Adipose splicing plasticity in response to nutrient and inflammatory inputs

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Background: The increasing worldwide prevalence of obesity has become a major public health concern. Obesity elevates the risk of metabolic disorders, including type II diabetes and cardiovascular disease. Given its high prevalence, healthcare burden and contribution to global morbidity, a deeper understanding of the molecular mechanisms underlying obesity and insulin resistance is essential for developing effective therapies. Method: To understand how dysfunction-associated changes emerge in vivo, we first analysed differentially expressed genes in perigonadal white adipose tissue (pgWAT) from C57BL/6 mice fed for 6- or 12-week high-fat diet, where pathway analysis revealed an enrichment of the spliceosome pathway, particularly after longer dietary interventions. RNA splicing is a fundamental mechanism regulating gene expression in eukaryotic cells, yet its modulation by macronutrients in pgWAT remains poorly understood. To investigate mechanistically alternative splicing (AS) in adipocyte dysfunction, we established an in vitro model of chronic obesity using 3T3-L1 adipocytes exposed to stimuli mimicking obesity and/or insulin resistance conditions, including TNFα to induce inflammation, hyperglycaemaia and hyperinsulinemia. Results: Splice-switching oligonucleotides were used to probe the functional impact of six identified TNFα-induced AS events, including Spag9 exon 30, Protein A exon X and Protein B exon G. ASO-mediated skipping of Protein A exon X impaired insulin-stimulated glucose uptake in 3T3-L1 adipocytes. ASO-mediated skipping of Spag9 exon 30 increased JNK activation, while ASO-mediated skipping of Protein B exon G decreased it. Moreover, we identified regulators of the splicing of the selected inflammation-induced events via downregulation of three wellknown splicing factors: Rbfox2, Srsf3 and Mbnl1. Conclusion: Together, our data identify AS events in obesity and/or insulin resistance conditions and their upstream regulators as potential RNA-based therapeutic targets in obesity-associated metabolic disease.