Biochimica et Biophysica Acta 1839 (2014) 444–451

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagrm

Review

The roles of Mediator complex in cardiovascular diseases

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ARTICLE INFO

Article history:
Received 27 December 2013
Received in revised form 19 March 2014
Accepted 11 April 2014
Available online 18 April 2014

Keywords:
Cardiovascular diseases
Mediator complex
Congenital heart diseases
Energy homeostasis
Pluripotency
Endothelial progenitor cells

ABSTRACT

Despite recent treatment advances, an increase in cardiovascular diseases (CVD) mortality is expected for the next years. Mediator (MED) complex plays key roles in eukaryotic gene transcription. Currently, while numerous studies have correlated MED alterations with several diseases, like cancer or neurological disorders, fewer studies have investigated MED role in CVD initiation and progression. The first finding of MED involvement in these pathologies was the correlation of missense mutations in MED13L gene with transposition of the great arteries. Nowadays, also MED13 and MED15 have been associated with human congenital heart diseases and others could be added, like MED12 that is involved in early mouse development and heart formation. Interestingly, a missense mutation in MED30 gene causes a progressive cardiomyopathy in homozygous mice suggesting a potential role for this subunit also in human CVDs. Moreover, several subunits like MED1, MED13, MED14, MED15, MED23, MED25 and CDK8 exert important roles in glucose and lipid metabolism. Although these evidences derive from *in vitro* and animal model studies, they indicate that their deregulation may have a significant role in human CVD-related metabolic disorders. Finally, alternative transcripts of MED12, MED19 and MED30 are differently expressed in circulating endothelial progenitor cells thus suggesting they can play a role in the field of regenerative medicine. Overall, further functional studies exploring MED role in human CVD are warranted. The results could allow identifying novel biomarkers to use in combination with imaging techniques for early diagnosis; otherwise, they could be useful to develop targets for novel therapeutic approaches.

1. Introduction

Regulation of gene expression in eukaryotes is an extremely complex process that is controlled by several different mechanisms and at multiple stages [1]. Alterations of transcription regulation are commonly involved in the pathogenesis of many human diseases and most of the signaling pathways finally target the core transcription machinery [2]. For eukaryotic protein-coding genes, this machinery comprises RNA polymerase II (Pol II), the general initiation factors TFIIA, -IIB, -IID, -IIE, -IIF, and -IIH, DNA-binding transcription factors (TFs) and transcription coactivators. Mediator (MED) is a multisubunit complex that joins transcription factors bound at the upstream regulatory elements, such as nuclear receptors (NRs), and the transcription machinery at the promoter region [1,3,4]. Mammalian MED is a large complex containing about 30 subunits arranged in four structurally distinct modules, named head, middle and tail modules, representing the main complex core, and kinase module, variably associated with the core (CDK module) (Fig. 1) [4,5]. Thus, two main subclasses can be distinguished, larger complexes containing all or some combinations of the kinase module subunits and smaller complexes lacking the kinase module [6]. The discovery of paralogs like MED13L has suggested that complexes with different kinase modules might regulate different subset of genes [6]. Moreover, we retain that MED complex composition is not yet fully described and novel putative variants can participate to the formation of further complexes. Indeed, our research group has already identified new alternative isoforms of some subunits and others are expected to exist based on bioinformatics studies [7,8]. Other subunits, like MED1 and MED26 are found in only a fraction of the total MED complexes [6]. Structural and biochemical studies have further refined the entire MED organization with the recognition of additional sub-modules [3–6,9]. Thus, current literature suggests that a minimal module may serve as a core enabling the cell to custom-design different MED complexes. Each complex is then designed in response to the signals from transcription factors, which are recruited to the complex in response to the continuous changes of the cellular environment [6].

It is well established that MED is crucial for the pre-initiation complex (PIC) since it interacts with Pol II thus influencing the transcription initiation process. However, to date, many studies strongly indicate that it functions in later transcription steps beyond initiation, for example in

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http://dx.doi.org/10.1016/j.bbagrm.2014.04.012
1874-9399/

