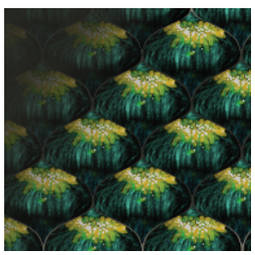
The DNA Damage Response Disrupts Chromosome Segregation during Mitosis

- Activation of the DDR in mitosis induces chromosome missegregation via increased k-MT stability.
- k-MT stability is regulated by ATM/CHK2–mediated activation of PLK1 and Aurora A kinases.
- Persistent mitotic DNA damage drives whole chromosome segregation defects in cancer cells.



Activation of the DNA damage response (DDR) induces cell-cycle arrest in order to provide sufficient time for DNA repair before mitosis. In addition, chromosome segregation errors can lead to DNA damage in mitotic cells, which lack overt DNA repair activity. Although a causative relationship between premitotic DNA damage and structural chromosomal instability (s-CIN), such as chromosome bridges and acentric chromosomes, has been established, the role of the DDR in whole chromosomal instability (w-CIN) remains less clear, prompting Bakhoum, Kabeche, and colleagues to study how activation of the DDR in mitosis affects chromosome segregation. Treatment of human cell lines with DNA damaging agents led to an increase in lagging chromosomes, despite normal mitotic spindle formation. Mechanistically, DNA damage induced whole chromosome segregation errors during mitosis by aberrantly stabilizing kinetochore–microtubule (k-MT) attachments. This effect was dependent on activation of the mitotic kinases polo-like kinase 1 (PLK1) and Aurora A. Inhibition of the DDR via pharmacologic means or genetic suppression of ataxia telangiectasia mutated (ATM) or CHK2 prevented DNA damage–induced chromosome missegregation, whereas constitutive activation of ATM promoted chromosome lagging. Furthermore, CHK2 inhibition in a panel of cancer cells that exhibit elevated endogenous DNA damage during mitosis decreased the incidence of lagging chromosomes. These results demonstrate that activation of the DDR during mitosis drives whole chromosome segregation errors and may provide a link between s-CIN and w-CIN in cancer. ■

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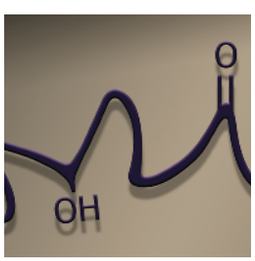
L-2-Hydroxyglutarate May Be a Renal Cell Carcinoma Oncometabolite

- Increased levels of L-2-hydroxyglutarate (L-2HG), not D-2HG, are found in renal cell carcinoma.
- Elevated L-2HG in renal cell carcinoma is associated with 5hmC loss and altered histone methylation.
- L-2HG accumulation is not due to *IDH* mutation but can be attributed to loss of L2HGDH expression.

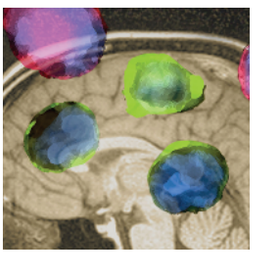


Mutations in metabolic enzymes that cause aberrant metabolite accumulation have been increasingly implicated in tumorigenesis. For example, neomorphic isocitrate dehydrogenase 1 and 2 (*IDH1/2*) mutations common in acute myeloid leukemias and gliomas lead to the production of the D-enantiomer of 2-hydroxyglutarate (D-2HG), whereas inactivating mutations in fumarate hydratase and succinate dehydrogenase in renal cell carcinoma (RCC) and other cancers lead to the accumulation of fumarate and succinate, respectively. Elevated levels of these "oncometabolites" are thought to promote tumorigenesis by inhibiting 2-oxoglutarate (2-OG)–dependent dioxygenases such as histone demethylases and 5-methylcytosine hydrolases, which can in turn lead to altered epigenetic states. In a metabolomic analysis of clear cell RCC (ccRCC), the most common RCC subtype, Shim and colleagues found that L-2HG, not D-2HG, was significantly elevated in ccRCCs compared with normal renal tissue. High L-2HG levels were associated with reduced 5-hydroxymethylcytosine (5hmC), indicative of inhibition of 2-OG–dependent enzymes. Recent sequencing efforts have not identified *IDH* mutations in RCC, but several RCC cell lines showed reduced expression of L-2HG dehydrogenase (*L2HGDH*), which is located on a chromosomal region commonly lost in RCC. L2HGDH knockdown increased L-2HG and decreased 5hmC levels in renal cells, whereas expression of L2HGDH in RCC cells lowered L-2HG levels, increased 5hmC levels, decreased histone H3 methylation, and reduced proliferation and colony formation. The identification of L-2HG as a putative oncometabolite and L2HGDH deficiency as an additional cause of 2-OG–dependent enzyme inactivation expands our understanding of how metabolic alterations in cancer can induce epigenetic modifications. ■

See article, p. 1290.

