

Review

Myocyte Enhancer Factor 2A Plays a Central Role in the Regulatory Networks of Cellular Physiopathology

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ABSTRACT: Cell regulatory networks are the determinants of cellular homeostasis. Any alteration to these networks results in the disturbance of cellular homeostasis and induces cells towards different fates. Myocyte enhancer factor 2A (MEF2A) is one of four members of the MEF2 family of transcription factors (MEF2A-D). MEF2A is highly expressed in all tissues and is involved in many cell regulatory networks including growth, differentiation, survival and death. It is also necessary for heart development, myogenesis, neuronal development and differentiation. In addition, many other important functions of MEF2A have been reported. Recent studies have shown that MEF2A can regulate different, and sometimes even mutually exclusive cellular events. How MEF2A regulates opposing cellular life processes is an interesting topic and worthy of further exploration. Here, we reviewed almost all MEF2A research papers published in English and summarized them into three main sections: 1) the association of genetic variants in MEF2A with cardiovascular disease, 2) the physiopathological functions of MEF2A, and 3) the regulation of MEF2A activity and its regulatory targets. In summary, multiple regulatory patterns for MEF2A activity and a variety of co-factors cause its transcriptional activity to switch to different target genes, thereby regulating opposing cell life processes. The association of MEF2A with numerous signaling molecules establishes a central role for MEF2A in the regulatory network of cellular physiopathology.

Key words: myocyte enhancer factor 2, MEF2A, MEF2C, MEF2D, GLUT4, CaMK

1. Introduction

Myocyte enhancer factor 2 is an important family of transcription factors comprised of four members, MEF2A-D. These have an almost identical N-terminus (amino acids (aa) 1-86), but distinct C-termini (Figure 1A). The N-terminus has a MADS (MCM1, Agamous, Deficiens and Serum response factor) domain (aa 2-57) followed by a MEF2-specific domain (aa 58-86). The

MADS and MEF2 domains mediate dimerization, high affinity binding to the DNA sequence CTA(A/T)4TAG/A and interaction with co-factors. The C-terminus functions mainly in transcription activation and in nuclear localization of the MEF2 proteins [1, 2]. Distinct amino acid sequences in the C-terminus of different MEF2 members may determine their specific activity of transcription activation. MEF2A is located on chromosome 15q26.3 and is composed of 12 exons with a

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total length of about 115 kb [3]. A region in its C-terminus and encompassing aa 472-507 harbors the key nuclear localization signal (Fig. 1B), while the aa 321-472 region is thought to be the transcriptional activation domain (TAD) [2]. TAD has four sub-domains: TAD1 (aa 289-296), TAD2 (aa 312-322), TAD3 (aa 338-348) and TAD4 (aa 364-373) (Fig. 1C). These are conserved among MEF2A, MEF2C and MEF2D and are important for the transcription activation activity [2]. The region between aa 274 and 288 is associated with repression of transcription activation and is thus an inhibition domain

(iD) (Fig. 1C) [2]. Variants generated by alternative splicing are frequently observed in the MEF2 family [4]. The alignment of eight MEF2A variants downloaded from NCBI shows that alternative splicing exons are commonly involved with the MADS box, the MEF2 domain and with downstream region (aa 87-136). aa 19-86 is missing in variants X7 and X8 compared with other variants, this may be the consequence of alternative splicing (Fig. 1D). Another common alternative splicing exon encodes TAD1 and was reported as β -exon in a previous study by Zhu et al [4].

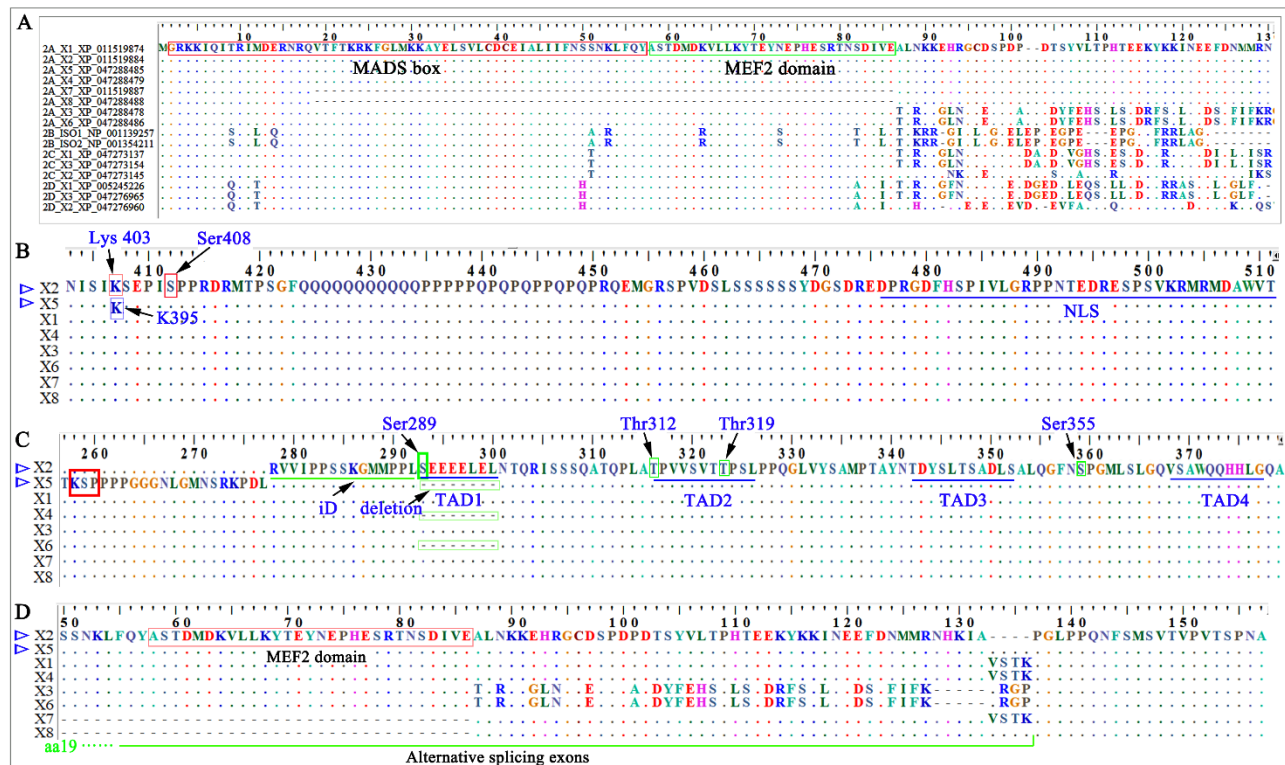


Figure 1. Core domains of MEF2 protein. A dot indicates that an amino acid is identical with that of the top reference sequence at the same position, and ‘-’ indicates that a residual is deleted at the same position compared to the top reference sequence. ‘X’ plus a number represents the MEF2 variants in the NCBI database. MEF2A X2 variant (XP_011519884) is used as the reference sequence for indicating the amino acid position. (A) sequence alignment for different variants of MEF2A-D downloaded from NCBI database, ‘XP_’ or ‘NP_’ plus numbers indicate the sequence accession number in NCBI database; (B) sequence alignment for the C-termini of eight MEF2A variants; (C) KSP, transcription inhibition, transcription activation domain, and phosphorylation sites in MEF2A variants; (D) the region in which alternative splicing commonly occurs.

Expression of the different MEF2 members overlaps in distinct pattern during embryogenesis and in adult tissues [5]. Second-generation sequencing results of the transcriptome of various human tissues show that the highest expression of MEF2A mRNA occurs in heart tissue followed by skin and brain tissue (Fig. 2A)[6]. The highest expression of MEF2C mRNA was seen in the brain, where it was almost three-fold that of lymph nodes (second highest expression) and 6-fold that of heart (Fig. 2B)[6]. The high expression of MEF2C in brain tissue

concurs with its important role in brain development. Conditional knockout of Mef2c in neuronal populations within the mouse brain causes many severe behavioral and synaptic phenotypes, while Mef2c haploinsufficiency caused by microdeletions in chromosome 5q14.3 have been linked to neurodevelopmental disorders [7]. Although redundant roles among MEF2A, MEF2C and MEF2D were observed during skeletal muscle regeneration [8], the distinct expression profiles of MEF2 members suggests that they have unique roles in different

tissues in addition to their common functions. Estrella et al [9] found that MEF2A was essential for the correct differentiation of myoblasts, whereas MEF2B, MEF2C and MEF2D were dispensable for this process. Different MEF2 members can specifically recognize distinct targets amongst the total cohort of MEF2-regulated genes in the muscle genome [9]. Desjardins et al [10] found that MEF2A and MEF2D, not MEF2C, was indispensable for cardiomyocyte survival, and the major role of MEF2C was involved in cardiac morphogenesis and direct

reprogramming, more than that, MEF2A and MEF2D antagonized the regulation of cell-cycle and differentiation gene programs by MEF2C. Mef2a loss of function mutations, or Mef2a knockout mice in a background of normal function for other MEF2 members, still results in an obvious disease phenotype [11, 12], indicating that MEF2A has irreplaceable functions. Indeed, dysfunction of MEF2A has been associated with cardiovascular disease, neurodegenerative disease and tumor progression [11, 13-16].