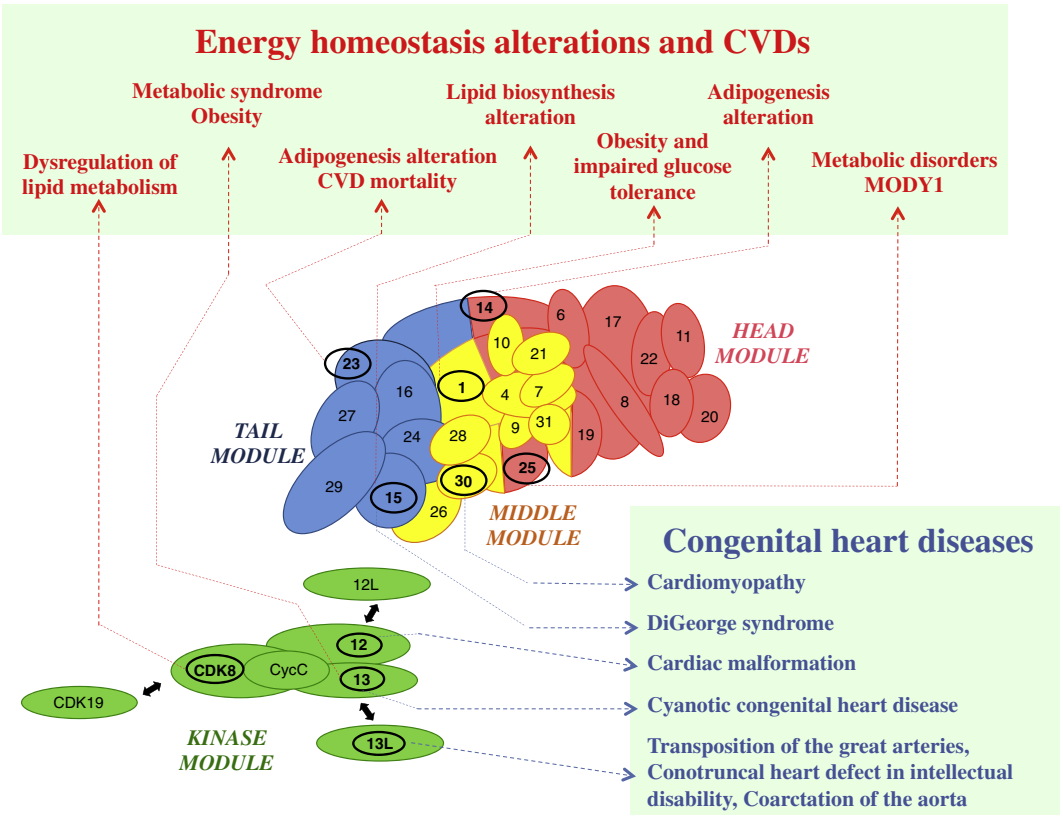

Fig. 1. Schematic representation of MED structure with the main sub-modules. Subunits that are involved in CVD are circled. Several MED subunits impair organ specific glucose, lipid and energy homeostasis and/or disturb the mitochondrial oxidative phosphorylation, thus producing CVD and related pathologies. Other subunits are altered by genetic modifications in many congenital heart diseases. Black arrows indicate the homologs of MED12 and MED13, MED12-like (MED12L) and MED13-like (MED13L), respectively, which are alternatively part of the kinase module. The relative position of all subunits arises from the current knowledge even though the precise structural localization of some subunits inside the complex is still debated.

the elongation phase [5], in termination of transcription [10], mRNA processing [11], as well as in chromatin remodeling [12,13]. Indeed, MED can connect enhancers with promoters through the recruitment of cohesin [12]. Noteworthy, since depletion of MED and cohesion subunits from embryonic stem cells resulted in altered differentiation this mechanism functions also in pluripotency maintenance [12]. Moreover, MED interacts with the RNA binding protein hnRNPL to regulate alternative mRNA processing through MED23 subunit [11].

Another important issue in the MED function is the role of the regulatory CDK module. This structure was previously assumed to act as a negative regulator of transcription; however, several latter studies have demonstrated it may also have a positive role in the transcription activation [4,6,9,14,15].

The alteration of MED components in their structure and function has important consequences in medicine. Currently, the number of studies reporting genetic mutations in different MED genes that are associated with specific human disease is greatly increasing [6,9,16]. However, these relationships between individual MED subunits and disease have been mainly described in cancer [6,9] and neurological disorders [6,16–19], whereas fewer studies have specifically investigated the role of MED in cardiovascular diseases (CVDs) [6,16]. Epidemiologic studies clearly demonstrate that a rise in CVD mortality rates is expected for the next 25 years in the developing countries, despite recent treatment advances [20]. The principal risk factors that negatively affect cardiovascular health are obesity, dyslipidemia, hypertension and diabetes mellitus [20]. Therefore, lifestyle modification and adequate pharmacotherapy can prevent the onset of CVDs.

In this review, we discuss the recent findings concerning MED alterations in CVDs to elucidate their biological implications and identify possible better diagnostic and therapeutic approaches in this critical area.

