Stay lean without dieting
Lose Sam68

Marc-Étienne Huot1, and Stéphane Richard2
1Laval University Cancer Research Center; Hôtel-Dieu de Québec; CHUQ; Quebec City, QC Canada; 2Terry Fox Molecular Oncology Group and Bloombfield Center for Research on Aging; Lady Davis Institute for Medical Research and Departments of Oncology and Medicine; McGill University; Montreal, QC Canada

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Introduction

Alternative splicing is well known to be tissue-specific. Although several genes have been shown to undergo alternative splicing in adipocytes, little is known about the mechanism that regulates alternative splicing during adipogenesis. We recently reported that Sam68−/− mice exhibit a lean phenotype and are protected against diet-induced obesity. Our genome-wide exon array analysis in white adipose tissue (WAT) from wild-type and Sam68−/− mice revealed that Sam68 deficiency leads to an abnormal splicing of the mTOR gene. This has been shown to reduce the overall mTOR protein content and activity during in vitro adipose differentiation. In Sam68−/− mice, this situation leads to an increased energy expenditure, decreased adipogenesis and WAT formation.

The Src-associated in mitosis 68 kDa protein (Sam68) is a member of the signal transduction and activation of RNA (STAR) family of proteins.1 The recent identification of Sam68-interacting proteins has shown that Sam68 is involved in several signal transduction pathways via its association with SH3- and SH2-containing signaling molecules, suggesting that Sam68 is an adaptor protein.1-3 The trademark of all STAR proteins is the presence of a single hnRNPK homology (KH) domain known to bind RNA with a high relative affinity.4 Sam68 has been shown to bind to a bipartite sequence of U(U/A)AA rich motifs.5,6 Sam68 is a known regulator of alternative splicing. Sam68 has been shown to regulate the inclusion of the variable exon v5 of CD44,7 a cell surface glycoprotein involved in tumor invasion.7,8 Sam68 also modulates the splicing of mRNAs encoding SF2/ASF, SMN2, the proapoptotic protein Bcl-x, the cancer-related splice isoform cyclin D1b and the neurexin 1 gene.10-13 More extensive studies have recently defined Sam68 as major regulator of neurogenesis,10,14 spermatogenesis,15 osteogenesis16 and more recently, adipogenesis.5 These studies clearly indicate that Sam68 regulates alternative splicing during cellular differentiation.

The availability of Sam68-deficient mice has led to the identification of some of the physiological roles of this KH-type RNA-binding protein. Sam68−/− mice do not display any overt phenotype, but male mice are infertile due to a spermatogenesis defect, while the females have reduced fertility due to defects in the proper expression of gonadotropin receptor transcripts in pre-ovulatory follicles in the adult ovary.15-18 Moreover, Sam68−/− mice are protected against age-induced osteoporosis.16 Indeed, aged Sam68−/− mice have been shown to preserve bone density via the Sam68-dependent promotion of osteoblast differentiation and are thus protected against age-related bone loss.16 These results suggest that Sam68 is directly involved in mesenchymal stem cell differentiation. The loss of Sam68 expression shifts mesenchymal stem cell differentiation toward the osteogenic lineage, instead of the adipocytic lineage. This was confirmed by our recent demonstration that Sam68-deficient mice are lean and protected against diet-induced obesity.5
Sam68 and Adipogenesis

To confirm a role for Sam68 in the regulation of adipocyte differentiation, we initially characterized the primary MEFs harvested from Sam68+/− and Sam68−/− embryos. Adipogenesis was monitored at days 0, 4, 6 and 12 following initial stimulation with standard adipogenic differentiation media. As expected, we found that primary Sam68−/− MEFs were unable to differentiate into adipocytes, unlike their Sam68+/− counterparts. Magnetic resonance imaging studies of both Sam68+/+ and Sam68−/− aging mice showed that Sam68 inactivation affects mouse weight independently of food consumption. We also determined the respiratory exchange ratio, which was found to be similar in Sam68+/+ and Sam68−/−, suggesting that both mouse genotypes primarily utilize fatty acids as an energy source. On the other hand, we have shown that Sam68−/− mice exhibit a higher energy expenditure than Sam68+/+ mice, likely due to increased physical activity, which contributes to the lean phenotype. This difference in whole body weight was more striking when mice were fed a high-fat diet. The latter experiment clearly showed that Sam68−/− mice are unable to gain weight under a high-fat diet, whereas the wild-type littermates significantly increased in weight. In-depth analysis of adipose tissue revealed that Sam68−/− mice had a reduced number of adult-derived stem cells (i.e., adipogenic progenitors), correlating with a decrease in the expression of pericyte markers (α-SMA and NG2). Taken together, these results show that Sam68 is required for normal adipogenesis.

