Cancer-associated mutations of the pre-mRNA splicing factor U2AF2 alter splice site signal recognition

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The essential splicing factor U2AF2 recognizes a polypyrimidine (Py) tract splice site signal and initiates spliceosome assembly. High-throughput sequencing has revealed that recurrent missense mutations of genes encoding auxiliary pre-mRNA splicing factors are associated with cancers, most commonly hematologic malignancies. At the molecular level, these mutations often subtly alter splicing factor – RNA binding characteristics. Although less frequent, cancer-associated mutations of the critical U2AF2 protein also reccur. To explore the extent and structural consequences of cancer-associated *U2AF2* mutations, we surveyed The Cancer Genome Atlas (TCGA), International Cancer, Genome Consortium (ICGC), and Catalogue of Somatic Mutations in Cancer (COSMIC) using the cBioportal and COSMIC interfaces. Most cancer-associated *U2AF2* defects were missense mutations, many of which recur at the Py tract RNA interface of the U2AF2 structure (PDB ID 5EV4). Based on the structural locations, we hypothesized that several mutations would alter Py tract recognition properties of U2AF2. By fluorescence anisotropy RNA binding assays, we show that a leukemia-associated N196K mutation enhances U2AF2 binding to a representative Py tract by four-fold, whereas a prostate/colon cancer-associated G301D mutation reduced binding by ten-fold. We are in the process of testing the functional and structural consequences of these and other mutations for splicing in cell lines and crystal structures of U2AF2 – oligonucleotide complexes. Altogether, these results suggest that altered Py tract recognition by mutant U2AF2 can contribute to neoplastic transformation.

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