Table 1 summarizes all the MED subunits that are thought to be involved in CVDs and related risk factors through diverse mechanisms, which will be detailed below.

2. MED alteration in congenital heart diseases

Since MED is a key component of the transcription machinery it was expected that deficiencies of MED complex subunits generally could result in embryonic lethality, precluding the study of its physiological function [3–6,21]. Indeed, most of the subunits belonging to the MED core are required for embryonic growth and cell viability. Specifically, knockout and mutant mice have been obtained for various individual subunits, such as MED1, MED12, MED21, MED23, MED24, MED31 and CDK8 [reviewed in 21]. Noteworthy, these mice are all embryonic lethal even though they display different phenotypes as well as mortality at different developmental stages [21]. These findings suggest that distinct subunits can have different roles and specificities during embryonic development [21]. Similar behaviors could be expected for other subunits. For example, genetic inactivation studies in yeast demonstrated that MED17 is responsible for the direct communication with Pol II [22]. This fundamental observation suggests that MED17 could be required for embryonic growth and cell viability in higher eukaryotes even though further investigation is needed to elucidate whether disruption of this subunit can alter also mammalian development. Moreover, studies on knockdown mice or targeted inactivation of other subunits, such as MED1, MED12, MED14, MED19, MED25, MED26 and MED28 and CDK8, indicated that these subunits are essential for specific gene expression programs during differentiation and organogenesis [21]. Thus, it is predictable that individual MED subunits can be associated with a range of human congenital malformations, including congenital heart diseases (CHD) (Fig. 1; Table 1).

Table 1
Involvement of specific MED subunits in CVDs and their risk factors.

MED subunit	CVDs or risk factors	Biological process	Molecular mechanism	Study model	References
MED1	Obesity and impaired glucose tolerance	Adipogenesis induction Glucose metabolism alteration	Interaction with NRs	Animal	[49,50]
MED12	Cardiac malformation	Perturbed cardiovascular system development	Involvement in Wnt/β-catenin signal pathway	*In vitro*/Animal	[8,24]
MED13	Cyanotic congenital heart disease	Hypoxia	A-to-I RNA editing	Human	[25]
	Obesity and metabolic syndrome	Metabolic homeostasis	Downregulation by miR-208a	Animal	[56]
MED13L	TGA	Defects in early heart development	Mutations in MED13L gene	Human	[27]
	Conotruncal heart defect in ID				[31]
	Coarctation of the aorta				[32]
MED14	Adipogenesis alteration	Lipid homeostasis	Interaction with PPARγ	*In vitro*	[60]
MED15	DiGeorge syndrome	Conotruncal heart malformations	22q11.2 deletion	Human	[34]
	Lipid biosynthesis alteration	Cholesterol and fatty acid biosynthesis	SREBP-1α target gene expression	Animal	[61]
MED23	Adipogenesis alteration	Lipid homeostasis	Regulation of insulin signal transduction pathway	*In vitro*	[62,63]
	Increased CVD mortality	Serum homoarginine reduction	SNPs on chromosome 6	Human	[64]
MED25	Metabolic disorders	Lipid homeostasis	HNF4α driven transcription	*In vitro*	[67]
	MODY1	Reduction of insulin secretion from β-cells	Defective MED25/HNF4α interaction	*In vitro*/Human	[65]
MED30	Cardiomyopathy	Mitochondrial deterioration and dysfunction	Missense mutation in Med30	Animal	[37]
CDK8	Dysregulation of lipid metabolism	Fatty acid and triglyceride liver accumulation	Transcription alteration of SREBP-1c target genes	*In vitro*/Animal	[69]

2.1. MED12

Studies in stem cells knocked down for MED12 allowed evidencing that MED12 interacts with Nanog in the regulation of Nanog-target genes, thus identifying a novel role for MED12 in embryonic stem cell regulation [23]. Interestingly, we identified an alternative transcript of MED12 that is feasibly involved in endothelial cell differentiation [8].

Moreover, MED12 is known to be essential for early mouse development since it is necessary for correct Wnt/b-catenin and Wnt/planar cell polarity signaling [24]. Indeed, embryos with altered *Med12* expression did not complete gastrulation while mutants that fail to develop beyond embryonic day 10 have severe defects in neural tube closure, somitogenesis and also heart formation [24]. Particularly, apoptotic cells of *Med12*-deficient embryos were mostly present in the heart and not generally widespread. All mutant embryos had an enlarged heart that did not loop and mostly stayed in the midline clearly indicating cardiac dysfunction [24].

