Review

The Mediator Complex and Lipid Metabolism

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Abstract: The precise control of gene expression is essential for all biological processes. In addition to DNA-binding transcription factors, numerous transcription cofactors contribute another layer of regulation of gene transcription in eukaryotic cells. One of such transcription cofactors is the highly conserved Mediator complex, which has multiple subunits and is involved in various biological processes through directly interacting with relevant transcription factors. Although the current understanding on the biological functions of Mediator remains incomplete, research in the past decade has revealed an important role of Mediator in regulating lipid metabolism. Such function of Mediator is dependent on specific transcription factors, including peroxisome proliferator-activated receptor-gamma (PPAR\textgamma) and sterol regulatory element-binding proteins (SREBPs), which represent the master regulators of lipid metabolism. The medical significance of these findings is apparent, as aberrant lipid metabolism is intimately linked to major human diseases, such as type 2 diabetes and cardiovascular disease. Here, we briefly review the functions and molecular mechanisms of Mediator in regulation of lipid metabolism.

Keywords: mediator, transcription, lipid, cofactor, metabolism

1. Introduction

To maintain normal functions, all living systems demand a precise control of gene expression. Dysregulation of gene expression may disrupt normal biological processes and often causes diseases in mammals. The expression level of a gene can be regulated at multiple steps, including transcription initiation and elongation, mRNA processing, translation, and protein stability. However, regulation at the step of transcription initiation is conceivably the most efficient in controlling the abundance of a gene and thus is critical for most genes. During transcription initiation, DNA-binding transcription factors decide which genes to be expressed, and the basal transcription machinery, which contains RNA Polymerase II (Pol-II) and a group of general transcription factors, are involved. In addition to these essential elements, transcription cofactors are usually required for modulating the gene expression quantities in eukaryotes [1]. One of the highly conserved transcription cofactors is the multi-subunit Mediator complex, which can bridge several transcription factors to Pol-II [1,2], and therefore, Mediator is likely involved in many biological processes. Mutations of Mediator subunits have been reported in diseases, such as cancer [3-6] and mental diseases [7,8].

Due to the prevalence of obesity, metabolic diseases are more common in modern society than before. Obesity is closely associated with major human diseases such as type 2 diabetes mellitus (T2DM), non-alcoholic fatty liver disease (NAFLD) and cardiovascular disease (CVD) [9]. According to the United States Center for Disease Control (CDC), nearly 24 million people in the United States had diabetes in 2007, and strikingly, up to 70% of diabetic patients were also diagnosed with NAFLD. In fact, a study reported that about one-third of the US population had NAFLD [10]. CVD is the leading cause of death in US. Data from the CDC show that currently one in every three deaths is from heart disease or stroke in US. One common feature of these obesity-associated diseases is dysregulation of lipid metabolism [9,11,12]. Although regulation of lipid metabolism is complicated, past research has identified some key regulators in this process, including transcription factors PPAR\textgamma [13] and SREBPs [14-16]. Recent studies have shown that Mediator is a pivotal cofactor for these transcription factors, suggesting a role of Mediator in lipid metabolism. In this short review, we will focus on the function of Mediator in adipogenesis and lipogenesis, and will also briefly discuss the roles of Mediator in other aspects of lipid metabolism.
2. Mediator and gene expression

Mediator is a multi-protein complex, and was first purified from yeast as a transcription cofactor that is required for transcription activators, such as VP16, -dependent transcription [17-19]. Similar complexes were later purified from mammalian cells as key transcription cofactors for a number of transcription factors, including nuclear hormone receptors and SREBPs [20-24]. Although not all Mediator subunits are present in the same purified complex, there are more than 30 distinct proteins (MED1 – MED31 etc.) belonging to the mammalian Mediator and most of them are conserved among various species, including yeast, C. elegans, Drosophila, human, mouse and plants [1]. Biochemical analyses have identified at least two distinct Mediator complexes: the small-Mediator that can stimulate transcription of Pol-II-dependent genes in vitro, and the large-Mediator that are inactive or repressive in vitro [25]. The large-Mediator has a kinase sub-module containing four subunits of cyclin-dependent kinase-8 (CDK8), Cyclin C (CycC), MED12, and MED13 [25]. Recent studies in cultured cells and in model organisms in vivo suggest that the Mediator subunits of the kinase sub-module can function to repress or activate gene transcription, depending on the biological contexts [1,2,26]. Since Mediator can interact with Pol-II, it was initially believed that this complex regulates all Pol-II-dependent genes. However, a number of studies have shown that several subunits display surprising pathway-specific functions in vitro, although in many cases the same subunits can also be recruited to the promoters of many other genes as parts of the Mediator complex [1]. The molecular basis of such specificity remains poorly understood, but it is likely due to the specific interactions between the subunits and the transcription factors that are critically involved in the specific biological pathways. In addition, not all transcription factors can recruit the Mediator complex. For example, the transactivation domain of Myb transcription factor does not interact with Mediator in vivo [27]. Furthermore, the specific biological contexts also determine the presence or activation of a unique set of transcription factors. In addition to the roles in transcription initiation, recent studies also revealed some interesting functions of Mediator in some later steps of gene expression, i.e. elongation, termination and pre-mRNA processing [2].

