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Regulation of Lipid and Glucose Metabolisms with ADD1/SREBP1 in Fat Cells

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Obesity and Adipocyte Biology

Obesity has increased at an alarming rate in recent years and in now a worldwide public health problems. Obesity is considered as a major risk factor to cause several serious medical conditions such as hyperlipidemia, hypertension, several cardiovascular disease and non-insulin dependent diabetes mellitus (NIDDM). Obesity results from a disorder of energy balance, occurring when energy intake chronically exceeds energy expenditure. Under normal physiological conditions, the two processes of energy balance, energy intake and expenditure, are tightly linked and controlled. An imbalance results in the deposition of excess energy in the from of increased adipose tissue. Obesity usually involves an increased amount of triglycerides per cell but also often includes an increased number of adipocytes. The most important function of adipose tissue is its ability to store an release fat during periods of feeding and fasting to maintain energy homeostasis in our body. Most of adipocyte differentiation occurs in late prenatal and early postnatal development in distinct anatomical locations. However, the number of adipocytes in an organism does not appear to be firmly fixed. Adipocyte differentiation from precursor cells (preadipocytes) is a process that is regulated in both space and time in response to specific hormonal or nutritional signals.

Despite extensive studies, the precise mechanisms that directly connect adipocyte differentiation to obesity are not well understood. As a first step to investigate these links, it is important to understand the molecular basis of adipogenesis and lipid metabolism. The molecular mechanisms controlling both adipocyte differentiation and fatty acid metabolism are therefore of considerable interest.

ADD1/SREBP1c

ADD1 is a member of basic-helix-loop-helix (bHLH) family of transcription

factors (Tontonoz et al. 1993). The DNA binding site for the bHLH proteins is a well conserved consensus CANNTG nucleotide sequence motif (or E-box) that is present in the regulatory cis-elements of many tissue-specific gene. ADD1 is expressed predominantly in white adipose tissue, brown adipose tissue and liver, and its expression is induced at a very early stage of adipogenesis (Kim and Spiegelman 1996). The human homolog of ADD1 has been independently identified as the sterol regulatory element binding protein (SREBP) 1c. , which binds the sterol regulatory element (SRE) 1 (ATCACCCCAC), a non-E-box DNA motif (Yokoyama et al 1993). This SRE1 motif has been found in the promoters of several genes involved in cholesterol homeostasis (Brown and Goldstein 1997). In fact, the observations relating to the DNA binding capabilities of ADD1 and SREBP1 have been reconciled: ADD1/SREBP1 have an unusual dual DNA binding specificity which includes certain E-box and non-E-box (SRE1) sequence motifs (Kim et al 1995). This dual DNA-binding specificity of the ADD1/SREBP1 homodimer is controlled by a single tyrosine residue at position 320 in the basic domain that replaces a conserved arginine residue found in all other known bHLH proteins. ADD1/SREBP1 contains two transmembrane domains which results in its localization to the endoplasmic reticulum and nuclear membrane (Wang et al. 1994, Kim and Spiegelman 1996). Depletion of cellular cholesterol leads to a specific proteolysis which releases SREBPs from the membrane, allowing them to enter the nucleus and direct transcription of cholesterol-responsive genes (Wang et al. 1994, Brown and Goldstein 1999).

Subsequent studies have demonstrated that there are three major SREBP isoforms encoded by two different genes. Among these isoforms, SREBP1a contains acidic rich amino acids in N-terminus, which makes it a potent transcriptional activation domain. SREPB1c, a human homolog of ADD1, has less transcriptional activity compared to SREBP1a. In liver and adipose tissue, ADD1/SREBP1c mRNA is 9 and 5 fold more abundant than SREBP1a respectively (Shimano et al. 1997, Shimomura et al. 1997).

