, Tsui DW, Murtaza M, Biggs H, Rueda OM, Chin SF, et al. Analysis of circulating tumor DNA to monitor metastatic breast cancer. N Engl J Med 2013;368:1199–209.

Chan KCA, Jiang P, Chan CWM, Sun K, Wong J, Hui EP, et al. Noninvasive detection of cancer-associated genome-wide hypomethylation and copy number aberrations by plasma bisulfite sequencing. Proc Natl Acad Sci U S A 2013;110:18761–8.

An Instructing Bone Marrow "Soil" Determines Dormancy of Disseminated Tumor Cells



Single disseminated tumor cells (DTC) already typically are spread throughout the body. After treatment of primary tumors, DTCs grow and form secondary tumors in certain organs, while remaining dormant in other organs. To provide mechanistic underpinnings into this dichotomy in a hepatic squamous cell carcinoma model of metastasis, Bragado and colleagues revealed that sustained dormancy in the bone marrow was maintained by high levels of TGFβ2, driving canonical (SMAD1/5-dependent) and noncanonical signaling via TGFβ-RI, -RII, -RIII, and p38 (MAPK) in tumor cells. Quiescence was maintained through induction of the CDK inhibitor p27 and inhibition of CDK4, with p38 signaling mediating changes in the circadian rhythm and metastasis suppressor transcription factor DEC2/SHARP-1 (BHLHE41). TGFβ2 was much less abundant in the lung, where dormancy was short-lived and DTCs rapidly formed metastases. Importantly, pharmacologic inhibition of TGFβ-RI or p38α/β (MAPK14/11) awakened dormant disease and fueled multiorgan metastasis. This work reveals a new "seed and soil" mechanism whereby TGFβ2 and TGFβRIII signaling through p38α/β regulates DTC dormancy and defines restrictive (marrow) and permissive (lung) microenvironments for metastases in squamous carcinoma. This study also provides novel markers to assay dormancy of DTCs (P-p38^{high}/TGFβ−RIII^{high}) and identifies microenvironmental markers (TGFβ2^{high} in bone marrow) that contribute to activation of dormant disseminated disease. (*Image courtesy of Wikimedia Commons and NIH.*)

Bragado P, Estrada Y, Parikh F, Krause S, Capobianco C, Farina HG, et al. TGF-β2 dictates disseminated tumour cell fate in target organs through TGF-β-RIII and p38α/β signaling. Nat Cell Biol 2013; 15:1351–61.

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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