3. Mediator and adipogenesis

Adipogenesis is an important aspect of lipid metabolism. Increased prevalence of obesity has attracted great interest in understanding adipogenesis, a developmental process of forming adipocytes from precursor cells or pre-adipocytes. A transcriptional cascade including at least two waves tightly controls adipogenesis [28]. In the first wave, extracellular adipogenic stimuli activate early adipogenic transcription factors, including CCAAT/enhancer-binding proteins (C/EBP-β and -δ), Kruppel-like factors (KLFs), early growth response 2 (Krox20), cAMP response element binding protein (CREB), SREBP-1c [28]. These transcription factors in turn activate the expression of the nuclear receptor PPARγ and C/EBPα in the second wave, and lead to the formation of mature adipocytes with the accumulation of lipid droplets [28]. Both in vitro and in vivo studies have demonstrated that PPARγ and C/EBPs are key regulators of adipocyte differentiation [29].

Nuclear receptors, such as PPARγ, estrogen receptor (ER), thyroid hormone receptor (TR), and vitamin D receptor (VDR), interact with the MED1 subunit (initially called TRAP220, DRIP205, ARC205, or PBP) of Mediator through the LXXLL motifs in a ligand-dependent manner [1]. Mouse embryonic fibroblasts (MEFs) derived from MED1-knockout embryos display defects on the expression of PPARγ-target genes and PPARγ-stimulated adipogenesis, suggesting that MED1 is required for adipogenesis [30]. Interestingly, PPARγ can also induce adipogenesis independently of the LXXLL motifs of MED1 [31], suggesting that Mediator can also be recruited to the promoters of PPARγ target genes independently of MED1. A later study shows that such recruitment is mediated through the MED14 subunit [32]. MED14 directly interacts with the N-terminus of PPARγ in a ligand-independent manner, and is required for the transcription activity of PPARγ [32]. In 3T3-L1 cells, MED14 knockdown represses adipogenesis [32]. In addition to the role in white adipocyte development, MED1 may also play roles in brown adipose tissues through activating brown adipocyte-specific UCP-1 [33]. MED1 in cooperation with another cofactor of PPARs, PGC-1α, dynamically regulates PPARγ and TRα -mediated activation of UCP-1 in brown adipocytes [33].

The MED23 subunit (initially called Sur2 or CRSP130) of Mediator directly interacts with the transactivation domains of the adenovirus E1A protein and the mitogen-activated protein kinase–regulated transcription factor Elk-1 [34]. Recent studies show that MED23 plays a critical role in deciding multi-potent mesenchymal stem cells differentiation into smooth muscles or adipocytes [35]. MED23-knockout MEFs are defective of insulin-induced adipogenesis in vitro [36]. Mechanistically, loss of MED23 or its interacting transcription factor Elk1 inhibits the transcriptional induction of Krox20, an insulin-stimulated immediate early gene during adipogenesis [36]. The adipogenic defect in MED23-deficient cells can be rescued by over-expression of Krox20 or one of its downstream transcription factors, C/EBPβ or PPARγ [36], although a previous study has shown that MED23 interacts with C/EBPβ and is functionally required for C/EBPβ-mediated transcription [37]. Thus, research has revealed important roles of MED1, MED14 and MED23 in activating the adipogenesis program.

4. Mediator and lipogenesis

The SREBP transcription factors are master regulators for lipid biosynthesis, and belong to the basic helix-loop-helix leucine zipper (bHLH-Zip) family [15]. SREBP activation is regulated at multiple steps, including gene transcription, proteolytic processing of precursor maturation, nuclear protein stability and transcriptional activity [38,39]. The transcriptional activity of mature SREBPs is controlled through recruiting
specific transcriptional cofactors, including CBP/p300 [40] and the Mediator complex [20,27].

Nuclear magnetic resonance (NMR) studies have shown that the MED15 subunit (initially called ARC105 or PCQAP) of Mediator harbors a domain that is conserved and highly similar to the KIX domain of CBP/p300 in structure [27,41]. The MED15-KIX domain is the docking surface on the Mediator complex for the physical and functional interaction with the transactivation domains of SREBPs [27]. Interestingly, the MED15-KIX domain is more selective in recognizing transcription factors than the CBP-KIX domain, which interacts with many transcription factors including SREBPs, Myb, CREB and NF-κB/p65. In cultured cells and C. elegans, MED15 is required for SREBP-target gene transcription [27]. In C. elegans, MED15 regulates the biosynthesis of oleic acids and subsequent biological phenotypes, and the defects caused by MED15-knockdown can be rescued by supplementation of oleic acids in diets [27]. Thus, in vitro and in vivo data support a pivotal role of MED15 in regulating SREBP-mediated gene expression and lipid biosynthesis.

