Regulation of alternative splicing in obesity and weight loss

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Alternative splicing (AS) is a mechanism by which multiple mRNA transcripts are generated from a single gene. According to recent reports approximately 95–100% of human multi-exon genes undergo AS. This increases the amount of functionally different protein isoforms, and in some cases leads to metabolic diseases. Herein we provide a brief overview of the basic aspects of splicing regulation in obesity and insulin resistance with specific examples. In addition, we review our recent findings demonstrating that weight loss regulates AS of TCF7L2 gene in both liver and adipose tissue, and that this splicing associates with changes in fatty acid and glucose metabolism. Future studies using global analysis of transcript variants and splicing regulators are needed for exploring the association of AS with metabolic alterations in obesity and type 2 diabetes (T2D). Understanding of the molecular mechanisms behind the aberrantly spliced transcripts may also provide opportunities for new diagnostic approaches.

Introduction

The relatively small number of protein coding genes creates the need for the generation of multiple proteins from one gene. Alternative splicing (AS) is a mechanism by which multiple transcripts are generated from a single gene. According to recent reports approximately 95–100% of human multi-exon genes undergo AS, compensating for the low number of genes present.1 AS increases the amount of protein isoforms leading to changes in interactions of proteins with other proteins, nucleic acids and membranes. Localization and enzymatic properties may also differ between isoforms.2

Regulation of Alternative Splicing

Precise and efficient removal of introns from pre-mRNA is a complicated process that requires recognition of RNA sequence elements by protein regulators. Regulatory elements in pre-mRNA sequence (exonic or intronic) exist in the form of splicing enhancers and silencers. These sequences are recognized by serine/arginine-rich splicing factors (SR proteins) and heterogeneous ribonucleoproteins (HNRNPs). Together with five small nuclear ribonucleoprotein particles (snRNPs) SR proteins and HNRNPs form the spliceosome. In most cases splicing of introns is efficient and leads to constitutive splicing of introns from pre-mRNA when mRNA is formed. AS occurs when the recognition of sequence elements is less efficient and thus splicing may depend on the abundance and activity of SR proteins and HNRNPs. SR proteins are generally considered to activate splicing whereas HNRNPs repress splicing. Typically, AS is tissue-specific, demonstrating that despite similar sequence elements splicing is regulated by variable function of splicing regulators in different tissues.

Sequence-dependent regulation of splicing. AS can be caused by point mutations within splicing enhancers and silencers in a gene.3 At least 15% of all point mutations responsible for genetic diseases have been estimated to affect splicing.4 In fact, a large number of human diseases result from abnormal regulation of AS.5 Genes involved in metabolism have also been described to have important splicing events. For instance, mutations in the ABCA1 gene, associated with Tangier disease and familial HDL deficiency,7 and in the GPD1 gene, associated with hereditary hypertriglyceridemia,8 result in aberrantly spliced mRNA variants and in truncated protein isoforms. In addition, single nucleotide polymorphisms (SNPs) have been shown to affect AS. For example, common SNP in the gene encoding HMG-CoA reductase (HMGCR), associated with LDL-cholesterol levels, change splicing and lead to altered enzymatic activity.9 Recently, we reported, that a common variant of the GIP receptor (GIPR) gene, influences splicing resulting in decreased expression of variant coding for fully active receptor.10 Finally, it has been suggested that SNPs associated with obesity at multiple loci are located near to sequences regulating AS,11 suggesting that AS may be one mechanism how obesity risk SNPs lead to disease. Importantly, AS regulated by the SNPs in the obesity loci may also be affected by changes in regulatory proteins that may be linked with environmental signals.

Environmental-dependent regulation of splicing. AS is regulated by physiological signals, allowing an organism to adapt to the alterations in the environment. For example, stress induces changes in AS of SMG-P12 and exposure to medications and chemicals can modulate splicing.13 Interestingly, AS was recently reported to be controlled by circadian clock and feeding in mice,14 suggesting that very common factors regulate splicing.

Although the exact pathways regulating the activity of regulatory proteins are only partially known, it is important to note
The insulin receptor exists in two alternatively spliced isoforms. INSR-A promotes growth while INSR-B regulates glucose homeostasis (for a review see ref. 18). AS of low density lipoprotein receptor, LDLR, triggered by an intronic mutation results in a isoform with impaired function due to misfolding of the protein.

