RNA Looping by ILF2 Dimerization Orchestrates Alternative Splicing Programs in Ovarian Cancer

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Background: Aberrant alternative splicing is a hallmark of cancer, yet how RNA-binding proteins (RBPs) structurally organize pre-mRNAs to control splice-site selection remains poorly understood. Interleukin Enhancer Binding Factor 2 (ILF2) is frequently overexpressed in cancers, but its mechanistic role in splicing regulation and tumor progression remains unclear. Method: We integrated patient genomics with paired CRISPR activation and interference screens to identify RBP dependencies in ovarian cancer. Transcriptome-wide RNA sequencing defined ILF2-regulated splicing programs. CRIC-seq was applied to map long-range RNA–RNA interactions associated with ILF2. Biochemical mutagenesis and functional assays were used to investigate the roles of ILF2 dimerization and RNA motifs in RNA looping and cancer phenotypes. Results: ILF2 emerged as a core RBP dependency in ovarian cancer and is required for tumor cell proliferation, invasion, and tumor growth. Transcriptome-wide analyses showed that ILF2 preferentially regulates cassette exon splicing. CRIC-seq revealed that ILF2 mediates long-range RNA–RNA interactions that loop pre-mRNAs to control exon inclusion in a positiondependent manner. Mechanistically, ILF2 homodimerization bridges distant RNA regions, while a poly-G motif guides ILF2- associated RNA looping. Disruption of ILF2 dimerization abolishes RNA looping, perturbs splicing regulation, and suppresses tumorigenicity. Conclusion: These findings define an ILF2-centered RNA architectural mechanism that regulates alternative splicing through RNA looping and suggest ILF2 dimerization interfaces as potential therapeutic targets in ovarian cancer.