Osteogenesis is driven by a kinase and alternative splicing supernetwork

RNA splicing networks remodel cell signalling during osteogenesis

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Alternative splicing drives mesoderm lineage-specific interactome remodelling

Introduction

Mesenchymal stem cells (MSCs) are multipotent stem cells which reside in and are maintained throughout adult life by stem cell niches distributed throughout the human body including bone marrow, adipose tissue, liver, and muscle.

Alternative splicing … there exists a network of splicing factors

The effects of alternative splicing at the proteome level is the subject of ongoing research. Recently, several important regulatory paradigms have emerged⚠️.

Protein kinases form one of the largest protein families in humans and play major roles in cellular signalling and signal transduction.

High-throughput analysis of AS and DS from RNA-Seq is a considerable bioinformatic challenge due to difficulties in accurately reconstructing the transcriptome from short reads⚠️(cufflinks) or grouping short reads by their parent transcript ⚠️. Recent breakthroughs in the development of tools have allowed accurate identification and quantification of AS/DS from short-read RNA-Seq in a completely *de-novo* manner⚠️(leafcutter). Abandoning transcriptome reconstruction in favour of a local view of transcriptome heterogeneity ⚠️, the concepts of alternative and differential transcripts have been recently re-defined as Local Splice Variants (LSVs) ⚠️(MAJIQ). LSVs are regions of the transcriptome with multiple possible transcript topologies, necessarily bound by two constitutive exonic regions except when directly adjacent to the 5’ cap or 3’ poly A tail. LSVs are pertinent as the insights offered by short-read RNA-Seq are largely local⚠️.

Here, we used two recently developed *de-novo* DS detection tools, JUM⚠️ and PSI-Sigma⚠️, to detect and quantify transcriptome-wide LSV changes in differentiating MSCs using RNA-Seq. We show that the functional consequences of alternative splicing are coherent, with many AS events validated in the proteome. Using CLIP-Seq to profile the transcriptome-wide binding sites of upstream SFs, many of which have been previously uncharacterised, we dissect the mechanistic regulation of alternative splicing events observed. The degree of cross-regulation between SF and kinase networks was investigated using SILAC-based temporal profiling of the phosphoproteome. Extensive crosstalk between SFs and kinases constitutes a highly connected network of networks – a “supernetwork” which signals for osteogenesis.