In addition, AS of several genes has been reported to be important in adipogenic pathways: PPARγ, Pref-1, MC2R, and ACBP. Peroxisome proliferator-activated receptor gamma (PPARγ) is a key regulator of adipogenesis and is expressed in two alternatively spliced isoforms, PPARγ1 and PPARγ2. PPARγ2 exhibits higher ability to induce adipogenesis in response to low ligand concentrations. Pref-1 (Pref-1) is specifically expressed in preadipocytes but not in adipocytes. There are four major alternative splicing products of Pref-1 (Pref-1 A–D). Only Pref-1A and Pref-1B severely inhibit adipogenesis whereas Pref-1C and Pref-1D do not affect adipocyte differentiation. The melanocortin 2 receptor (MC2R) stimulates lipolysis in mouse adipocytes. Additional splice variant, including exon 3, was detected during adipogenesis. Acyl-CoA binding protein (ACBP), a key player in fat metabolism, is expressed in various alternative isoforms. Splicing pattern of ACBP changes during differentiation in SGBS human cell strain, suggesting particularly important function of ACBP-1C isoform in lipogenic processes during fat cell differentiation in humans.

We recently published that expression of several RNA processing genes was decreased in liver and skeletal muscle of obese humans, suggesting that there may be a common dysregulation of AS in obesity. More specifically, 46 out of 199 RNA splicing genes were downregulated in the liver, and 41 were downregulated in the skeletal muscle. Downregulation of RNA processing and splicing genes was also observed in a mouse model of diet-induced obesity. Downregulation of splicing factor correlated with insulin levels suggesting that it may be related to hyperinsulinemia in obesity. The specific example in our publication demonstrated downregulation of splicing factor SFRS10 in both obese individuals and in a mouse model of diet-induced obesity. Reduction in SFRS10 associated with hyperinsulinemia, increased lipogenesis and cellular accumulation of triglycerides. Reduction of SFRS10 expression led to increased expression of the lipogenic β isoform of LPIN1, described above, both in cells and in SFRS10 heterozygous knockout mice. In fact, the lipogenic effect of SFRS10 siRNA in HepG2 cells depended on LPIN1 splicing.

Alternative Splicing in Obesity and Insulin Resistance

There are many obesity related genes that are regulated by AS, such as Lipin 1 (gene LPIN1), insulin receptor (INSR), low density lipoprotein receptor (LDLR), and GIPR described above. LPIN1 has two isoforms (α and β) based on AS of exon 6. The β isoform is generated by the inclusion of exon 6 and associates with increased expression of lipogenic genes while the α isoform, excluding exon 6, promotes proliferation. The insulin receptor exists in two alternatively spliced isoforms. INSR-A promotes growth while INSR-B regulates glucose homeostasis (for a review see ref. 18). AS of low density lipoprotein receptor, LDLR, triggered by an intronic mutation results in a isoform with impaired function due to misfolding of the protein.

Figure 1. Proposed mechanisms for changes in alternative splicing in obesity. Changes in phosphorylation (p in the figure) and transcription of serine/arginine-rich splicing factors (SR in the figure) may alter splicing leading to protein isoforms with different function. These effects may interact with single nucleotide polymorphisms (SNPs) in critical intron-exon boundaries. Dashed arrows refer to our own recently published data in Cell Metabolism.
adipogenesis. mTOR has been identified as a splicing target of Sam68, the Src-associated substrate during mitosis of 68 kDa. Sam68 acts as an intronic splicing enhancer for mTOR. Sam68-deficient mice have been recently reported to exhibit a lean phenotype and resistance to obesity induced by diet. In this model depletion of Sam68 resulted in altered splicing of mTOR and expression of truncated form of mTOR.25

Interestingly, AS does not only regulate the accumulation of fat but also the type of lipids accumulated. Δ6 desaturase, encoded by FADS2, plays an essential role in omega-3 and omega-6 fatty acid synthesis is regulated by AS of exons 1–4. HNRNP I, a known regulator of AS in several genes, has been shown to alter splicing of FADS2, by binding to exonic splicing silencer, favoring expression of variant FADS2AT1, characterized by truncation of exons 1 and 4 and skipping of exons 2 and 3. Knockdown of HNRNP I in HepG2 cells was shown to result in a decrease of both omega-3 and omega-6 fatty acids and change in fatty acids profile.26