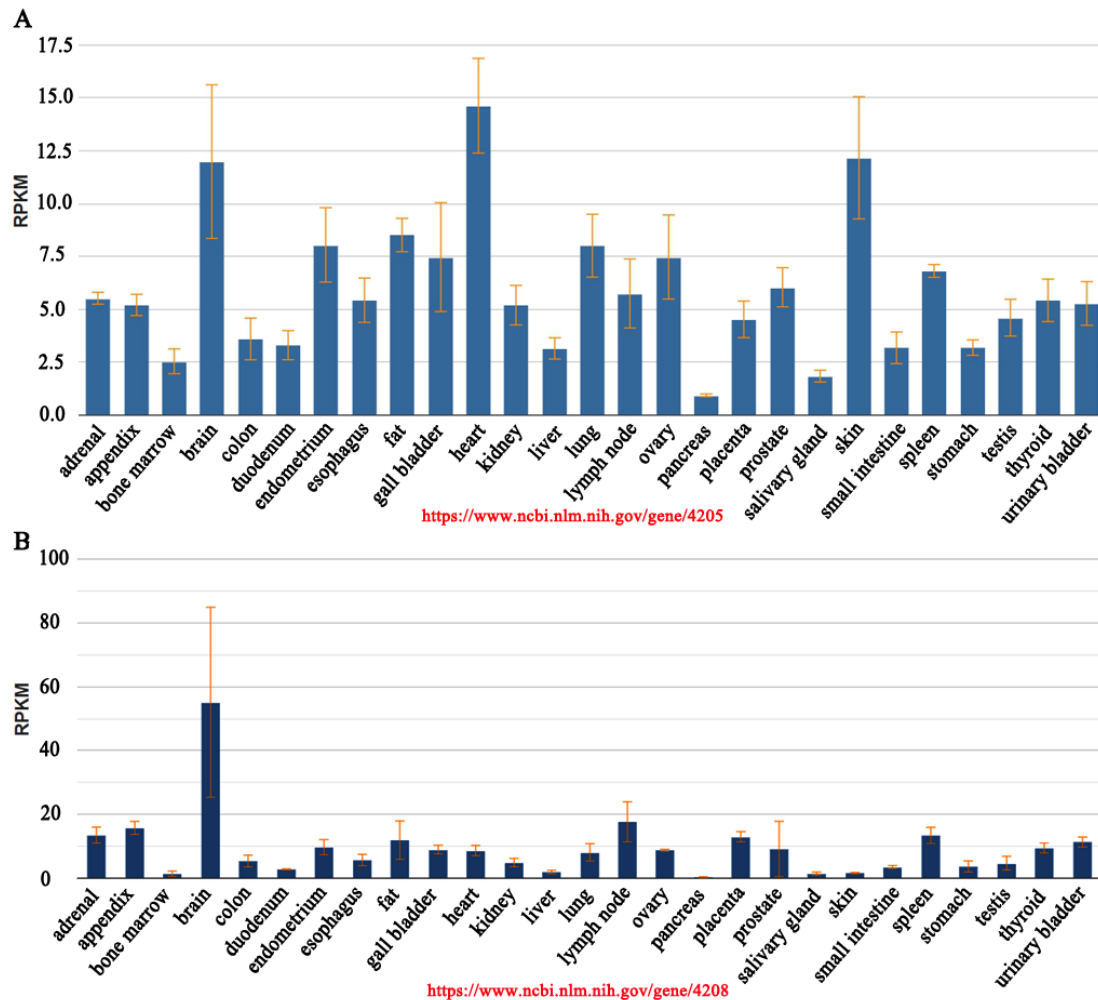


Figure 2. The expression of MEF2A and MEF2C in different human normal tissues determined by RNA-sequencing (Fagerberg, L. et al. 2014) [6]. RPKM, Reads Per Kilobase per Million mapped reads.

MEF2A is expressed in non-muscle cells as well as in muscle cells [17]. It is also highly expressed in myocardium, skeletal muscle, smooth muscle, neuronal cells and coronary artery endothelial cells [2, 11]. The expression pattern of MEF2A in endothelial cell precursors is similar to that of vascular endothelial growth factor receptor 2 and von Willebrand factor (endothelial cell marker) [18]. Mef2a mRNA can be detected in the

blood vessels of early mouse embryos [19], while MEF2A protein can be detected in cells of the embryonic vascular system as early as 8.5 days after oocyte fertilization. These results support the notion that MEF2A plays an important role in controlling vascular morphogenesis. There are also reports that MEF2A can mediate many detrimental cellular events, including senescence, death and apoptosis [20-23]. Hence, it appears that MEF2A is

widely involved in the regulation of cell proliferation, differentiation, survival, and death. However, it is still not clear how these different or opposing cell fates are regulated by MEF2A. Here, we summarize the physiological functions of MEF2A in different cells and tissue types, we also review the regulatory mechanism and regulatory network of MEF2A transcriptional activity in order to better understand the physiological functions of MEF2A.

2. Association of genetic variants in MEF2A with cardiovascular disease

Wang et al. [11] reported that a 21-bp deletion (Δ 21bp-del) in the last exon of MEF2A co-segregated with coronary artery disease (CAD)/myocardial infarction (MI) in patients with a family pedigree. This study sparked the interest of many researchers in cardiovascular disease. Since then, a large number of studies have reported on the correlation between genetic variants in the MEF2A coding region and CAD/MI. In subsequent MEF2A re-sequencing studies in the Chinese Han population, the MEF2A Δ 21bp-del was found in a small number of CAD/MI patients. However, its low population frequency means that it cannot account for the etiology of the sporadic CAD/MI [24-26]. However, several new mutations were found during MEF2A re-sequencing studies, including N263S and P279L in exon 7 [26] and a repeat number variation of CAG and CCG in exon 11 [24]. Li et al. [27] found several mutations in exon 11 of MEF2A that were unique to CAD patients, but their population frequency was low and they showed no statistical correlation with CAD susceptibility. Dai et al. [28] reported that the TT genotype of the 1353 G/T locus of MEF2A, and the haplotype of 1291-1293 CCG D + 1305 G + 1353T, may be associated with early-onset CAD. Xu et al. [29] also reported that a novel 6-bp deletion co-segregated with CAD in a family pedigree study, but this variant was not found in sporadic cases.

Weng et al. [30] re-sequenced the MEF2A gene in sporadic cases of CAD. Only one older patient was found to carry the Δ 21bp-del mutation amongst 300 patients. Further study on 1500 individuals without CAD revealed two Δ 21bp-del carriers. Genotyping of 19 family members of the above three probands found the Δ 21bp-del did not co-segregate with CAD, demonstrating that it was not a genetic risk factor for CAD, at least not in Caucasians, and thus not a common cause of CAD. However, the conclusion of this study was questioned because the non-CAD individuals were determined without the use of angiography [31]. Nevertheless, studies on Japanese [32], Italian [33], German [34], Iranian [35] and Irish [36] populations also found no significant

correlation between the Δ 21bp-del in MEF2A and CAD/MI. It should be borne in mind however that the Δ 21bp-del is rare in human populations and therefore it is difficult to reach statistical significance with limited sample size.