Glioblastomas Shed Invasive Mesenchymal Tumor Cells into the Circulation

- CTCs were identified using a microfluidic device and GBM-specific markers in patients with GBM.
- GBM CTCs exhibit a mesenchymal profile that is also present in a subset of primary tumor cells.
- Rare metastatic GBM lesions are primarily mesenchymal and exhibit additional mutations.

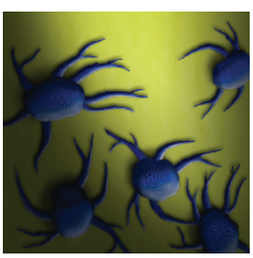


Despite being locally invasive and highly angiogenic, glioblastoma (GBM) seldom metastasizes to other organs, and circulating brain tumor cells (CTC) have not been isolated in patients with GBM. It remains unclear whether this reflects an inability of GBM cells to invade the vasculature or a failure of GBM cells to proliferate extracranially. Sullivan, Nahed, and colleagues applied the CTC-iChip, a microfluidic platform that uses magnetically tagged antibodies to deplete blood samples of leukocytes and enrich for CTCs, to two orthotopic patient-derived GBM xenograft mouse models and samples from 33 human patients with GBM. Rare CTCs were identified based on expression of a panel of GBM-specific markers in approximately half of tumor-bearing mice and in at least one blood sample from 39% of patients, and were detected at higher frequency in patients with progressive disease. Analysis of individual CTCs revealed decreased expression of neural lineage markers and elevated expression of genes characteristic of the mesenchymal GBM subtype, which is associated with poor prognosis. Analysis of primary GBM xenografts identified a subpopulation of mesenchymal, migratory tumor cells in white matter tracts and surrounding necrotic foci. Furthermore, the visceral lesions in a rare patient with metastatic GBM were exclusively mesenchymal and harbored additional mutations that were absent from the primary tumor, suggestive of clonal evolution. These findings indicate that GBMs do shed CTCs into the blood, which provides insight into GBM invasion and may eventually prove clinically useful in monitoring this aggressive disease. ■

See article, p. 1299.

TMPRSS2 Links AR Signaling and Prostate Cancer Metastasis through HGF

- Deletion of the AR target *Tmprss2* reduces metastasis in a prostate cancer mouse model.
- TMPRSS2 activates c-MET signaling via HGF cleavage, leading to enhanced invasion and metastasis.
- Inhibition of TMPRSS2 may decrease metastatic progression in patients with prostate cancer.



The androgen receptor (AR)–regulated transmembrane protease serine 2 (TMPRSS2) is primarily expressed in the prostate epithelium, but its role in prostate cancer progression is not known. Lucas, Heinlein, and colleagues found that TMPRSS2 expression correlated with AR positivity and was elevated in localized high-grade human prostate cancers and in the majority of distant metastases. Targeted deletion of *Tmprss2* in the transgenic autochthonous mouse model for prostate cancer (TRAMP) background resulted in larger primary tumors but significantly reduced rates of distant metastases. *Tmprss2* expression increased tumor cell invasion *in vitro* and was associated with induction of genes involved in epithelial–mesenchymal transition. Hepatocyte growth factor (HGF), which binds c-MET and has been implicated in prostate cancer invasion and metastasis, was identified through a peptide cleavage screen as a potential substrate of TMPRSS2. Consistent with this finding, exposure to ectopic or endogenous TMPRSS2 induced the proteolytic cleavage of HGF and activated c-MET in prostate cancer cell lines. Intriguingly, TMPRSS2-induced HGF–c-MET signaling also suppressed prostate cancer cell proliferation in a cell context–dependent manner. Importantly, chemical library screens identified bromhexine hydrochloride (BHH) as a potent bioavailable inhibitor of TMPRSS2, and BHH treatment reduced prostate cancer cell invasion and metastatic frequency in the TRAMP model without toxicity. Taken together, these results indicate that TMPRSS2 is a potential therapeutic target that links AR signaling to prostate cancer metastasis through activation of the HGF–c-MET axis. ■

See article, p. 1310.