**Alternative Splicing of TCF7L2 in Response to Weight Loss**

TCF7L2 is a transcription factor playing an essential role in canonical Wnt signaling pathway. Common intronic variants within TCF7L2 gene have been associated with type 2 diabetes (T2D) but the molecular mechanisms how variations in the gene lead to increased risk of developing T2D have remained elusive. SNP at rs7903146, that is associated with increased T2D risk, has been shown to alter splicing of FADS2, by binding to exonic splicing silencer, favoring expression of variant FADS2AT1, characterized by truncation of exons 1 and 4 and skipping of exons 2 and 3. Knockdown of HNRNP I in HepG2 cells was shown to result in a decrease of both omega-3 and omega-6 fatty acids and change in fatty acids profile.26

AS of TCF7L2 has also been shown to explain the association between TCF7L2 SNPs and impaired TCF7L2 function in T2D. Tissue-specific splicing pattern of TCF7L2 in several human tissues (pancreas, pancreatic islets, colon, liver, monocytes, primary lymphocytes, skeletal muscle, subcutaneous and visceral adipose tissue, peripheral blood mononuclear cells, and lymphoblastoid cell lines) has been described. In several studies the association between T2D risk genotypes and TCF7L2 splicing pattern has been investigated. Most of the studies found no association between splicing and genetic variants.28–30 Mondal et al. found an association between rs7903146 genotype and TCF7L2 splicing pattern in adipose tissue31 and study by Prokunina et al. demonstrated an association between splicing and genetic variants.32–34 We were invited to write this mini-review due to our recent findings linking obesity and weight loss related regulation of AS with the risk of T2D.40 Mondal et al. suggested a link between TCF7L2 splicing in subcutaneous fat and obesity, but the authors acknowledged that the significance level was marginal and results were not corrected for multiple correlated traits and splice variants.35 Because it is known, that inhibition of Wnt signaling induces adipogenesis and that protein isoforms generated by
alternative splicing of TCF7L2 exhibit different ability to activate Wnt signaling pathway we investigated AS of TCF7L2 in the context of obesity surgery induced weight loss, and the association of TCF7L2 splicing with the levels of plasma glucose and serum free fatty acids (FFAs) in three independent cohorts (n = 216). Moreover, the possible association between splicing pattern and common SNP variant associated with T2D was assessed. Expression of the shorter mRNA variant, lacking exons 12, 13, and 13a (Fig. 2), was reduced after weight loss both in subcutaneous fat (n = 46) and also in the liver (n = 11). Additionally, the short mRNA variant was more common among individuals with type 2 diabetes than in subjects with normal glucose tolerance and there was a positive correlation between this variant with type 2 diabetes than in subjects with normal glucose tolerance and there was a positive correlation between this variant with hyperglycemia and impaired insulin action in adipose tissue (Fig. 2), whereas no association with insulin secretion or insulin-stimulated whole body glucose uptake was observed. Interestingly, the expression of short variant increased during adipocyte differentiation in SGBS cell strain with known inhibition of Wnt signaling.42 No association between splicing and common risk SNP was detected, which can be explained by relatively small sample size and by fact that the common genetic variant is linked to T2D and not to obesity. No change in total TCF7L2 expression was found leading to the conclusion that effect on splicing is independent of regulation of transcription.

In summary, our study showed that the short TCF7L2 mRNA variant in subcutaneous fat is regulated by weight loss and is associated with hyperglycemia and impaired insulin action in adipose tissue. These results suggested that the regulation of TCF7L2 splicing in adipose tissue by weight loss may be important in adipocyte biology, leading to increased diabetes risk (Fig. 2).

Future Perspectives

AS is a common regulatory mechanism that increases the amount of biologically significant protein isoforms. It can be regulated both by genetic variation and environmental cues, as demonstrated in this mini-review and elsewhere.3 Therefore, regulation of AS should also be investigated in metabolic disease. Large cohorts of tissue biopsies from individuals with detailed clinical phenotype will be needed for exploring the association of AS with metabolic alterations in obesity and T2D. In combination with genome wide association studies, exploring the genetic risk of diseases, these databases can be utilized to investigate potential gene–environment interactions behind AS and metabolic diseases.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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