Reported correlations between other polymorphic loci in the exonic region of MEF2A and CAD/MI are also controversial. In the Saudi population, several SNPs located in exon 11 showed weak correlations with CAD or MI. Among these, haplotype 1A-2G-3G-4A-5C-6G-7G-8A comprised of eight SNPs was associated with a significantly increased risk of CAD (OR = 6.39) [37]. In a Spanish population, Gonzalez P et al. [38] found the 279 Leu allele frequency was three-fold higher in CAD/MI patients compared to the control group. Another Spanish population study found the CAG repeat (poly Q) polymorphism (CAG)₁₁ allele of MEF2A was a risk factor for left ventricular hypertrophic cardiomyopathy, and was second only to the risk from hypertension [39]. A study of an Iranian population investigated two SNPs: rs34851361 (A/G) and rs325400 (T/G). The authors found that rs325400 (T/G) and the haplotype A-T composed of these two loci were significantly correlated with CAD susceptibility [40]. A case-control study of a Chinese population found that a CAG repeat polymorphism in exon 11 of MEF2A was associated with CAD susceptibility. The (CAG)₉ allele frequency in CAD patients was significantly higher than in the control group ($P < 0.001$), and was also associated with the severity of CAD [41]. However, a large case-control study of a German population did not find any mutations or polymorphisms that were associated with MI, including Pro279Leu in exon 7 and the (CAG)_n repeat in exon 11 [36]. In addition to the observation that the (CAG)₁₁ allele was associated with the risk of left ventricular hypertrophic cardiomyopathy in the Spanish population, a heterozygous nonsense mutation (Gly240*) in MEF2A also co-segregated with dilated cardiomyopathy in a Chinese family pedigree [13]. A nonsense mutation in MEF2C (p.Y157*) was associated with dilated cardiomyopathy in another Chinese family pedigree [42]. The cardiomyopathy phenotype induced by loss of function mutations in MEF2A and MEF2C indirectly supports the important role of these genes in the development of cardiac tissue, as demonstrated also in animal models [12, 43, 44].

To date, no MEF2A genetic variation has been consistently associated with sporadic CAD/MI. Several genetic variants found in pedigree studies, which result in severe dysfunction of MEF2A have not been linked to CAD/MI susceptibility in sporadic cases. However, the frequency of these variants in the population is very low and hence it may be difficult to achieve statistical significance with a limited sample size. This also shows

that mutations causing serious dysfunction of MEF2A are difficult to spread in the population, although they do occasionally occur as heterozygotes in adult individuals of some family pedigrees. In such individuals, the function of MEF2A may be compensated by other genes. These observations also indirectly show that MEF2A function is very important for the entire life process of human beings. In light of the apparent functional redundancy of MEF2, an interesting question is whether other MEF2 members have high expression levels in individuals who carry loss-of-function mutations in MEF2A and survive to adulthood.

3. Physiopathological functions of MEF2A

3.1 MEF2A functions in myocardial formation, differentiation and pathology

MEF2A is necessary for cardiac development and myocardial differentiation. It is the main MEF2 gene product expressed in postnatal myocardium. Most mice that lack MEF2A die suddenly in the first week after birth and show significant right ventricular dilation, myofibril rupture, mitochondrial disorder, and fetal heart gene program activation. The few MEF2A knockout mice that survive into adulthood also show myocardial mitochondrial deficiency and susceptibility to sudden death [12]. These findings reveal specific roles for MEF2A in maintaining the appropriate mitochondrial content and cellular structural integrity of the postnatal heart, roles which cannot be compensated by other MEF2 sub-types [12]. The phenotype of *Mef2a*-mutant mice is clearly distinct from that of *Mef2c* mutants, which die from severe cardiovascular abnormalities at embryonic day 9.5 [12, 45]. This observation suggests that MEF2A and MEF2C possess distinct functions beyond their redundancy. The promoter regions of *COX6A_H* and *COX7A_H* lack NRF sites but have a conserved MEF2 domain. Chromatin immunoprecipitation (ChIP) assay shows that NRF1 binds to the promoter of *MEF2A*, and that MEF2A binds to the promoter of *COX6A_H*, *PPARGC1A* and to that of *MEF2A* itself. *MEF2A* mRNA and promoter-reporter-signal are regulated by the expression level of NRF1 and show a positive association. PGC1 α stimulates increased MEF2A promoter-reporter activity. This effect is mediated by the NRF1 and MEF2 elements of the MEF2A promoter, since mutation of either site results in attenuation of the PGC1 α effect [46]. These results indicate that MEF2A coordinates the expression of cardiac and muscle respiratory chain subunits through the NRF1 \rightarrow MEF2A \rightarrow COX_H transcription cascade [46]. Destruction of this cascade regulatory loop may explain the lack of mitochondria in the striated muscle of MEF2A deficient mice [46]. The

DNA-binding transcription factors MEF2A, NKX2.5, GATA4 and SRF play key roles in the differentiation, maturation, and homeostasis of cardiomyocytes [34]. MEF2A can bind to the Gata4 and Nkx2.5 promoters and depletion of MEF2 decreases the expression of these genes [47, 48]. Schlesinger et al [47] found that among hundreds of target genes with transcription factor binding sites (TFBS) for GATA4, MEF2A, NKX2.5 and SRF in mouse HL-1 cardiomyocytes, many overlap with and are co-regulated by at least two of the GATA4, MEF2A, NKX2.5 and SRF transcription factors. The Gene Ontology terms of 'muscle cell differentiation' and 'heart looping', are significantly over-represented amongst MEF2A and NKX2.5 targets, respectively. This indicates that MEF2A plays an independent and central role in cardiac development and function. It also functions in coordination with GATA4, NKX2.5 and SRF, and all of these factors are mutually regulated. In addition, MEF2A plays a role in cardiomyocyte survival by regulating costomere integrity [49]. Activation and elevated expression of MEF2A and MEF2C in the adult heart is often closely related to myocardial hypertrophy [50]. MEF2A increases the expression of COX-2 by up-regulating the expression of heparin-binding EGF-like growth factor (HB-EGF), which is related to cardiomyocyte hypertrophy induced by MEK5-ERK5 signal [51]. Abnormally elevated calcium signal induces myocardial hypertrophy. Calcium/calmodulin-dependent protein kinase (CaMK) is an effective activator of MEF2 activity (reviewed in ref. [5]). CaMKs promotes the expression of several fetal heart genes by increasing the transcriptional activity of MEF2A, thus inducing myocardial hypertrophy (reviewed in ref. [5]). Ventricular hypertrophy in rats exposed to arsenic trioxide may be related to the up-regulation of MEF2A, CAMKK2, CALM3 and TNNT3 in cardiomyocytes induced by this agent [52]. The long noncoding RNA *Ahit* prevents myocardial hypertrophy by down-regulating MEF2A expression in synergy with SUZ12 [53]. Suppressors of MEF2 (class II HDACs and MTR) are highly expressed in the heart, brain and skeletal muscle, thus mirroring the tissues in which high levels of MEF2 expression are observed (reviewed in ref. [5]). Together, the above findings suggest that maintaining a physiological level of MEF2A activity is critical, and that either hypo- or hyper-activity may lead to deleterious consequences.

3.2 The role of MEF2A in vascular endothelial cell function and angiogenesis

The main MEF2 members expressed in vascular cells are MEF2A and MEF2C. MEF2 protein has been shown to control vascular integrity by promoting endothelial cell (EC) survival. This is essential for EC homeostasis and

for the regulation of protective genes against atherosclerosis [54]. However, Materna et al [43] found that almost complete early deletion of Mef2c in the vascular endothelium resulted in viable offspring that were indistinguishable from wild type mice and had no overt defects in their vascular system, suggesting a less important role for Mef2c in vascular development. MEF2A may therefore be more important than MEF2C for the development and normal functioning of the vascular endothelium. MEF2A is a transcriptional effector for vascular endothelial growth factor signal, and thus a key regulator of angiogenesis [55]. The expression of KLF2 in ECs is induced by many factors, including laminar shear stress and angiopoietin-1 (Ang1). The flow-dependent induction of KLF2 is mainly mediated through the MEK/ERK/MEF2 pathway [56]. However, the induction of KLF2 by Ang1 is via the PI3K/Akt/MEF2 pathway and is not mediated by the signaling pathways involving ERK5 and HDAC5 [57]. We previously found that inhibiting the expression of MEF2A in vascular endothelial cells (VECs) resulted in accelerated cell aging, and therefore MEF2A over-expression could rescue VECs from senescence induced by oxidative stress [58]. Resveratrol has been shown to delay the aging and apoptosis of VECs. Our previous study found that treatment of VECs with resveratrol significantly up-regulated the expression of MEF2A, thereby preventing apoptosis induced by oxidative stress [59]. When siRNA was used to specifically silence MEF2A and the VEC were then treated with resveratrol, the apoptosis induced by silencing MEF2A could not be reversed [59]. Knockout of MEF2A or MEF2C in human pulmonary artery endothelial cells (PAECs) results in cellular dysfunction. The transcriptional activity of MEF2 in PAECs from pulmonary artery hypertension (PAH) patients was impaired by the increased nuclear localization of HDAC4 and HDAC5. Moreover, the restoration of MEF2A activity reversed the PAH phenotype in experimental models [60]. Evidence for the protective effect of MEF2A on blood vessels also includes the observation that inhibition of MEF2A in ApoE^{-/-} mice promotes a vascular inflammatory response and atherosclerosis [59, 61]. Together, the above studies suggest that MEF2A plays an important role in maintaining the normal physiological function of VECs. However, several studies have also reported harmful effects of MEF2A on endothelium. For example, inhibition of MEF2A in the myocardium of a type 1 diabetes mouse model reduced myocardial fibrosis by partially regulating the transformation of endothelium to mesenchyme [20]. Additionally, up-regulation of MEF2A was associated with HUVEC dysfunction and oxidative stress injury induced by glucose deprivation [62, 63]. Finally, inhibition of MEF2A enhanced neovas-

cularization and promoted blood flow recovery after ischemia in vivo via post-transcriptional regulation of the miR-329 and miR-494 microRNAs [64]. The above studies demonstrate the dual and opposing roles of MEF2A in vascular endothelium and vascular tissue, while highlighting the complex regulation of vascular endothelial function by MEF2A. More in-depth research is needed to fully understand the mechanisms by which MEF2A mediates different vascular endothelial functions.