2.2. MED13

MED13 has been recently involved in cyanotic CHD [25]. Indeed, cyanotic children manifested significantly higher rates of Adenosine-to-Inosine (A-to-I) RNA editing in an intronic *Alu* segment of MED13 gene compared to acyanotic children, together with a more difficult surgical course. RNA editing is a site-specific RNA modification that leads to a difference between RNA and the starting DNA sequence [26]. A-to-I RNA editing is mediated by the adenosine deaminase acting on RNA (ADAR) family of enzymes [26]. These posttranscriptional RNA changes in MED13 are hypothesized to affect cellular and metabolic pathways and to influence the perioperative course following hypoxia [25]. Interestingly, RNA editing in *Alu* elements may influence gene expression through different mechanisms, including altered RNA splicing and mRNA nuclear retention [25,26]. Nevertheless, further studies are needed to understand the detailed mechanism of gene regulation by this subunit [25].

2.3. MED13L

MED13L is a Mediator complex subunit13-like of the kinase module [27,28]. The involvement of MED in human CVDs was showed for the first time in a study reporting missense mutations and gene interruption in MED13L gene in patients with congenital heart defect, in particular in those displaying transposition of the great arteries (TGA) and mental retardation. This finding suggested that MED13L is involved in early development of both heart and brain [27]. Indeed, MED13L is broadly expressed in several human tissues and it is highly expressed in the aorta and cerebellum [27]. TGA represents the most common severe heart defect diagnosed in humans at birth and accounts for 5–7% of all CHDs [29]. It occurs when the two main arteries, the pulmonary artery and the aorta, go out of the heart switched in position [29]. In this condition there are two separate blood circulations instead of a single connected one [29]. Thus, blood with oxygen from the lungs does not get to the rest of the body [29]. TGA determines a cyanotic heart defect that leads to a bluish-purple coloring of the skin and shortness of breath [30]. TGA is not compatible with healthy survival and postnatal palliative treatment and corrective surgery are required [30]. Recently, in addition to the chromosomal balanced translocation of the region containing MED13L gene, a homozygous missense mutation in this gene was found in two siblings with non-syndromic intellectual disability (ID) [31]. Moreover, copy number changes of MED13L have been described [31]. Particularly, two intragenic *de novo* frameshift deletions, probably leading to haploinsufficiency, were identified in two patients with a similar phenotype of hypotonia, moderate ID, cono-truncal heart defect and facial anomalies [31]. A further patient with hypotonia and learning difficulties, showed a triplication in 12q24.2, including MED13L [31]. These findings showed that MED13L haploinsufficiency, in contrast to the previously observed missense mutations, cause a distinct syndromic phenotype [31]. Additionally, a MED13L copy number gain resulted in a milder phenotype [31]. A further paper has recently reported the association of MED13L gene duplication also with coarctation of the aorta [32]. Taken together, these genetic data suggest that MED13L is involved in early heart development, but future studies are required to establish how its reduced expression produces TGA, heart defects and ID. Interestingly, MED13, also belonging to the CDK module, has been recently implicated also in cyanotic congenital heart disease (see above) [25].

2.4. MED15

Haploinsufficiency and reduced expression of MED15 contribute to the clinically heterogeneous phenotypes associated with the chromosome 22q11.2 deletion syndrome [33]. This pathology, called DiGeorge syndrome (DGS) or velo-cardio-facial syndrome (VCFS), represents one of the most common multiple congenital anomaly syndromes in humans, occurring in approximately 1/3000 live-births [34]. The DGS is characterized by an extremely heterogeneous phenotype impacting nearly every organ system and developmental function [35]. Common

clinical manifestations include cardiac defects, palatal anomalies, characteristic facial dimorphisms, immune dysfunction and hypocalcemia arising from underdevelopment of the thymus and parathyroid glands, respectively, and neuropsychiatric anomalies, including schizophrenia and psychoses [34,35]. Particularly, patients with 22q11.2 deletion syndrome are associated with a high rate (74%) of congenital heart defects, especially conotruncal malformations, tetralogy of Fallot, interrupted aortic arch, ventricular septal defect and truncus arteriosus [35]. The broad range of clinical manifestations associated with 22q11.2 deletion syndrome suggests the involvement of multiple genes [36]. As expected, the typically deleted region, found in most of patients (>90%), comprises about 60 genes, while a minor number of patients (<10%) show a smaller deletion, called microdeletion, including about 28 genes representing the most common interstitial deletions known to occur in humans [36]. MED15 is one of the genes deleted in the microdeletion associated with 22q11.2 deletion syndrome [36].

