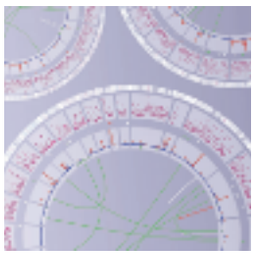
Non-V600E *BRAF* Mutations Are Actionable in Melanoma

- A subset of "*BRAF*–wild-type" melanomas harbor non-V600E *BRAF* mutations.
- MEK/ERK signaling induced by the L597 and K601 mutants was suppressed by MEK inhibitors.
- A patient with $BRAF^{L597S}$-mutant metastatic melanoma responded to MEK inhibitor therapy.

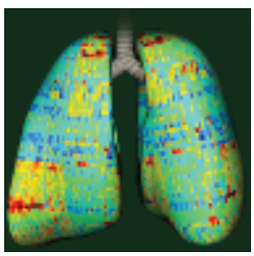


Kinase inhibitors have become accepted treatments for the 40% to 50% of metastatic melanomas harboring $BRAF^{V600E}$ or *KIT* exon 11 and 13 mutations. To identify other potential targets in patients lacking these mutations, Dahlman and colleagues performed whole-genome sequencing on an aggressive, $BRAF^{V600E}$-negative, *KIT*-negative metastatic melanoma and matched blood. Interestingly, a $BRAF^{L597R}$ mutation was identified that affects the same exon (exon 15) as the V600E mutation. Screening of *BRAF* exon 15 in an additional 49 melanomas revealed 2 other tumors with L597 mutations (L597Q and L597S), a third with a K601E mutation, and a fourth with a D594N mutation, indicating that as many as 8% of melanomas classified clinically as "*BRAF* wild-type" may actually harbor other less common *BRAF* mutations. To determine whether these BRAF mutants were functionally similar to $BRAF^{V600E}$, which causes constitutive MEK/ERK pathway activation, the authors ectopically expressed the L597Q, L597R, L597S, and K601E mutations in 293H cells and observed that all mutants led to elevated phospho-MEK and phospho-ERK levels. Furthermore, phospho-ERK was significantly decreased by a MEK inhibitor, suggesting that patients with metastatic melanoma harboring these *BRAF* mutations may also benefit from MEK inhibitor therapy. Indeed, a patient with $BRAF^{L597S}$-mutant metastatic melanoma who was enrolled in a phase I trial of an allosteric MEK inhibitor experienced a partial radiographic response and was progression-free for more than 24 weeks. These findings suggest that expanded *BRAF* mutational testing may benefit additional patients with metastatic melanoma. ■

See article, p. 791.

PARP1 Is a Potential Therapeutic Target in SCLC

- PARP1 is expressed more highly in SCLC cells than NSCLC or other solid tumor cells.
- PARP inhibitors suppress SCLC cell growth and potentiate the effects of cytotoxic agents.
- PARP inhibition may augment existing DNA repair defects in SCLC cells.

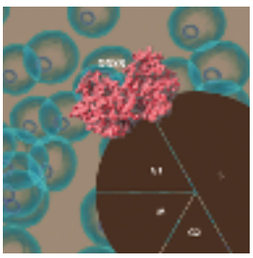


Small cell lung cancer (SCLC) is a highly lethal malignancy with a distinct metastatic potential and treatment response from the more common non–small cell lung cancer (NSCLC). Common genetic abnormalities identified in SCLC to date have not been amenable to targeted therapy, and clinical outcomes remain poor. Byers and colleagues analyzed the levels of 193 total and phosphorylated proteins in a large panel of SCLC and NSCLC cell lines to identify potential SCLC therapeutic targets. Compared with NSCLC cells, SCLC cells had significantly higher levels of the DNA repair protein poly (ADP-ribose) polymerase (PARP1). At the mRNA level, SCLC cells had the highest median *PARP1* expression of any solid tumor cells tested, and PARP1 protein levels were significantly higher in primary tumor samples from patients with SCLC or other neuroendocrine lung cancers than those from patients with NSCLC. SCLC cell lines were also highly sensitive to small-molecule PARP inhibitors, which reduced DNA repair protein levels and therefore possibly exacerbated underlying DNA repair defects in SCLC cells. Combining PARP1 inhibition with double-strand break-inducing chemotherapeutic agents commonly used in SCLC therapy led to greater decreases in SCLC cell viability than either treatment alone, further suggesting that PARP inhibitors may sensitize SCLC cells to DNA damage. These preclinical data provide support for targeting PARP1 in SCLC and suggest that PARP inhibitors may have efficacy in SCLC in combination with standard chemotherapy. ■

See article, p. 798.

Amplification of *DDX5* Promotes DNA Replication in Breast Cancer

- A screen for DNA replication factors identified the DEAD-box protein DDX5.
- DDX5 recruits RNA polymerase II to the promoters of genes required for DNA replication.
- The *DDX5* locus is amplified and DDX5 is essential in ~25% of human breast cancers.

