Splicing

Major finding: Lineage-specific splicing of *ANXA7* enhances EGFR signaling and glioblastoma progression.

Mechanism: PTBP1 mediates *ANXA7* exon skipping in precursor cells, which impairs EGFR endosomal targeting.

Impact: Posttranscriptional mechanisms reprogram developmental processes to activate oncogenic signaling.

TISSUE-REGULATED SPLICING PROMOTES EGFR SIGNALING IN GLIOBLASTOMA

Regulation of mRNA splicing is critical for normal developmental processes such as neuronal differentiation in the brain, and aberrant alternative splicing can generate variant protein isoforms that promote oncogenic transformation and glioblastoma progression. However, the role of tissue-specific splicing of alternative exons in tumorigenesis remains unclear. Ferrarese and colleagues hypothesized that alternative splicing of annexin A7 (*ANXA7*), a membrane-bound tumor suppressor that undergoes tissue-specific exon skipping in the brain, contributes to glioblastoma progression. In support of this idea, expression of ANXA7 isoform 2, which lacks the alternative exon, was elevated in glioblastoma tissue as well as in neural and glial progenitor cells, which represent potential glioblastoma cells of origin. In contrast to the full-length ANXA7 isoform 1, ANXA7 isoform 2 lacked the ability to terminate EGFR signaling via endosomal targeting and degradation, suggesting that alternative splicing of *ANXA7* enhances oncogenic EGFR activity. Exon skipping in *ANXA7* was mediated by polypyrimidine tract-binding protein 1 (PTBP1), a heterogeneous nuclear ribonucleoprotein that was upregulated in glioblastomas compared with normal brain tissue via gene amplification and loss of the neuron-specific miRNA miR-124. Depletion of PTBP1 in glioblastoma or brain tumor stem cells increased ANXA7 isoform 1 expression, diminished EGFR activation, and inhibited glioblastoma invasion and angiogenesis; these phenotypes were rescued by concomitant silencing of ANXA7 isoform 1, suggesting that PTBP1-driven splicing promotes brain tumor progression by disrupting the tumor-suppressive function of ANXA7 in endocytosis. In addition, high *PTBP1* expression was associated with shorter survival in patients with glioblastoma, similar to patients with *EGFR* amplification, further underscoring the importance of PTBP1-dependent activation of EGFR in glioblastoma progression. These results provide insight into how posttranscriptional, lineage-specific splicing of a tumor suppressor can reprogram normal developmental processes to stimulate oncogenic signaling and tumorigenesis. ■

Ferrarese R, Harsh GR, IV, Yadav AK, Bug E, Maticzka D, Reichardt W, et al. Lineage-specific splicing of a brain-enriched alternative exon promotes glioblastoma progression. J Clin Invest 2014;124:2861–76.

Melanoma

Major finding: MC1R-driven cAMP–PKA signaling promotes ATR phosphorylation and repair of UV-induced DNA damage.

Mechanism: Phosphorylation of ATR at Ser435 by PKA enhances its interaction with XPA and localization to UV damage.

Impact: Pharmacologic cAMP activation may enhance NER and limit UV mutagenesis in MC1R-defective individuals.

MC1R PROTECTS AGAINST MELANOMA BY AUGMENTING NUCLEOTIDE EXCISION REPAIR

Loss-of-function polymorphisms in melanocortin 1 receptor (MC1R), a G_S-coupled receptor that signals through cAMP and protein kinase A (PKA), are major genetic risk factors for UV sensitivity and melanoma development. MC1R activation in melanocytes protects against UV-induced DNA damage via pigment production, but has also been suggested to suppress melanoma via independent mechanisms such as DNA repair. Jarrett and colleagues found that stimulation of cAMP via MC1R or forskolin enhanced the clearance of UV-induced DNA photolesions via nucleotide excision repair (NER). This increase in NER efficiency was dependent on direct phosphorylation of ataxia telangiectasia and RAD3-related protein (ATR) at Ser435 by PKA in response to UV irradiation and MC1R–cAMP signaling. PKA-mediated phosphorylation of ATR accelerated its interaction with the NER core protein xeroderma pigmentosum complement group A (XPA) in the nucleus and promoted the recruitment of XPA to sites of UV damage on chromatin; phosphomimetic mutation of ATR Ser435 enhanced formation of the ATR–XPA complex at damaged DNA, whereas ATR depletion or mutation of Ser435 to alanine blocked NER, suggesting that this posttranslational modification is necessary and sufficient to optimize repair of UV damage. Consistent with this idea, pharmacologic stimulation of cAMP with forskolin suppressed the accumulation of UV-induced mutations in both wild-type and *MC1R*-mutant cells in an ATR–XPA-dependent manner. Of note, PKA-driven ATR phosphorylation enhanced NER independent of CHK1 activation and cell-cycle arrest, which are typically induced by ATR following DNA damage. These findings delineate the mechanism by which the MC1R–cAMP–PKA pathway regulates NER to maintain genomic stability in melanocytes and provide support for the idea that pharmacologic induction of cAMP signaling may enhance NER and reduce melanoma risk in individuals with defective MC1R. ■

Jarrett SG, Horrell EM, Christian PA, Vanover JC, Boulanger MC, Zou Y, et al. PKA-mediated phosphorylation of ATR promotes recruitment of XPA to UV-induced DNA damage. Mol Cell 2014;54:999–1011.