2.5. MED30

A missense mutation in *Med30* gene causes a progressive cardiomyopathy in homozygous mice that show precipitous lethality 2–3 weeks after weaning [37]. Through the analysis of gene expression profile, pleiotropic modifications occurred in the transcription of cardiac genes required for oxidative phosphorylation and mitochondrial integrity [37]. In contrast, a ketogenic diet extended viability to 8.5 weeks [37]. This intervention was found to increase the expression of cardiac genes, as *Pgc1a* and *Sod2*, and to reduce oxidative stress [37]. These observations allowed associating the MED complex and mitochondrial oxidative phosphorylation mechanism, with a specific impact on cardiac function. Although the role of MED30 in early cardiac function during the period of weaning has been demonstrated only in a preclinical model, these data indicate that genetic lesions affecting MED subunits can be considered as a potential cause of primary cardiomyopathies in humans, with particular attention to pediatric age. Finally, cardiopathy morbidity and mortality might be mitigated modifying diet composition.

3. MED-dependent alteration of energy homeostasis and CVDs

Several MED subunits and complexes have been co-purified with NRs, which are transcription factors broadly involved in many biological processes such as, development, reproduction and homeostasis. Importantly, MED has been established as an essential center for NR signaling pathways in response to a variety of signals [38,39]. Thus, as coactivator for a broad range of NRs, MED itself has been functionally associated with most of the NR activities, including lipid and glucose homeostasis [38,39]. Noteworthy, it is well recognized that deregulation of energy homeostasis is a risk factor for CVDs [20]. A huge number of studies have linked MED subunits and NRs, as well as other key transcription factors, to their related pathways and biological processes, like adipogenesis and glucose balance (Fig. 1; Table 1). However, their involvement in CVDs has been established mainly through *in vitro* and *in vivo* studies on animal models, whereas studies directly connecting MEDs with human CVDs are still lacking.

3.1. MED1

The activity of MED1 subunit depends on its interaction with several co-regulators. Indeed, MED1 represents the main MED component interacting with several NRs [38–40]; among them, peroxisome proliferator-activated receptor-gamma (PPARγ), estrogen receptor, thyroid hormone receptor (TR), and vitamin D receptor [41–45]. All these receptors interact with MED1 through the LXXLL motifs in a ligand-dependent manner [38–42,44,45]. Furthermore, it is also a crucial interaction hub for other gene-specific activators, cofactors and chromatin modifying enzymes [39]. Examples are p53, BRCA1 and GATA family members [39,46–48]. Finally, it is also an important regulatory target for signal transduction pathways through posttranslational modifications [38–40,49]. Since MED1 interacts with this variety of transcription factors, it is expected to be implicated in many functions like cell development, energy homeostasis, as lipid and glucose metabolism and consequently in those pathologies where these biological processes are disrupted [38]. Mostly, MED1 exerts an important role in energy homeostasis [50]. Particularly, it is required for PPARγ-stimulated adipogenesis and exerts a considerable influence on the functions of this class of NRs and their target genes [41,42]. *Med1* mutant mice are embryonically lethal at E11.5 days demonstrating its requirement during development [51]. Because of this embryonic lethality, several conditional *Med1* knock-out lines and *Med1* knock-in lines with mutated *Med1* have been developed in order to investigate the important functions of MED1 in NR pathways [38,50]. The principal tissue where fatty acid and glucose metabolism occur is skeletal muscle that, therefore, represents also a key site of energy homeostasis with implications for possible therapeutical intervention both in type 2 diabetes, obesity and CVDs [52]. Moreover, NRs and coregulators play central roles in the maintenance and function of normal muscle [53]. Noteworthy, skeletal muscle-specific *Med1* knockout mice showed enhanced insulin sensitivity and improved glucose tolerance as well as resistance to high-fat diet-induced obesity [50]. Furthermore, deletion of the iNOS gene in *ob*/*ob* mice significantly increased *Med1* gene expression, suggesting an improvement in energy homeostasis [54].

Overall, the advantageous metabolic phenotype observed in mice with muscle specific *Med1* ablation, or with *Med1* mutations disrupting the NR-MED interactions, indicates the possibility to generate new therapeutic approaches for CVD risk factors through modulation of MED1-NRs interactions [50,55].