mTOR Splicing

Using a genome-wide exon expression array, we found that the loss of Sam68 influences the splicing of a large number of genes in white adipose tissue. We showed that the splicing of the mTOR gene is greatly influenced by the levels of Sam68. mTOR belongs to the phosphatidylinositol 3-kinase-related protein family and is known to regulate key cellular processes, such as cell size, cell proliferation, cell motility and cell survival. mTOR is the catalytic subunit found in two different complexes (mTORC1 and mTORC2). mTORC1 is characterized by its association with the protein Raptor and its sensitivity toward rapamycin, whereas mTORC2 is characterized by the presence of the protein Rictor and its resistance to rapamycin. The inhibition of mTORC1 is known to prevent the adipogenic differentiation of pre-adipocytes, and to downregulate fat deposition in rodents. Moreover, the inhibition of mTORC1 signaling in mice leads to lean phenotypes.

For the above-mentioned reasons, we decided to investigate the Sam68-dependent alternative splicing of mTOR in greater detail. We found that the loss of Sam68 activates the usage of an intronic polyadenylation signal within intron 5 of the mTOR gene. This novel isoform that we designate as mTORi5, includes exons 1 to 5 plus a readthrough of intron 5, thereby generating a ~1 kb mRNA (Fig. 1A). We showed that Sam68 associates with two short UUUUA sequences within intron 5. Mutations within each of the two UUUUA elements of the sequences influence Sam68 binding and lead to an increase in mTORi5 isoform protein level.

mTORi5

Using qRT-PCR, we observed that mTORi5 mRNA is abundant in Sam68-deficient 3T3-L1 cells but absent in control 3T3-L1 cells. As mTOR intron 5 harbors an in-frame premature termination codon as well as a polyadenylation signal, the intron retention event is expected to lead to an mTOR protein of ~25 kDa with a large C-terminal truncation. The protein predicted to be expressed from the mTORi5 mRNA would contain most of the N-terminal HEAT domains, a type of protein-protein interaction domain found in a number of cytoplasmic proteins. This led us to believe that the mTORi5 protein might exhibit dominant-negative behavior via its binding to a component of the mTORC1/2 complexes, thus forming a catalytically inactive complex. To assess this possibility, we introduced a vector that expresses a FLAG-tagged mTORi5 protein in HeLa cells. We then monitored the activity of the mTORC1/2 complexes, using ribosomal protein S6 phospho-specific antibodies (to detect mTORC1 activity) and AKT phospho-specific antibodies (to assess mTORC2 activity). Although the ~25 kDa FLAG-mTORi5 protein was effectively expressed in these cells, we found that it had virtually no influence on the levels of either ribosomal protein S6 S240/S244 or AKT S473 phosphorylation following insulin stimulation. Moreover, we were unable to detect the mTORi5 protein in cells in which Sam68 expression was abrogated using N-terminal-specific commercial antibodies that recognized the ectopic expression of FLAG-mTORi5. These results allowed us to rule out any dominant-negative function for the mTORi5 protein and suggested that the mTORi5 mRNA or protein might be rapidly degraded via an as yet unidentified mechanism.

Sam68 Regulates mTOR Activity

One major effect of generating the mTORi5 isoform is a decrease in mTOR mRNA and protein levels. Since, mTORC1 is essential for normal adipogenesis, and as depletion of the major component of the complex, namely mTOR, affects pre-adipocyte differentiation, a deficiency in Sam68 expression leads to a decrease in mTORC1 activity. The overall effect of reduced mTORC1 activity is an inhibition of pre-adipocyte differentiation (Fig. 1B). Interestingly, we were able to pre-empt the latter phenotype by reintroducing full-length mTOR in Sam68-deficient 3T3-L1 cells. The latter result indicates that Sam68 contributes in adipogenesis by insuring the proper splicing of intron 5 in the course of mTOR mRNA synthesis, thus allowing the expression of full-length mTOR.

Conclusion

Our work suggests that Sam68 is a key regulator of alternative splicing in white adipose tissue. Our results offer new therapeutic opportunities for blocking adipogenesis and diet-induced obesity, which could prevent the long-term complications related to diet-induced obesity, one of the leading preventable causes of death in North America.
Figure 1. Schematic representation of the function of Sam68 during adipogenesis. (A) Effect of Sam68 on mTOR mRNA splicing. Sam68 inactivation or impairing its binding to specific elements present in mTOR intron 5 increases read-through and inclusion of intron 5, thereby activating an intronic polyadenylation signal. This leads to a novel variant of mTOR mRNA whose translation predicts the synthesis of a truncated version of mTOR, termed mTORi5. (B) Sam68 inactivation blocks the progression of adipogenesis at the preadipocyte level, due to a decreased pool of full-length mTOR protein, which results in the lack of mTORC1/2 activation.
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