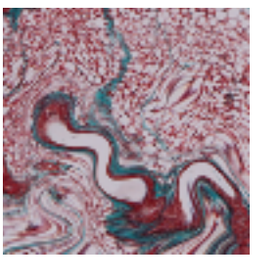


Identifying additional factors required for DNA replication might reveal potential vulnerabilities of rapidly proliferating cancer cells that are dependent on increased DNA synthesis. Mazurek and colleagues hypothesized that an inability to stably maintain a plasmid with only one origin of replication would distinguish cells lacking an essential DNA replication factor. An shRNA screen of a library of poorly characterized G_1 and S-phase genes identified *DEAD (Asp-Glu-Ala-Asp) box helicase 5 (DDX5)*, encoding an ATP-dependent RNA helicase, as a significant determinant of plasmid stability. The authors observed that only cancer cell lines overexpressing *DDX5* or with amplification of the *DDX5* gene were sensitive to *DDX5* knockdown, which reduced cell proliferation in association with slowed progression through S-phase, decreased levels of known DNA replication factors, and impaired DNA replication pre-initiation complex assembly. In these *DDX5*-amplified or overexpressing cells, DDX5 physically interacted with E2F1, a key regulator of S-phase gene expression, and recruited RNA polymerase II to the promoters of E2F target genes, indicating that *DDX5* overexpression indirectly supports DNA synthesis in a subset of cancer cells by upregulating the expression of DNA replication factor genes. Importantly, an analysis of 2 independent breast cancer databases revealed that the *DDX5* locus was amplified in 25% to 28% of samples, suggesting that an acquired role of DDX5 in DNA replication may be a common oncogenic mechanism. Inhibition of DDX5 may thus be an effective therapeutic strategy in the subset of cancers that are dependent on DDX5 activity. ■

See article, p. 812.

CD36 Repression Generates a High-Risk, Protumor Microenvironment

- CD36 is suppressed in multiple cell types in tumors and high mammographic density tissue.
- CD36 is a critical regulator of adipocyte content and extracellular matrix accumulation.
- Low CD36 expression is correlated with increasing tumor grade and size in breast cancer.



High mammographic density is a strong risk factor for breast cancer characterized histologically by reduced adipocyte differentiation and enhanced extracellular matrix deposition, which are also observed in tumor-associated desmoplastic stroma. To investigate the molecular mechanisms underlying these features, DeFilippis and colleagues examined fibroblasts derived from high and low mammographic density breast tissue. High-density associated fibroblasts (HDAF) exhibited impaired adipocyte differentiation and increased secretion of desmoplastic matrix proteins, thus recapitulating both aspects of the high mammographic density phenotype. Gene expression profiling identified the transmembrane receptor *CD36* as the most significantly downregulated gene in HDAFs; CD36 expression was also decreased in invasive ductal carcinoma (IDC) samples compared with normal tissue, suggesting a functional role for this protein in regulating the shared desmoplastic phenotypes. Indeed, CD36 expression in normal fibroblasts was necessary and sufficient to modulate fat and matrix protein accumulation *in vitro* and *CD36* deficiency resulted in decreased fat content and augmented matrix production *in vivo*. Reduced CD36 expression was observed in multiple stromal cell types in both nonmalignant high mammographic density tissue and in breast tumors, suggesting that widespread CD36 repression may precede and promote tumor formation. Furthermore, CD36 expression was inversely correlated with tumor grade and size in IDC. These results demonstrate that CD36 repression generates a proactive microenvironment that increases cancer risk and facilitates tumor initiation and progression, and suggest that CD36 modulation may be therapeutically useful. ■

See article, p. 826.

An IL-1/PGE_2 Signaling Loop Elicits a Stem-Cell Phenotype via EMT

- IL-1 secreted by carcinoma cells induces PGE_2 production in mesenchymal stem cells.
- Autocrine PGE_2 and paracrine IL-1 signaling stimulate cytokine expression in MSCs.
- Activation of β-catenin signaling promotes cancer stem cell formation and tumor progression.

Mesenchymal stem cells (MSC) recruited to tumor-associated stroma modulate carcinoma cell behavior to facilitate epithelial–mesenchymal transition (EMT) and tumor progression. Induction of EMT is associated with acquisition of a stem cell–like phenotype that correlates with enhanced tumor-initiating capacity. To elucidate the mechanisms that generate this favorable tumor microenvironment, Li and colleagues investigated the interactions between tumor cells and MSCs. Secretion of interleukin (IL)-1 by colon carcinoma cells was necessary and sufficient to stimulate expression of the COX-2 enzyme and subsequent production of prostaglandin E_2 (PGE_2) by cocultured MSCs. Autocrine PGE_2 signaling in turn cooperated with carcinoma cell–derived IL-1 to induce the expression of other cytokines, including IL-6 and IL-8, in MSCs. Treatment of carcinoma cells with PGE_2 and these cytokines resulted in decreased expression of the epithelial marker E-cadherin and increased protein levels of mesenchymal markers, indicative of EMT, and promoted tumor cell invasion. Furthermore, this EMT program was associated with an increased frequency of aldehyde dehydrogenase (ALDH)–expressing cancer stem cells (CSC) and augmented tumor initiation, both of which were dependent on PGE_2 production by COX-2. Additionally, PGE_2-mediated paracrine stimulation of tumor cells triggered nuclear localization and activation of β-catenin via AKT, which contributed to the induction of a CSC phenotype. Importantly, IL-1 and COX-2 expression were elevated in aggressive human colon and breast carcinomas, suggesting that inhibition of this signaling pathway may be useful in treating advanced tumors. ■

See article, p. 840.

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