3.2. MED13

A clear link exists between MED13 and the thyroid hormone receptor signaling. Indeed, a recent paper has recently demonstrated that MED13 functions in the heart to control metabolic homeostasis and energy expenditure [56]. In this study pharmacological and genetic tools were used to modulate the expression of MED13, in the heart [56]. On the one hand, genetic deletion of MED13 specifically in cardiomyocytes enhanced obesity in response to high-fat diet and caused metabolic syndrome [56]. On the other hand, cardiac-specific overexpression of MED13 in mice conferred resistance to high-fat diet-induced obesity, improved systemic insulin sensitivity and glucose tolerance and lowered plasma lipid levels [56]. Interestingly, pharmacologic inhibition of miR-208a led to a similar metabolic phenotype; miR-208a is a cardiac-specific miRNA encoded by an intron of the cardiac specific α-myosin heavy chain gene [56]. These important findings not only show that MED13 is negatively regulated by miR-208a, but also explain miR-208a metabolic regulation in the heart [56]. Indeed, several studies indicate an important role for microRNAs (miRNAs) in heart disease and metabolism [57]. Moreover, in this study metabolic defects were further linked to MED13-specific repression of genes regulated by nuclear receptors, particularly thyroid hormone receptor [56]. Indeed, metabolic control by MED13 was associated with altered expression of several TR and NR responsive genes in the heart [56]. Overall these findings revealed an important role of the heart in the metabolic regulation with MED13 and miR-208a as key components of this regulatory mechanism [56]. Remarkably, in a previous study, the alteration of miR-208a expression by antimiR-208a subcutaneous delivery during hypertension-induced heart failure in a rat model protected against CVDs and metabolic syndrome [58]. Similarly, genetic deletion of miR-378 and 378* protected against diet-induced obesity in mice [58]. These miRNAs target multiple mRNAs (also including MED13), which regulate systemic energy homeostasis [59]. These studies indicate the potential of oligonucleotide-based therapies to modulate cardiac miRNAs and validate miR-208 and miR-378 as potent therapeutic targets in cardiac

function regulation during CVD progression. Moreover, it would be of interest to investigate the role of MED13 in human CVDs, or in its predisposition conditions, through genetic studies exploring possible MED13 mutations/polymorphisms and gene expression alterations.

3.3. MED14

It is known that PPARγ can also induce adipogenesis independently of MED1 [42]. Indeed, a later study demonstrated that MED could also be recruited to the promoters of PPARγ target genes through the MED14 subunit [60]. MED14 directly interacts with the N-terminus of PPARγ in a ligand-independent manner, and is required for the transcription activity of PPARγ [60]. Moreover, *in vitro* studies also showed that MED14 knockdown could repress adipogenesis suggesting a role of this subunit in lipid homeostasis, whose alteration is a risk factor for cardiovascular related disorders [60].

3.4. MED15

MED15 subunit is an important controller of fatty acid biosynthesis, whose deregulation is another key risk factor for the onset of CVDs [61]. In detail, MED15 is a transducer of the sterol regulatory element-binding protein 1-alpha (SREBP1α) that controls lipid metabolism [61]. Indeed, MED15 knockdown by siRNA or shRNA resulted in a decrease of SREBP target gene expression, such as fatty acid synthase, in mammalian cells [61]. This finding suggests that MED15 and SREBP work in the same signal pathway. Interestingly, the addition of oleic acid allowed recovering most of the MED15 knockdown phenotypes in the *in vivo* model [61]. This evidence corroborated that the observed phenotypes were due to decreased production of oleic acid [61]. Thus, MED15 seems to be a necessary factor for SREBP-mediated gene expression in controlling lipid biosynthesis [61]. Similarly to SREBP encoding genes, it may be also linked to the development of obesity, atherosclerosis, type 2 diabetes, fatty liver and lipodystrophy also in humans, but further studies are necessary to better understand the molecular events correlating MED15 with these pathologies.

3.5. MED23

MED23 can be considered a pivotal regulator of adipogenesis [62]. Indeed, it was demonstrated to act as a critical molecule in the regulation of insulin signal transduction pathway during adipogenesis [62]. Moreover, this subunit controls the cell fate preference that directs differentiation into smooth muscle cells or adipocytes [63]. Strikingly, cardiovascular abnormalities, like heart edema and lower heart rate, were found from Med23 zebrafish morphants. Since the imbalance of fat and vasculature tissue is involved in many metabolic diseases and CVDs, these findings suggest that a better understanding on the opposing role of MED23 in adipocyte and smooth muscle cell differentiation may provide novel therapeutic strategies for these diseases.

Recently, in order to identify genomic loci associated with homoarginine serum levels, a genome-wide association study has reported a suggestive association with single nucleotide polymorphisms (SNPs) on chromosome 6 at the MED23/arginase (ARG1) locus [64]. Noteworthy, low serum levels of homoarginine have been associated with increased risk of total and cardiovascular mortality [64].