3.3 Role of MEF2A in the proliferation, differentiation, and pathology of smooth muscle and skeletal muscle

Regeneration of skeletal muscle requires the participation of MEF2A, MEF2C and MEF2D [8]. A study reported that MEF2A can antagonize the expression of Hspb7 in mouse skeletal muscle and this may benefit preventing muscle atrophy [65]. MEF2A promotes the proliferation and differentiation of myoblasts through direct transcriptional regulation of myozenin2 (MYOZ2) [66]. It also participates in myocyte differentiation through direct regulation of calpain 3 (Capn3) [67], and plays a role in myofibrogenesis by regulating the expression of Myospryn [68]. Depending on the p38 and calmodulin activity levels, static stretch promotes the nuclear localization of MEF2A in C2C12 myocytes, thereby inducing the expression of myosin heavy chain (MHC) [69]. However, MEF2A appears to have different physiological functions in vascular smooth muscle cells (VSMC). Treatment of VSMCs with H₂O₂ promotes cell senescence by inducing significant up-regulation of MEF2A [21]. However, the opposite result is obtained after H₂O₂ treatment of VECs, wherein it promotes cellular senescence by significantly down-regulating MEF2A [58, 70]. Inhibition of MEF2A induces the phenotypic transformation, proliferation, and migration of VSMC [71]. Reported pathological factors that induce MEF2A up-regulation in VSMC also include AngII and balloon injury [72, 73]. The different functional roles observed for MEF2A in VECs and VSMCs has resulted in considerable confusion regarding its importance in vascular diseases, especially atherosclerotic vascular diseases.

3.4 The role of MEF2A in neuron survival, differentiation, and neuro-degenerative diseases

Accumulating evidence points to an important physiological function for MEF2A in the nervous system. MEF2A and MEF2D are highly expressed in hippocampal CA1 neurons, while MEF2C expression is located mostly in the dentate gyrus and neocortex [74]. Redundant function between MEF2A and MEF2D was revealed in granule neurons, as one of them loss function,

that will be at least partially compensated by the other [75]. MEF2A plays a key role in the differentiation and maturation of rat neural stem cells into neurons, while also being necessary for the survival of cerebellar granule neurons. Chromatin immunoprecipitation (ChIP) sequencing has revealed that MEF2A-specific binding sites in mouse neuronal cells were mainly enriched in MAPK signaling pathway, axon guidance and oxytocin signaling pathway [76]. Specific silencing of MEF2A significantly reduces MEF2 response element-mediated transcription in granule neurons and inhibits the survival of granule neurons [77]. Phosphorylation-dependent degradation of endogenous MEF2A in rat cerebellar granule neurons induces neuronal apoptosis [78, 79]. Neurotoxin-induced granule neurons apoptosis is caused by caspase-dependent degradation of MEF2A and MEF2D, not of MEF2C, that are phosphorylated by cyclin-dependent kinase 5 (CDK5) [80]. Under physiological conditions, MEF2A is degraded through chaperone-mediated autophagy (CMA). This mechanism can protect primary neurons from oxidative stress-induced cell damage. Under pathological conditions, mild oxidative stress (200 μM H_2O_2) increases the degradation and activity of MEF2A. Excessive oxidative stress (> 400 μM H_2O_2) halts its degradation, resulting in the accumulation of non-functional MEF2A and harmful effects on cells [81]. By inhibiting the activity of proteasome, neurotoxin stops the degradation of ubiquitin-modified MEF2A and the accumulation of inactive MEF2A in the nucleus, thereby inducing cell damage [82]. These indicate that the decreased expression, the disruption of the functional MEF2 and the accumulation of function-impaired MEF2 are pernicious for neuron survival. The functional role of MEF2A, MEF2C and MEF2D in the cellular and non-cellular autonomous control of adult hippocampal neurogenesis is different to their role in the process of neural development. Although the number of induced neurons in MEF2 knockout mice was increased, the dendrites poorly developed, demonstrating a lack of coupling between neuronal survival and dendritogenesis [83].

The expression of MEF2A and the regulation of its activity both play a role in neuronal differentiation. MEF2A is highly expressed in differentiated neurons in the brain throughout synaptogenesis [74, 84, 85], and plays a key role in neural presynaptic development in mammals. Down regulation of MEF2A in vivo significantly increases the density of orphan presynaptic sites in primary neurons and rat cerebellar cortex [86]. However, covalent modification of MEF2A at lysine 403 by small ubiquitin related modifier (SUMO) protein inhibits the activity of MEF2A and induces the postsynaptic dendritic differentiation in the mammalian brain [84]. The sumoylated transcription inhibitor form

of MEF2A mediates the inhibition of orphan presynaptic sites. Synaptic aggregation protein 1 (Syt1) is a direct inhibitory target gene of sumoylated MEF2A in neurons. Inhibition of Syt1 eliminates the role of orphan sites downstream of MEF2A. The elimination of orphan presynaptic sites induced by MEF2A triggers the maturation of presynaptic boutons in the brain [86]. MEF2A has been implicated in the morphogenesis of dendritic claws of granule neurons in the cerebellar cortex [84, 85]. The modification of MEF2A required for postsynaptic differentiation occurs via a phosphorylation-regulated sumoylation to acetylation switch (SAS) peptide motif, which activates MEF2A and in turn inhibits dendritic claw differentiation [87, 88]. MEF2C, but not MEF2A and -D, plays major role in hippocampal synaptic function [89]. However, observation that sudden discharge induces the expression of MEF2A/D-dependent target gene *Arc* that contributes to synaptic silencing and elimination indicates involvement of MEF2A/D in synaptic function [90].

The plasticity thresholds of brain neurons during neural circuit formation are fine-tuned via altering MEF2 levels [91]. Abnormal expression and functional defects of MEF2A are often associated with neurodegenerative diseases. Hypermethylation of the MEF2A promoter [14] or genetic variants in MEF2A exons [15] have been linked to Alzheimer's disease. Experiments with a mouse model show that deletion of *Mef2a* can lead to behaviors related to autism and drug addiction [92, 93]. The neuropeptide oxytocin (OT) induces neurite contraction in neurons that express normal levels of MEF2A and neurite growth in neurons that lack MEF2A. Therefore, MEF2A is believed to determine the responsiveness of neuronal cells to OT and to be involved in the pathological mechanism of autism [94, 95]. MEF2A may also regulate the expression of genes related to thought disorders. Mutations in the MEF2 binding sites of the two schizophrenia related genes QKI (HGNC: 21100) and PDE10A (HGNC: 8772) have been associated with formal thought disorder [96]. Halting the degradation of the ubiquitinated MEF2A by neurotoxins induces the characteristics of Parkinson's disease in an animal model [82], indicating that accumulation of inactive MEF2A is also one of the causes of neurodegenerative diseases. Degenerated cognitive ability is one of the hallmarks of neurodegenerative diseases. Clinical brain transcriptomic data shows the expression level of MEF2C is positively correlated with cognitive ability, while over-expression of MEF2A/C in PS9 mice remarkably improves the cognition and reduces hyperexcitability [97]. MEF2A also regulates the bidirectional interference of activity-dependent AMPA receptors, which constitute a major foundation of learning and memory [98]. However, two studies reported that MEF2A is associated with reduction of the ability for

spatial learning and memory in mice [93, 99]. These findings about the roles of MEF2 in cognition and learning abilities seem to be contradictory. Since the expression of MEF2A promotes neurite contraction and reduces hyperexcitability, is the negative correlation between MEF2A and spatial or sport learning abilities observed in mice caused by the increase of neural hyperexcitability resulted from reduction of MEF2 expression?