Recently, we have shown that the CDK8 subunit of Mediator inhibits lipid biosynthesis by promoting nuclear SREBP-1a/1c protein degradation [42]. CDK8 and its activating partner CycC are among the most conserved subunits of Mediator. CDK8 or CycC-null Drosophila larvae display an “obese” phenotype, primarily due to increased lipogenic gene expression and lipid biosynthesis [42]. Loss of function of CDK8 or CycC increases lipogenic gene expression in a SREBP-dependent manner in both Drosophila and mammalian cells, and CDK8 knockdown in mouse liver also caused an elevation of triglycerides in liver and plasma [42], suggesting the importance of hepatic CDK8 in controlling lipid levels. There are three isoforms of SREBPs in mammals. Biochemical analyses reveal that CDK8 can directly phosphorylate SREBP-1c at the conserved Threonine-402 residue (T402) [42], which is known to facilitate the binding to the E3 ligase, SCF<sub>Pbw7</sub>, and thus controls its nuclear SREBP-1c degradation [43]. CDK8 knockdown inhibits poly-ubiquitination and subsequent degradation of nuclear SREBP-1c without affecting the level of mRNA or precursor of SREBP-1c [42]. Thus, CDK8-CycC regulation of lipogenesis is primarily through the control of nuclear SREBP-1c protein stability.

5. Mediator and other pathways of lipid metabolism

Nuclear receptors play critical roles in several aspects of lipid metabolism. MED1 serves as an anchor for the interaction between Mediator and nuclear receptors. MED1 knockout is embryonic lethal by affecting placental, cardiac, hepatic, and bone marrow development [44]. Liver-specific knockout of MED1 in mice first revealed its functions on ligand-dependent activation of PPARα target genes, which are key players in fatty acid oxidation [44]. Liver-specific knockout of MED1 in mice also demonstrated its role in the regulation of hepatic constitutive androstane receptor (CAR), whose target genes are involved in various aspects of steroid and xenobiotic metabolism [45]. MED1 functionally interacts with glucocorticoid receptor (GR) [46]. GR agonist dexamethasone induces hepatic steatosis and enhances CAR expression in the liver. Liver-specific knockout of MED1 in mice abolished dexamethasone-induced hepatic steatosis due to the loss of induction of the GR and CAR-target genes [47]. High-fat diet (HFD) is known to induce fatty liver in mice and is frequently used to generate models for insulin resistance and NAFLD. Liver-specific knockout of MED1 inhibited HFD-induced fatty liver, which is largely due to inactivation of PPARγ [48]. Skeletal muscle-specific knockout of MED1 in mice increased mitochondrial density in white muscles and expression of genes involved in respiratory uncoupling, such as UCP-1 and Cidea [49]. As a result, the knockout mice enhanced insulin sensitivity and glucose tolerance and inhibited HFD–induced obesity [49]. Through regulation of liver X receptors (LXR) [50] and farnesoid X receptor (FXR) [51], MED1 also plays important roles in steroids and bile acids.

Several recent studies have also shown the functions of other Mediator subunits in regulating lipid metabolism. Cardiac-specific knockout and over-expression of MED13 in mice revealed the role of MED13 in metabolic regulation by controlling the activation of transcription factors, such as TR [52]. Through selective activation of hepatocyte nuclear factor 4α (HNF4α)-target genes, MED25 plays a role in the regulation of lipid and xenobiotic metabolism in hepatocytes [53]. In addition, MED25-HNF4α interaction is also critical in regulating glucose-stimulated insulin secretion in β-cells [54]. Mice with a missense mutation in MED30 displayed pleiotropic changes in transcription of cardiac genes required for oxidative phosphorylation and mitochondrial integrity, suggesting the role of MED30 in induction of a metabolic program for oxidative phosphorylation and fatty acid oxidation [55]. Moreover, it has been reported that MED2, MED3, MED15, MED18 and MED19 are required for inositol biosynthesis in yeast through activator protein Ino2 [56], though it is unclear whether these subunits also regulate phospholipid biosynthesis in other organisms.

6. Conclusions

In vitro and in vivo studies have demonstrated the important roles of Mediator subunits in regulating several aspects of lipid metabolism. MED1, MED14, and MED23 are clearly required for the development of adipocytes. MED15, CDK8 and CycC are involved in SREBP-mediated gene transcription and subsequent de novo lipogenesis. The Mediator complex is also involved in other aspects of lipid metabolism, including fatty acid oxidation, phospholipid biosynthesis, and bile acid metabolism. In most cases, the Mediator subunits function as the bridge, in the context of the Mediator complex, between specific transcription factors and the basal transcription machinery. However, CDK8 and its activating partner CycC function to repress lipogenic gene expression and de novo lipid biosynthesis by promoting the degradation of nuclear SREBP-1c proteins. Future research will reveal a more comprehensive understanding on Mediator functions in lipid metabolism and its roles in metabolic diseases.
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References