3.6. MED25

MED25 is recruited by hepatocyte nuclear factor 4α (HNF4α) in a ligand-independent manner, and this physical interaction is essential for HNF4α-mediated transcription [65]. HNF4α is a member of the NR superfamily and it is known to regulate the expression of a wide variety of crucial genes, including those involved in embryogenesis and early development, liver and pancreatic cell differentiation, as well as glucose and amino acid metabolism and lipid homeostasis [66]. Moreover, an impaired HNF4α-driven transcription in human liver is responsible for many pathological conditions, such as altered drug metabolism, fatty liver, and diabetes [67]. Noteworthy, MED25 has been recognized as one of the HNF4α binding partners in pancreatic ß-cells leading to insulin secretion [66]. HNF4α mutations are monogenic causes of a dominant inherited form of diabetes, the Maturity Onset Diabetes of the Young 1 (MODY1) [68]. Interestingly, MED25/HNF4α interaction is disrupted by the two MODY mutations, positioned at the LXXLL motif binding pocket, thus impairing normal insulin secretion [65]. These findings indicate a key role for MED25 as an active MED component in HNF4α signaling, and suggest it could be a novel target for therapeutic intervention in the related pathologies [65]. Moreover, these results also advise to screen patients with MODY1 for the presence of possible MED25 gene mutations, thus elucidating its involvement in this disease.

3.7. CDK8

A recent study revealed that, as MED15, also CDK8 subunit is a key negative regulator for lipid biosynthesis [69]. It acts by directly phosphorylating SREBP-1c at the threonine-402 residue (T402) [69]. *In vitro* and *in vivo* data demonstrate that CDK8 promotes the polyubiquitination and subsequent degradation of nuclear SREBP-1c without affecting the level of mRNA or precursor of SREBP-1c and producing a triglyceride accumulation in hepatocytes [69]. This study showed that CDK8 and CycC protein levels are negative regulators of the lipogenic pathway in *Drosophila*, mammalian hepatocytes, and mouse liver. Moreover, their levels were negatively regulated by food intake in mouse liver and by insulin in the hepatocytes [69]. Thus, it was hypothesized that insulin could stimulate *de novo* lipogenesis by downregulating CDK8 and CycC proteins, which normally inhibit *de novo* lipogenesis through promotion of nuclear SREBP-1c degradation. This new mechanism of insulin-induced lipogenesis is in addition to the well-documented function of insulin in stimulating SREBP-1c at the mRNA level [69]. Noteworthy, *cdk8* knockdown in mouse liver *in vivo* caused a fatty liver-like phenotype and a dramatic elevation of triglycerides in plasma, suggesting a critical role of hepatic CDK8 in controlling blood lipid levels [69].

Interestingly, CDK8 is also required for Kes1-mediated repression of Gcn4 transcription factor activity, a pathway involved in membrane and lipid trafficking through trans-Golgi network and endosomal systems [70].

According to these findings, CDK8 could be a new target to regulate lipid biosynthesis in the related pathologies.

4. Mediator complex and regenerative medicine

The understanding of the molecular mechanisms underlying pluripotency and reprogramming has a great importance in the field of regenerative medicine [71]. Properly, an interesting and essential MED biological function is its key role in the expression of genes that control and maintain the pluripotent state [23,72–74]. For instance, depletion of MED subunits from embryonic stem cells resulted in altered differentiation implying also a role of this complex in the maintenance and induction of pluripotency [72]. The mechanism involved Nanog (or other reprogramming factors) interactions through MED and cohesin complexes in the formation of promoter-enhancer chromatin loops [72,73]. Besides, several studies have shown that master transcription factors Oct4, Sox2, and Nanog bind unusual enhancer domains (the so-called super-enhancers) and recruit MED complex to activate the gene expression program that controls the pluripotent state [74]. These super-enhancers consist of clusters of enhancers and differ from typical enhancers for several features like size, transcription factor density and content, ability to activate transcription, and sensitivity to perturbation [74,75]. Moreover, they play key roles in human cell identity in both health and disease [75]. Remarkably, reduced levels of Oct4 or

Mediator led to a preferential loss of expression of super-enhancer-associated genes [74].