The protective effect of some drugs on cerebral ischemia injury was shown to depend on an increase in MEF2A activity. Dexmedetomidine combined with netrin-1 can reduce cerebral ischemic injury by inhibiting endoplasmic reticulum stress via activation of the ERK5/MEF2A pathway [100]. Moreover, alternative splicing of MEF2A is associated with some neurological diseases. Significant differences have been reported in the MEF2A mRNA splices observed in muscle tissues from patients with myotonic dystrophy (DM) and neuromuscular disorder (NMD) compared to normal muscle tissue [101].

The current evidence indicates that a normal level of MEF2A expression and activity is necessary for the development, differentiation and survival of neurons, as well as for communication in the nervous system. MEF2A is an important regulator that ensures the health of the nervous system. All of these highlight the potential of MEF2A as therapeutic target or biomarker for neurodegenerative disease.

3.5 The role of MEF2A in tumors

MEF2 can support oncogenic or tumor suppressive activity, depending on its interactions with co-activators or with co-suppressor chaperones. In leiomyosarcoma (LMS), the expression level of class II HDACs determines whether MEF2 plays a tumor inhibitory or promoting role. Knockout of HDAC9 inhibits the transformed phenotype of LMS cells by restoring the transcription of some MEF2 target genes [102]. Transforming growth factor (TGF)- β plays a role in the invasion, metastasis and growth of cancer cells by promoting HDAC degradation and histone hyper-acetylation at the MEF2 site of the matrix metalloproteinase (MMP-10) promoter, and by activating MEF2A and up-regulating the expression of MMP-10 [103]. MEF2A expression is thought to promote the progression of multiple myeloma [104] and of colorectal cancer [105], with higher expression correlating with poor prognosis. P38 MAPK activation in the gastric cancer cell line MKN45 up-regulates GLUT-4 in a MEF2A-dependent manner and promotes glucose uptake and cell growth [106]. This suggests that a high expression level for MEF2A promotes the growth and metastasis of gastric cancer cells. However, other studies have reported tumor

suppressive activity for MEF2A. For example, epigallocatechin-3-gallate (EGCG) promotes the expression of KLF4 via MEF2A, thereby inhibiting the growth of gastric cancer cells [107]. Chen et al [108] found that MEF2A transcriptionally activates expression of the lncRNA HCP5, thus inhibiting the progression of gastric cancer. Houltuynia cordata was reported to induce the apoptosis of human HepG2 hepatoma cells by activating the HIF1a-FOXO3 and MEF2A pathways [109]. Clearly, additional research is required to allow more accurate conclusions about the role of MEF2A in tumor development and progression. The studies to date have investigated only one aspect of the activation and regulation pathway of MEF2A. However, MEF2A is part of a complex regulatory network, and its activity for transcriptional activation is affected by many factors including its expression level, protein modification and interacting co-factors. The final physiological and pathological roles of MEF2A are likely to be the result of multi-dimensional synergies or competitions.

3.6 Other physiological roles

The observation that Mef2a-knockout female instead of male mice are osteopenic suggests the role of MEF2A in osteoclast differentiation is associated with sex [110]. Suppression of MEF2 signal was reported to inhibit activation of human primary hepatic stellate cells [111]. MEF2A is expressed much higher than other three MEF2 members in primary human cytotrophoblasts, and induces cytotrophoblast differentiation and syncytium formation, suggesting dysregulation of MEF2A may be associated with placenta-related pregnancy disorders [112]. Negative role of MEF2A against survival of retinal ganglion cells was observed in mice after optic nerve crush [22]. These suggest that MEF2A may have many unique but distinct from the well-studied physiological functions in different tissues.

4. MEF2A: regulation of activity and regulatory targets

4.1 Regulation of MEF2A activity by post-translational modifications

Post-translational modifications are an important way to regulate MEF2A activity, with phosphorylation, acetylation and sumoylation being the most common. MEF2 protein is thought to be a nuclear target of the signal cascade in response to serum stimulation and to some cellular stressors [113]. Its transcriptional activity is increased following phosphorylation by p38 MAPK, and to a lesser extent after phosphorylation by the PKC isoforms δ and ϵ [113]. Depending on the sites,

phosphorylation of MEF2A can either inhibit or enhance its activity. Ser255 in MEF2A (variant X2, Figure 1C) is located within an evolutionarily conserved KSP motif and contains phosphorylation receptor sites for MAPK members (ERK1, ERK2 and stress activated protein kinase), Cdc2-like kinase (including Cdk5) and glycogen synthase kinase-3 (GSK3). The phosphorylation of Ser255 in MEF2A is independent of p38 MAPK, while the KSP motif is important for regulating the stability and function of MEF2A. The neuronal apoptosis induced by GSK3 may be related to excessive degradation of MEF2A following phosphorylation of Ser255. Thr312 and Thr319 in MEF2A are p38 MAPK-dependent phosphorylation sites (Fig. 1C). Their phosphorylation markedly increases the transcriptional activity of MEF2A [114]. ERK5 phosphorylation of MEF2A also increases its transcriptional activity [115]. GSK3 β can inhibit p38 MAPK activity. Drugs that suppress GSK3 β therefore attenuate the inhibition of p38 MAPK activity and promote the transcriptional activity of MEF2A/D in skeletal muscle and cardiomyocytes [116], thus demonstrating MEF2 activation through phosphorylation by p38 MAPK. The epidermal growth factor (EGF)-induced activation of MEF2A is specifically mediated by big mitogen-activated protein kinase (BMK1) that increases the transcriptional activation activity of MEF2A via phosphorylating its Ser-355, Thr-312 and Thr-319 [117]. Ser289 in MEF2A is a protein kinase CK2 site (Figure 1C). There is also a homologous region of this CK2 site in MEF2C and it is a known alternative splice site in different tissues [114]. The region surrounding this site could therefore determine the specific functional role of MEF2 in different tissues. Phosphorylation and dephosphorylation of Ser408 in MEF2A (Fig. 1B) is one of the switches that control the transition between inhibition and activation of MEF2A [22]. In the lower organism *Xenopus laevis*, anterior formation during development depends on the phosphorylation-based activation of MEF2A transcriptional activity by Nemo-like kinase (NLK) [118].

Some studies have shown that acetylation of MEF2 is associated with its DNA binding and transcriptional activity [119, 120]. A high acetylation level of MEF2A enhances its DNA binding activity in skeletal muscle. Exercise increases the acetylation level of MEF2A, thereby increasing its binding to the muscle carnitine palmitoyl transferase 1b (*Cpt1b*) promoter and promoting its transcription [121]. Exercise increases the acetylation level of MEF2A largely by promoting the phosphorylation of HDAC5. This results in its translocation from the nucleus to the cytoplasm, hence reducing its interaction with MEF2A [121]. Calmodulin promotes the acetylation of MEF2A at Lys403 (Fig. 1B)

through the dephosphorylation of Ser408, thereby enhancing MEF2A transcriptional activity [87].

Sumoylation of MEF2A leads to inhibition of its activity. Decreased transcriptional activity caused by sumoylation of K395 in MEF2A (variant X5, Fig. 1B) has been demonstrated both *in vivo* and *in vitro*. The nuclear E3 ligase PIAS1 was reported to promote sumoylation of MEF2A [122], while SUMO-specific proteases (SENPs) are thought to function by removing sumoylation of MEF2A. SENP2, a member of the SENPs, removes MEF2A sumoylation, thereby attenuating the inhibition of MEF2A activity [70]. Desumoylation of MEF2A may be very important in maintaining the functional role of MEF2A during embryonic development [87].

Different reversible modifications can regulate the activity of MEF2A for transcriptional activation. This allows MEF2A to have different functional roles in different tissues or cells, as well as showing large differences in the response to different stimuli in the same cell.

4.2 Effect of co-factors on MEF2A activity

MEF2A binds to specific MEF2 elements and interacts with many co-factors in order to activate gene transcription, thereby showing both promoter specificity and cell specificity [123]. The most typical MEF2 co-factors are muscle-specific transcription factors and chromatin remodeling enzymes, including histone acetyltransferases (HATs) and histone deacetylases (HDACs). Developmental and stress signals can positively or negatively regulate MEF2 activity by controlling its phosphorylation state and its association with co-factors (reviewed in ref. [5]). Class IIa HDACs are the most well studied inhibitors of the transcriptional activity of MEF2A. The interaction between HDACs and MEF2A in the nucleus reduces the DNA binding activity of MEF2A. HDAC5 can interact with MEF2A both *in vivo* and *in vitro* to strongly inhibit its ability for transcriptional activation [124]. The inhibition of MEF2A activity by HDACs can be attenuated by promoting their degradation and translocation from the nucleus to the cytoplasm. Apelin-APJ signaling mediates phosphorylation of HDAC4 and HDAC5 to promote their nuclear export, thus activates endothelial MEF2A and MEF2C [125]. Calcium/calmodulin dependent protein kinase (CaMK) phosphorylates two conserved serine residues at the N-terminal of HDAC-4, -5, -7 and -9 (reviewed in ref. [5]). Phosphorylation of these residues creates a docking site for the intracellular chaperone 14-3-3, which destroys the HDAC-MEF2 complex by binding to phosphorylated HDACs and releasing MEF2 from inhibition (reviewed in ref. [5]). HDACs suppress the transcription of gene promoters by deacetylating the

N-terminal tails of core histones to promote chromatin condensation. This inhibition can be attenuated by HATs (such as p300 and PCAF) that make the chromatin relax through acetylation of the N-terminal tails of core histones (reviewed in ref. [5]). The MEF2A/D:P300 complex increases the expression of C-Jun in macrophages, but the activity of MEF2A/D is suppressed when it is bound to HDAC1 and HDAC7. MEF2A/D can therefore have a dual role in the differentiation of monocytes into macrophages through its binding to different co-factors [126].

