Osteogenesis is driven by a kinase and alternative splicing supernetwork

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 or grouping short reads according to the transcript from which they were derived ⚠️. 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). As such, the concepts of alternative or differential transcripts have been recently re-defined as Local Splice Variants (LSVs) ⚠️(MAJIQ). LSVs are regions in the transcriptome with multiple transcript topologies, which means they are necessarily bound by two constitutive exons unless the LSV resides on the ends of the DNA-coding portion of the transcript. The concept of the LSVs is relevant to short-read RNA-Seq as it excels in the reconstruction of local transcript topology and furthermore, recent DS analysis tools have abandoned quantification via transcriptome reconstruction in favour of a localised view of transcriptome heterogeneity ⚠️.

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, reflecting in the overrepresentation of pro-osteogenic genes, transcript regions and domains, some of which were 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 dissected the mechanistic regulation of alternative splicing events observed. Striking changes in the alternative splicing of both SF and kinase transcripts prompted us to investigate the degree of cross-regulation between SF and kinase networks using SILAC-based MS/MS temporal profiling of the phosphoproteome. Extensive crosstalk between SFs and kinases represents a highly connected network-of-networks, that is, a “supernetwork” which signals for osteogenesis.