Cell therapy is a promising option for tissue engineering and regenerative medicine [76–78]. In the last years, several preclinical, and, more recently, also clinical studies, have shown promising results on cell therapy in the treatment of CVDs and their ischemic complications requiring therapeutic revascularization and vascular repair [76,77]. Particularly, studies using bone marrow-derived progenitor cells and circulating endothelial progenitor cells (EPCs) have allowed the translation of such therapies into clinical trials although with some controversy [76–79]. It is well recognized that aging and cardiovascular risk factors such as diabetes affect these cells thus limiting their therapeutic potential [78,79]. However, the effect of the above risk factors on these stem cell populations at molecular level still remains unclear. Moreover, since these cells are being considered for clinical therapies, it is fundamental to validate their identity and effectiveness. Current knowledge indicates that there are many circulating blood cells that can contribute to new blood vessel formation and vascular repair and to date no specific marker for an EPC has been identified [79].

Interestingly, we have characterized the expression profile of all the genes coding for MED subunits during EPC differentiation and reported novel transcripts of MED30, MED12 and MED19, which are differently expressed during differentiation of these progenitors into endothelial cells [7,8]. These isoforms are generated by alternative splicing, a mechanism that is known to increase transcript diversification in embryonic stem cells and other progenitor cells thus affecting also cell differentiation [80–82]. These findings suggested that specific MED isoforms might be used as additional markers for EPC identification [7,8]. Further studies in this area are requested to elucidate which EPC population participates to endothelium repair and which MED alteration could be involved in the repair capacities of these progenitors. Finally, the finding of alternative MED transcripts during endothelial cell differentiation [7,8] opens new roads of investigation in the field of regenerative medicine.

5. Conclusions and future perspectives

MED is a central regulator of transcription mechanism in eukaryotes. To date, alterations in MED complex genes have been associated with both developmental defects and several human diseases, ranging from congenital malformations to cancer. However, there are currently fewer studies correlating specific MED alterations to CVDs. Some of these alterations represent genetic modifications leading to heart malformation. Particularly, MED13L is associated with several congenital heart defects [27,31,32]. MED15 belongs to the region of chromosome 22q11.2 whose deletion leads to DiGeorge syndrome [34,35]. Moreover, although in a mouse model, a MED30 missense mutation causes progressive cardiomyopathy [37] and embryos with reduced MED12 expression died with cardiac malformations [24]. Finally, a post-transcriptional regulation mechanism, like A-to-I RNA editing, altering MED13 transcript affect the surgical course of children with cyanotic CHD [25]. Besides, as expected, several MED subunits, which are involved in the regulation of glucose and lipid metabolism, have been associated to CVDs; these include MED1, MED13, MED14, MED15, MED23, MED25 and CDK8 (see Fig. 1 and Table 1 and references therein). However, most of the studies reporting the role of these subunits in CVDs and related risk factors have been performed mainly *in vitro* or in animal models.

Thus, in order to gain insights about the impact of MED in human CVDs further studies are warranted, including genome-wide association and whole expression studies also through innovative technologies like Next-Generation Sequencing (NGS). Indeed, the introduction of this technology has allowed whole-transcriptome analysis to be a selected approach for the comprehension of mechanisms involved in many complex diseases [83]. Particularly, the possible identification, in a single experiment, of potentially novel genes/exons and splice isoforms, RNA editing, noncoding RNAs, fusion transcripts and allele-specific expression are some of the important advantages of NGS [83].

Altogether, these genetic analyses, followed by functional and preclinical studies, may also provide new advances on the understanding of MED-induced CVD mechanisms, with the aim of developing novel therapeutic strategies. Indeed, a striking biological property of MED, that renders it suitable as therapeutic target, is that its different subunits regulate different sets of genes and/or pathways. Thus, targeting a specific MED subunit should disrupt only a specific pathway without affecting the remaining gene transcription and, in turn, cellular functions. As described above, some MED subunits interact with cell-type specific transcription factors or cofactors suggesting that also their action can be cell-type specific [3,21]. Thus, a future challenge could be the use of small molecules as a strategy to target these protein–protein interactions between MED subunits and transcription factors. For instance, small molecule inhibitors of CDK8 kinase activity have been developed for use in cancer therapy, even though such inhibitors could affect also other CDKs or they could produce adverse effects on cell viability [84,85]. Thus, structural data from NMR analysis could help to add novel information on these interactions in order to develop other molecules.

Another approach was used in cancer cells to reduce the protein levels of CDK8 or CCNC, by delivering specific shRNAs that target their mRNAs [86]. However, this alternative strategy is still far from the clinical usage. Prospectively, if successful, these approaches may be beneficial for diseases where increased levels of CDK8 or CCNC subunits represent the pathophysiological mechanism. Similarly, they can be also extended to patients with diseases where other specific MED subunits contribute to their pathogenesis.

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