Mammalian spinous homologous protein 1 (MASH1) is necessary for the early development of the nervous system. MASH1 can regulate the expression of specific genes essential for neuronal differentiation through its synergistic effect with MEF2A. MEF2A and MASH1 are simultaneously induced during neuronal lineage differentiation. In transient transfection experiments, MEF2A and MASH1 act synergistically to activate gene expression through a mechanism based on specific physical interaction between these two factors [127]. Chicken ovalbumin upstream promoter-transcription factor II (NR2F2, COUP-TFII) is found in the mouse Leydig cells, and can form a complex with MEF2A, which binds to *Akr1c14* gene promoter to potentiate its expression [128]. Early growth response-1 (EGR1) is also a MEF2 co-regulator and interacts with MEF2A to specifically repress its activity. This may be the mechanism to maintain costamere gene expression at levels commensurate with cardiomyocyte contractile activity [129].

4.3 Regulation of MEF2A expression

The activity of MEF2A for transcriptional activation is regulated by post-translational modifications and co-factors, as well as by transcriptional and translational regulation of MEF2A. Mitochondrial specific nuclease (*EndoG*) is thought to be a negative regulator of *Mef2a* expression, while protein kinase B2 (AKT2) promotes *Mef2a* expression by inhibiting *EndoG* [130]. Bone morphogenetic protein-2 (BMP-2) promotes the expression of sarcomeric myosin heavy chain (MHC) and *Mef2a* by increasing PI3K activity. Moreover, ectopic expression of *Mef2a* can increase the transcription of BMP-2 depending on the level of PI3K activity, suggesting the existence of a regulatory loop that involves BMP-2/PI3K/MEF2A/PI3K /BMP-2 [131]. The expression of MEF2A may also be self-regulating, since its promoter region contains MEF2 binding sites. In addition, phosphorylation-induced activation of MEF2A by ERK5 and p38 MAPK promotes the expression of MEF2A [132]. Exercise increases the expression of *Nrf-1* and enhances the activity of CaMK II, resulting in hyper-

acetylation of H3 histone. This causes increased transcription of *Mef2a* by enhancing the binding of NRF1 to the *Mef2a* promoter [133]. MEF2A has a very long 3'-untranslated region (3'-UTR) which contains many miRNAs binding sites that play a cis-regulatory role in translational inhibition [134]. Many miRNAs that inhibit the expression of MEF2A have been identified. MiR-155 for example inhibits the expression of MEF2A, while the lncRNA *malat1* up-regulates the expression of MEF2A by adsorbing miR-155, thus promoting cardiomyocyte proliferation [135]. miR-144-3P was shown to have anti-tumor properties by inhibiting the expression of MEF2A [104], while miR-194-5p delays the progression of sepsis by down-regulating MEF2A [136].

The expression of MEF2A is also affected by various chemical agents. In the diabetes model of insulin deficiency induced by streptozotocin, the expression of *Mef2a* in heart and skeletal muscle decreases significantly [137]. Decreased expression of *Mef2a* and *Mef2d* in myocardium of rat is induced by chronic ethanol exposure [138], and the reduction of their expression in adipose-derived mesenchymal stem cells is induced by treating with indoxyl sulfate [139]. In contrast, resveratrol significantly up-regulates the expression of MEF2A in vascular endothelial cells [59]. Although regulation of the expression of MEF2A is the most direct way to control its activity for transcriptional activation, research in this area is still very limited and the relevant regulatory mechanisms have yet to be identified.

4.4 Downstream regulatory pathways and target genes of MEF2A

It was initially thought that downstream target genes that are transcriptionally regulated by MEF2A were mainly myogenesis-related genes. However, more recent studies have shown that MEF2A also regulates gene expression related to cell proliferation, development, survival and apoptosis [56]. The link between MEF2 and cell proliferation was shown by the finding that MEF2 regulates serum-induced c-Jun expression, which in turn regulates the cell cycle [140]. The MEF2 binding site in the c-Jun promoter is a common MEF2A/D motif that regulates cell proliferation in many cell types [140, 141]. The target genes regulated by MEF2A vary in different tissues and cell types, depending mainly on the local co-factors or MEF2A modification status. For example, in the atrium, MEF2A regulates genes involved in fibrosis and adhesion, whereas in the ventricle it controls genes related to inflammation and endocytosis [142]. In mouse myocardium, MEF2A regulates the expression of a large number of genes linked to costamere integrity and function [49]. Multiple MEF2 motifs occur in the core promoter regions of PIK3CA and PIK3CG. We

previously reported that MEF2A up-regulates the expression of PIK3CA and PIK3CG, which may be directly regulated by the binding of MEF2A to their promoters [58].

MEF2A positively regulates the expression of several important transcription factors including KLF4 [60, 107], KLF2 [21, 57, 60], GATA4 [48] and NKX2.5 [47], thereby controlling major cellular processes such as proliferation and differentiation. Vascular endothelial protective genes, such as KLF2 and KLF4, are transcriptionally activated by MEF2A [54]. MEF2A may also be involved in muscle regeneration and differentiation via transcriptional regulation of

microRNA (miRNA) expression. It promotes skeletal muscle regeneration by directly regulating the largest known mammalian miRNA cluster that encodes a subset of the Gtl2-Dio3 miRNAs. Gtl2-Dio3 miRNAs such as miR-410 and miR-433 suppress the WNT signaling inhibitors called secreted Frizzled-related proteins (sFRPs) [143]. Gtl2-Dio3 miRNAs are also referred to as maternally expressed 3 (MEG3)-iodothyronine deiodinase 3 (DIO3) miRNAs. These are up-regulated by MEF2A to promote bovine skeletal muscle differentiation by inhibiting protein phosphatase 2A (PP2A) signaling [144].

Table 1. The downstream target genes regulated by MEF2A.

Gene names	Tissue/cell	Physiopathological roles	References
KLF2	Vessel/endothelial cell	Vascular quiescence	54, 56,57
KLF4	Vessel/endothelial cell	Vascular protection	54,107
GLUT-4	Muscle, adipo	Glucose metabolism	121,137,145-152
Gata4	Cardiomyocytes	Maturation and homeostasis of Cardiomyocytes	47,48
c-Jun	Macrophages and others	Cell proliferation	126,139,140
Cpt1b	Skeletal muscle	β -oxidation of mitochondrial fatty acids	121
PIK3CA	Endothelial cell	Delay senescence	58
PIK3CG	Endothelial cell	Delay senescence	58
ZEB2	Colorectal cancer	Tumor formation and metastasis	105
CTNNB1	Colorectal cancer	Tumor formation and metastasis	105
Capn3 (calpain 3)	L6 myoblasts	Myoblast differentiation	67
COX6A_H	Striated muscle	Mitochondrial defects	46
PPARGC1A	Striated muscle	Mitochondrial defects	46
HB-EGF	Cardiomyocyte	Cardiomyocyte hypertrophy	51
MYOZ2 (myozenin2)	Myoblasts	Differentiation	66
Myospryn	Striated muscle	Myofibrogenesis	68
MHC (Myosin heavy chain)	C2C12 myocytes	Differentiation	69
MMP-10	Mammary epithelial cells	Invasion, metastasis and growth of cancer cells	103
Akr1c14	Mouse Leydig cells	Male reproductive development and health	128
Nkx2.5	Cardiomyocytes	Differentiation, maturation and homeostasis	47
p75^{NGFR}	Myocytes	Myogenic differentiation	153
Dusp6	C2C12 myocytes	Myocyte proliferation	154
Gtl2-Dio miRNA cluster	Rat skeletal muscle	Promote regeneration	143
MEG3-DIO3 miRNA cluster	Bovine skeletal muscle	Promote differentiation	144
lncRNA HCP5	Gastric cancer	Inhibits the progression of gastric cancer	108