ADD1/SREBP1c in Adipocyte Differentiation

ADD1 plays a role in adipocyte differentiation per se (Kim and Spiegelman 1996, Shimano et al. 1996). Overexpression of ADD1/SREBP1c in 3T3-L1 cells in the presence of hormonal inducers of differentiation results in elevated adipocyte marker gene expression and lipid accumulation as compared to control cells. On the other hand, ectopic expression of wild-type ADD1/SREBP1c in undetermined fibroblasts

results in some adipose conversion, but only under conditions strongly permissive for adipogenesis. Interestingly, expression of a dominant-negative ADD1/SREBP1c (a non-DNA-binding mutant targeted to the nucleus) completely abolishes the ability of preadipocytes to undergo differentiation and significantly represses adipocyte-specific gene expression (Kim and Spiegelman 1996).

The effects of ADD1/SREBP1c on adipocyte differentiation are probably due, at least in part, to an influence on PPARY activity, as cotransfection of PPARY with ADD1 causes a threefold to fivefold increase in the transcriptional activity of PPARY through the PPARY DNA-binding site (Kim and Spiegelman 1996). This augmentation of PPARY transcriptional activity could occur through several mechanisms, but the effects of ADD1/SREBP1 on the genes of fatty acid metabolism suggest that the connection could be through the generation of endogenous ligands (or activators) for PPARY. This notion was corroborated by the finding that the block in adipocyte differentiation mediated by dominant-negative ADD1/SREBP1c could be completely overcome by addition of a ligand for PPARY(Kim et al. 1998a). Further support came from the observation that conditioned media from fibroblasts transfected with ADD1/SREBP1c contained a factor(s) that could both bind to and activate a GAL4-PPARY ligand binding domain fusion protein. Taken together, these results suggest that ADD1/SREBP1c is involved in the production of an endogenous PPARY ligand or activator(s) (Kim et al. 1998a). More recently, it has been demonstrated that PPARY itself is a direct target gene of ADD1/SREBP1c, providing alternative but not mutually exclusive mechanisms by which ADD1/SREBP1c and PPARY could cooperate to enhance adipogenesis (Fajas et al. 1999).

In addition, ADD1/SREBP1c has an important role in the regulation of fatty acid metabolism rather than cholesterol homeostasis. It has been demonstrated that ADD1/SREBP1c stimulates the expression of FAS (fatty acid synthase), LPL (lipoprotein lipase), GPAT (glycerol-3-phosphate acyl transferase), SCD (stearoyl coA desaturase) 1 & 2 in several cell lines (Kim and Spiegelman 1996, Ericsson et al. 1997, Tabor et al. 1998, Tabor et al. 1999). In contrast, it appears that SREBP2 is more selective for genes involved in directly in cholesterol homeostasis.

ADD1/SREBP1c in Insulin Action

The other important function of ADD1/SREBP1c in vivo is that ADD1/SREBP1c mediates the transcriptional effect of insulin in adipocytes and liver tissue. Insulin levels rise postprandially to mediate anabolism and energy storage and

fall between meals to permit the release of adipose energy stores and the switch from glucose use to production. The relevant actions of insulin take places in adipose tissue muscle and liver where insulin receptor signaling exerts tissue-specific effects on metabolic pathways that impact on carbohydrate, lipid and protein metabolism. It has been demonstrated that expression of ADD1/SREBP1c mRNA was suppressed by fasting and restored by feeding in adipose tissue that closely paralleled the expression of FAS and leptin (Kim et al. 1998b). As a nutrition sensitive hormone, insulin significantly stimulates the expression of ADD1/SREBP1c mRNA in adipose and liver tissue (Kim et al. 1998b). Especially, dominant negative ADD1/SREBP1c blocks the ability of insulin to induce the glucokinase gene (Foretz et al, 1999a, Foretz et al. 1999b), and ADD1/SREBP1c mRNA was dramatically diminished in the liver of mice made diabetic with the βcell toxin streptozotocin (Shimomura et al. 1999). Taken together, ADD1/SREBP1c appears to be a strong candidate to be a general mediator of the action of insulin to regulate metabolism via effects on gene expression.

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