Ewing Sarcoma Harbors Few Targetable Genetic Aberrations

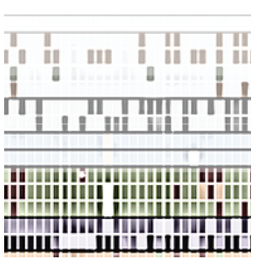
- Somatic mutations in pediatric Ewing sarcoma were defined using massively parallel sequencing.
- Tumors are genetically stable at diagnosis but exhibit an increased mutation rate at relapse.
- Recurrent mutations in *STAG2* are associated with loss of STAG2 expression and metastatic disease.

Pediatric Ewing sarcoma is a highly aggressive bone tumor characterized by recurrent rearrangement of genes encoding ETS family transcription factors, most commonly resulting in expression of the oncogenic fusion proteins EWS–FLI1 and EWS–ERG. However, there are currently no effective therapeutic strategies to directly target these chimeric transcription factors, emphasizing the importance of identifying additional therapeutic targets. To define the genomic landscape of somatic alterations in pediatric Ewing sarcoma, Crompton, Stewart, and colleagues performed integrative next-generation sequencing of human tumor samples and Ewing sarcoma cell lines. Other than recurrent *EWS–ETS* translocations, Ewing sarcoma tumors exhibited copy-number alterations, such as gain of chromosome 8 and focal deletion of *CDKN2A*, but harbored very few significantly mutated genes, indicating that these tumors are relatively genetically stable. Consistent with previous studies, recurrent mutations in *TP53* were specifically acquired in relapsed tumors. In addition, mutations or rearrangement of stromal antigen 2 (*STAG2*), which encodes a cohesin complex subunit, resulted in loss of STAG2 protein expression and were associated with *TP53* mutations and progression to metastatic disease. Intriguingly, compared with primary tumors sampled at diagnosis, tumors that relapsed after treatment exhibited an increased mutation rate and a distinct mutational profile, including unique *TP53* and *STAG2* mutations in different relapsed tumors from one patient. These findings underscore the role of tumor heterogeneity at diagnosis in clonal evolution and suggest that STAG2 may be a prognostic marker and potential therapeutic target in Ewing sarcoma. ■

See article, p. 1326.

STAG2 Mutation Is Associated with Aggressive Ewing Sarcoma

- Whole-genome sequencing revealed a low somatic mutation rate in Ewing sarcoma tumors.
- Mutation of *STAG2* is common in Ewing sarcoma and is mutually exclusive with *CDKN2A* deletion.
- *STAG2* mutations are associated with *TP53* mutations and poor outcome and may expand at tumor relapse.



Recurrent translocations involving *EWSR1* and genes encoding ETS family transcription factors have been implicated as oncogenic drivers of Ewing sarcoma, a primary bone tumor in children and adolescents. Although several copy-number alterations have also been identified, secondary genetic alterations in Ewing sarcoma remain poorly understood, prompting Tirode, Surdez, and colleagues to perform whole-genome sequencing of 112 Ewing sarcoma tumors and matched germline DNA. Strikingly, Ewing sarcoma tumors were characterized by a low frequency of single-nucleotide variants, structural variants, and copy-number alterations, which included gain of chromosomes 8, 12, and 1q, loss of chromosome 16q, and deletion of cyclin-dependent kinase inhibitor 2A (*CDKN2A*). The most frequently mutated gene was stromal antigen 2 (*STAG2*), which encodes a component of the cohesin complex and was somatically mutated in 17% of tumors. In addition, recurrent mutations in *TP53* and genes encoding epigenetic regulators, including *EZH2*, *BCOR*, and *ZMYM3*, were identified at lower frequencies. *STAG2* mutation and *CDKN2A* deletion were mutually exclusive in both Ewing sarcoma tumors and cell lines. In contrast, mutations in *STAG2* and *TP53* often coexisted in the same tumor and were associated with poor outcome in an extended patient cohort. Furthermore, a subset of relapsed tumors exhibited expansion of subclonal *STAG2*-mutant cells and loss of STAG2 protein expression compared with primary tumor samples. These findings identify an aggressive subtype of Ewing sarcoma characterized by *STAG2* and *TP53* mutations. ■

See article, p. 1342.

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