Solute carrier family 2 member 4 (SCF2A4), also known as glucose transporter 4 (GLUT4), is the most studied MEF2A target gene. The transcriptional activation of GLUT4 by MEF2A is affected by multiple factors. CaMK promotes the expression of GLUT4 by promoting H3 acetylation and increasing the binding of MEF2A at the MEF2 site in the GLUT4 promoter. This pathway mediates coffee- and exercise-induced expression of GLUT4 [145-147]. Exercise-induced up-regulation of GLUT4 may also depend on the exercise-induced expression of MEF2A. Silva et al [148] found that up-regulation of GLUT4 induced by muscle contraction *in vitro* is associated with increased expression and binding activity of MEF2A/D. Dimerization of the GLUT4 enhancer factor (GEF) enhances its affinity for hypo-phosphorylated MEF2A. Binding of MEF2A to the GLUT4 promoter increases the binding of GEF to domain I, thereby increasing the transcriptional expression of GLUT4 [149]. In the absence of MEF2 protein, the transcription co-repressor HDAC5 can interact with GEF and specifically inhibit GLUT4 promoter activity [149]. AMP-activated protein kinase phosphorylates GEF to promote nuclear translocation of it and MEF2A, thus enhances them binding to GLUT4 promoter and increases the transcription of GLUT4 [150]. In primary dispersed adipocytes, hyper-phosphorylation of MEF2A can enhance its binding to MEF2D, thus reducing its binding to the GLUT4 promoter and resulting in down-regulation of GLUT4 [114, 151]. Expression of GLUT4 in striated muscle depends on the insulin-induced up-regulation of MEF2A [137]. However, over-expression of MEF2A in adipocytes does not affect the expression of GLUT4, nor is it sufficient to prevent down-regulation of GLUT4 in the state of insulin deficiency [152].

The exercise-induced increase in the activity of MEF2A for transcriptional activation promotes the expression of its target genes and plays an important role in cell metabolism. MEF2A promotes glucose transport and maintains the normal physiological functions of glucose metabolism by up-regulating the expression of GLUT4. CPT1B plays an important role in the β -oxidation of mitochondrial fatty acids in skeletal muscle. On one hand, exercise training induces the acetylation of MEF2A, thus enhancing its DNA binding ability. On the other hand, exercise training decreases the binding of HDAC3 and HDAC5 to MEF2A, thereby increasing its activity for transcriptional activation. Exercise therefore increases the binding of MEF2A to the promoter region of Cpt1b and enhances its transcription [121]. The above studies highlight the important regulatory roles played by MEF2A in various metabolic pathways.

Direct inhibition of gene promoter activity by MEF2A has also been reported. The down-regulation of

p75^{NGFR} expression triggered by cell density phenomenon prior to the start of myogenic differentiation leads muscle progenitor cells to their ultimate fate. A mechanism is needed to counteract the effect of MyoD on p75^{NGFR} promoter activity. High expression levels of MEF2A abolish the MyoD-induced p75^{NGFR} promoter activity, suggesting that cell-specific regulation of the p75^{NGFR} gene may depend strictly on the intracellular composition and balance of appropriate bHLH transcription factors and their regulators [153]. This result shows that MyoD and MEF2A mediate the activation and inhibition of the p75^{NGFR} gene during muscle development. One possible mechanism is that MEF2A binding to MyoD occupies or affects the bHLH domain of MyoD, thus preventing MyoD from binding to the p75^{NGFR} promoter. The activity of dual specificity phosphatase 6 (Dusp6) promoter in C2C12 cells can be suppressed by MEF2D binding depending on p38MAPK [154]. These show the role of MEF2 as a direct suppressor.

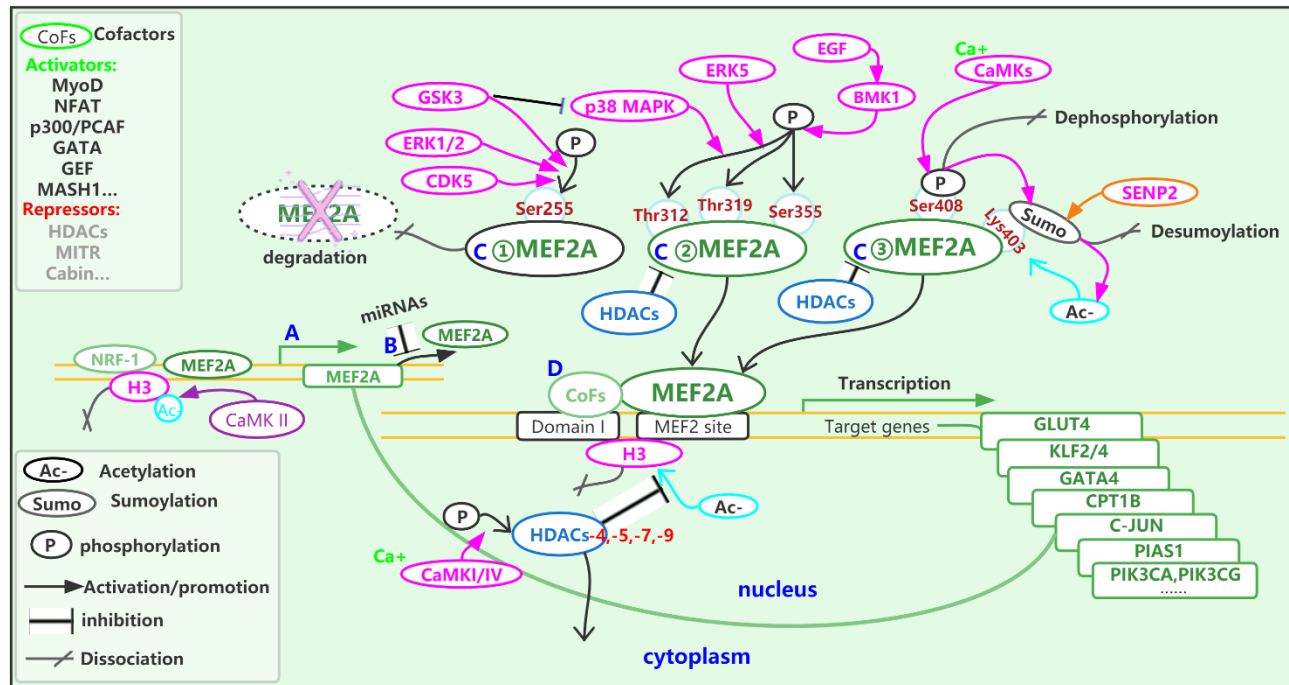
In mammals, thousands of genes contain MEF2 motifs, making them potential targets for MEF2A. However, the increased expression of MEF2A alone does not necessarily lead to a change in the expression of its target genes. Many other factors can affect the transcriptional activation of target genes, including the modification, degradation and activation of MEF2A itself, and the association and dissociation of MEF2A with co-activators or co-inhibitors. In addition, condensation and relaxation of the chromatin surrounding the promoter regions are affected by the acetylation status of core histones (Fig. 3). The multi-layered regulation and control of MEF2A activity for transcriptional activation means that MEF2A can regulate opposing cell physiological processes. Furthermore, MEF2A can have completely opposite functional roles in different cells or tissues. Figure 3 shows the main regulatory pathways or molecules involved in MEF2A-mediated transcriptional activation, together with the target genes regulated by MEF2A. Table 1 indicates the reported MEF2A target genes and their physiological or pathylogical roles.

5. Conclusion

Under physiological conditions, MEF2A usually acts as a cytoprotective factor to promote the proliferation, differentiation and survival of cardiomyocytes, vascular endothelial cells, smooth muscle cells and neuronal cells. MEF2A is essential for the formation of mitochondria in cardiomyocytes. However, under certain pathological conditions, MEF2A may also have detrimental functions by promoting SMC aging, myocardial hypertrophy and fibrosis, and tumor progression. Depending on the co-factors that it interacts with, MEF2A can promote either tumor inhibition or progression. The activity of MEF2A

for transcriptional activation is determined by its expression level, genetic variation, alternative splicing, post-translational modification (eg. phosphorylation, acetylation, sumoylation), and interaction with co-factors.

This multi-layered regulation establishes a central role for MEF2A in regulatory networks and in multifaceted physiological functions.



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Competing interests

All authors declare no conflict of interest.

