

## Oncogenes

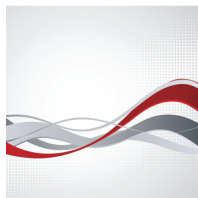
**Major finding:** Alternative transcription initiation results in a novel *ALK* transcript, *ALK<sup>ATI</sup>*, in melanomas.

**Clinical relevance:** *ALK* inhibitors suppress *ALK<sup>ATI</sup>* activity and may benefit patients with *ALK<sup>ATI</sup>*-expressing tumors.

**Impact:** These results define a previously uncharacterized mechanism for oncogene activation in cancer.

### A NOVEL *ALK* ISOFORM ARISES VIA ALTERNATIVE TRANSCRIPTION INITIATION

Oncogenes have been shown to be activated by various genetic alterations, including mutations, translocations, and amplifications. However, whether oncogenes can also be activated by other mechanisms remains unclear. To address this question, Wiesner and colleagues used RNA sequencing to identify differential expression of exons encoding the kinase domains of receptor tyrosine kinases in metastatic melanoma and thyroid carcinoma. This analysis revealed a previously uncharacterized transcript of anaplastic lymphoma kinase (*ALK*) consisting of a fragment of intron 19 followed by exons 20–29 that resulted from alternative transcription initiation (ATI) via an active ATI site in intron 19. Analysis of tumor samples from The Cancer Genome Atlas indicated that this *ALK* isoform, referred to as *ALK<sup>ATI</sup>*, was expressed in approximately 11% of melanomas and sporadically in other cancers, but not in normal tissues. Comprehensive genetic studies showed that *ALK<sup>ATI</sup>* was expressed from both *ALK* alleles, independent of genomic aberrations at the *ALK* locus. Transcriptional activation of the *ALK<sup>ATI</sup>* isoform resulted in expression of three distinct proteins, each of which contained the intact *ALK* intracellular



tyrosine kinase domain but lacked the extracellular and transmembrane domains. These *ALK<sup>ATI</sup>* proteins were activated via homodimerization and autophosphorylation and localized to both the nucleus and cytoplasm, in contrast to other *ALK* mutants that localized primarily to the cytoplasm. Expression of *ALK<sup>ATI</sup>* was sufficient to induce growth factor-independent proliferation *in vitro* and promoted tumor growth *in vivo*, consistent with an oncogenic function. Pharmacologic *ALK* inhibitors such as crizotinib suppressed *ALK<sup>ATI</sup>* phosphorylation and signaling and triggered regression of *ALK<sup>ATI</sup>*-driven tumors. Furthermore, crizotinib treatment led to rapid tumor shrinkage and symptomatic improvement in a patient with *ALK<sup>ATI</sup>*-expressing metastatic melanoma. These findings suggest an alternative mechanism for oncogene activation in cancer and support the notion that *ALK* inhibitors may be beneficial in patients with *ALK<sup>ATI</sup>*-driven tumors. ■

Wiesner T, Lee W, Obenauf AC, Ran L, Murali R, Zhang QF, et al. Alternative transcription initiation leads to expression of a novel *ALK* isoform in cancer. *Nature* 2015;526:453–7.

## Epigenetics

**Major finding:** WEE1 inhibition selectively kills H3K36me3-deficient tumor cells by RRM2 loss and dNTP starvation.

**Mechanism:** H3K36me3 promotes RRM2 transcription and WEE1 inhibition induces RRM2 degradation via CDK activation.

**Impact:** The WEE1 inhibitor AZD1775 causes tumor regression in H3K36me3-deficient xenografts.

### H3K36me3-DEFICIENT CANCERS ARE SENSITIVE TO WEE1 KINASE INHIBITION

Loss of histone H3 trimethylation at lysine 36 (H3K36me3) is frequently observed in tumors and is associated with a poor outcome. H3K36me3 loss can occur via multiple mechanisms, including loss of the tumor suppressor methyltransferase SET domain-containing 2 (SETD2). However, there are currently no therapies to specifically target H3K36me3-deficient cancers. Pfister and colleagues found that H3K36me3-deficient cancer cell lines were sensitive to treatment with AZD1775, an inhibitor of WEE1 kinase, which suppresses cyclin-dependent kinases 1 (CDK1) and CDK2. Depletion of SETD2 in wild-type cells sensitized them to AZD1775 treatment, confirming a synthetic lethal interaction between H3K36me3 loss and WEE1 inhibition in human cells. This selective killing of H3K36me3-deficient cells resulted from inhibition of DNA replication, with SETD2 loss leading to fork stalling and WEE1 inhibition causing stalled fork collapse. Expression of the RRM2 subunit of ribonucleotide reductase, which generates dNTPs for DNA synthesis, was reduced by AZD1775 treatment and SETD2 knockdown. Mechanistically, H3K36me3 loss decreased the transcription of *RRM2* via impaired recruitment of transcription initiation factors, whereas WEE1 inhibition triggered unchecked CDK1/2

activity that enhanced aberrant origin firing and promoted CDK-dependent phosphorylation and ubiquitin-mediated degradation of RRM2, resulting in dNTP starvation and cell death. Consistent with this mechanism, overexpression of exogenous RRM2 rescued cells from death. WEE1 inhibition was effective *in vivo* and induced regression of SETD2-deficient, but not SETD2-proficient, tumor xenografts. Furthermore, a monoclonal antibody against H3K36me3 distinguished SETD2-deficient from SETD2-proficient tumors, suggesting that it may be possible to determine which patients might benefit from WEE1 inhibitors based on H3K36me3 immunohistochemistry. These findings indicate that WEE1 inhibition selectively kills H3K36me3-deficient cancer cells through RRM2 depletion and dNTP starvation, and suggest that WEE1 inhibition may be a promising strategy for treating H3K36me3-deficient tumors. As AZD1775 is already in clinical trials, these results have the potential to be rapidly translated to patient care. ■

Pfister SX, Markkanen E, Jiang Y, Sarkar S, Woodcock M, Orlando G, et al. Inhibiting WEE1 selectively kills histone H3K36me3-deficient cancers by dNTP starvation. *Cancer Cell* 2015;28:557–68.