References

- [1] Borghi S, Molinari S, Razzini G, Parise F, Battini R, Ferrari S (2001). The nuclear localization domain of the MEF2 family of transcription factors shows member-specific features and mediates the nuclear import of histone deacetylase 4. *J Cell Sci*, 114(Pt 24): 4477-4483.
- [2] Yu YT (1996). Distinct domains of myocyte enhancer binding factor-2A determining nuclear localization and cell type-specific transcriptional activity. *J Biol Chem*, 271(40): 24675-24683.
- [3] Kent WJ, Sugnet CW, Furey TS, Roskin KM, Pringle TH, Zahler AM, et al. (2002). The human genome browser at UCSC. *Genome Res*, 12(6): 996-1006.
- [4] Zhu B, Ramachandran B, Gulick T (2005). Alternative pre-mRNA splicing governs expression of a conserved acidic transactivation domain in myocyte enhancer factor 2 factors of striated muscle and brain. *J Biol Chem*, 280(31): 28749-28760.
- [5] McKinsey TA, Zhang CL, Olson EN (2002). MEF2: a calcium-dependent regulator of cell division, differentiation and death. *Trends Biochem Sci*, 27(1): 40-47.
- [6] Fagerberg L, Hallstrom BM, Oksvold P, Kampf C, Djureinovic D, Odeberg J, et al. (2014). Analysis of the human tissue-specific expression by genome-wide integration of transcriptomics and antibody-based proteomics. *Mol Cell Proteomics*, 13(2): 397-406.
- [7] Harrington AJ, Bridges CM, Berto S, Blankenship K, Cho JY, Assali A, et al. (2020). MEF2C Hypofunction in Neuronal and Neuroimmune Populations Produces MEF2C Haploinsufficiency Syndrome-like Behaviors in Mice. *Biol Psychiatry*, 88(6): 488-499.
- [8] Liu N, Nelson BR, Bezprozvannaya S, Shelton JM,

- Richardson JA, Bassel-Duby R, et al. (2014). Requirement of MEF2A, C, and D for skeletal muscle regeneration. *Proc Natl Acad Sci U S A*, 111(11): 4109-4114.
- [9] Estrella NL, Desjardins CA, Nocco SE, Clark AL, Maksimenko Y, Naya FJ (2015). MEF2 transcription factors regulate distinct gene programs in mammalian skeletal muscle differentiation. *J Biol Chem*, 290(2): 1256-1268.
- [10] Desjardins CA, Naya FJ (2017). Antagonistic regulation of cell-cycle and differentiation gene programs in neonatal cardiomyocytes by homologous MEF2 transcription factors. *J Biol Chem*, 292(25): 10613-10629.
- [11] Wang L, Fan C, Topol SE, Topol EJ, Wang Q (2003). Mutation of MEF2A in an inherited disorder with features of coronary artery disease. *Science*, 302(5650): 1578-1581.
- [12] Naya FJ, Black BL, Wu H, Bassel-Duby R, Richardson JA, Hill JA, et al. (2002). Mitochondrial deficiency and cardiac sudden death in mice lacking the MEF2A transcription factor. *Nat Med*, 8(11): 1303-1309.
- [13] Qiao Q, Zhao CM, Yang CX, Gu JN, Guo YH, Zhang M, et al. (2021). Detection and functional characterization of a novel MEF2A variation responsible for familial dilated cardiomyopathy. *Clin Chem Lab Med*, 59(5): 955-963.
- [14] Li H, Wang F, Guo X, Jiang Y (2021). Decreased MEF2A Expression Regulated by Its Enhancer Methylation Inhibits Autophagy and May Play an Important Role in the Progression of Alzheimer's Disease. *Front Neurosci*, 15: 682247.
- [15] Gonzalez P, Alvarez V, Menendez M, Lahoz CH, Martinez C, Corao AI, et al. (2007). Myocyte enhancing factor-2A in Alzheimer's disease: genetic analysis and association with MEF2A-polymorphisms. *Neurosci Lett*, 411(1): 47-51.
- [16] Di Giorgio E, Hancock WW, Brancolini C (2018). MEF2 and the tumorigenic process, hic sunt leones. *Biochim Biophys Acta Rev Cancer*, 1870(2): 261-273.
- [17] Dodou E, Sparrow DB, Mohun T, Treisman R (1995). MEF2 proteins, including MEF2A, are expressed in both muscle and non-muscle cells. *Nucleic Acids Res*, 23(21): 4267-4274.
- [18] Subramanian SV, Nadal-Ginard B (1996). Early expression of the different isoforms of the myocyte enhancer factor-2 (MEF2) protein in myogenic as well as non-myogenic cell lineages during mouse embryogenesis. *Mech Dev*, 57(1): 103-112.
- [19] Edmondson DG, Lyons GE, Martin JF, Olson EN (1994). Mef2 gene expression marks the cardiac and skeletal muscle lineages during mouse embryogenesis. *Development*, 120(5): 1251-1263.
- [20] Chen XY, Lv RJ, Zhang W, Yan YG, Li P, Dong WQ, et al. (2016). Inhibition of myocyte-specific enhancer factor 2A improved diabetic cardiac fibrosis partially by regulating endothelial-to-mesenchymal transition. *Oncotarget*, 7(21): 31053-31066.
- [21] Zhao W, Zheng XL, Peng DQ, Zhao SP (2015). Myocyte Enhancer Factor 2A Regulates Hydrogen Peroxide-Induced Senescence of Vascular Smooth Muscle Cells Via microRNA-143. *J Cell Physiol*, 230(9): 2202-2211.
- [22] Xia X, Yu CY, Bian M, Sun CB, Tanasa B, Chang KC, et al. (2020). MEF2 transcription factors differentially contribute to retinal ganglion cell loss after optic nerve injury. *PLoS One*, 15(12): e0242884.
- [23] Tobin SW, Hashemi S, Dadson K, Turdi S, Ebrahimian K, Zhao J, et al. (2017). Heart Failure and MEF2 Transcriptome Dynamics in Response to beta-Blockers. *Sci Rep*, 7(1): 4476.
- [24] Dai DP, Zhou XY, Xiao Y, Xu F, Sun FC, Ji FS, et al. (2010). Structural changes in exon 11 of MEF2A are not related to sporadic coronary artery disease in Han Chinese population. *Eur J Clin Invest*, 40(8): 669-677.
- [25] Liu Y, Niu W, Wu Z, Su X, Chen Q, Lu L, et al. (2012). Variants in exon 11 of MEF2A gene and coronary artery disease: evidence from a case-control study, systematic review, and meta-analysis. *PLoS One*, 7(2): e31406.
- [26] Bhagavatula MR, Fan C, Shen GQ, Cassano J, Plow EF, Topol EJ, et al. (2004). Transcription factor MEF2A mutations in patients with coronary artery disease. *Hum Mol Genet*, 13(24): 3181-3188.
- [27] Li J, Chen HX, Yang JG, Li W, Du R, Tian L (2014). MEF2A gene mutations and susceptibility to coronary artery disease in the Chinese population. *Genet Mol Res*, 13(4): 8396-8402.
- [28] Dai Y, Zhang S, Wu W (2013). Analysis of MEF2A mutations in a Chinese population with premature coronary artery disease. *Genet Test Mol Biomarkers*, 17(4): 352-355.
- [29] Xu DL, Tian HL, Cai WL, Zheng J, Gao M, Zhang MX, et al. (2016). Novel 6-bp deletion in MEF2A linked to premature coronary artery disease in a large Chinese family. *Mol Med Rep*, 14(1): 649-654.
- [30] Weng L, Kavaslar N, Ustaszewska A, Doelle H, Schackwitz W, Hebert S, et al. (2005). Lack of MEF2A mutations in coronary artery disease. *J Clin Invest*, 115(4): 1016-1020.
- [31] Wang Q, Rao S, Topol EJ (2005). Miscues on the "lack of MEF2A mutations" in coronary artery disease. *J Clin Invest*, 115(6): 1399-1400; author reply 1400-1391.
- [32] Kajimoto K, Shioji K, Tago N, Tomoiike H, Nonogi H, Goto Y, et al. (2005). Assessment of MEF2A mutations in myocardial infarction in Japanese patients. *Circ J*, 69(10): 1192-1195.
- [33] Guella I, Rimoldi V, Asselta R, Ardissino D, Francolini M, Martinelli N, et al. (2009). Association and functional analyses of MEF2A as a susceptibility gene for premature myocardial infarction and coronary artery disease. *Circ Cardiovasc Genet*, 2(2): 165-172.
- [34] Altschuler D, Hirschhorn JN (2005). MEF2A sequence variants and coronary artery disease: a change of heart? *J Clin Invest*, 115(4): 831-833.
- [35] Inanloo Rahatloo K, Davaran S, Elahi E (2013). Lack of Association between the MEF2A Gene and Coronary Artery Disease in Iranian Families. *Iran J Basic Med Sci*, 16(8): 950-954.
- [36] Lieb W, Mayer B, König IR, Borwitzky I, Gotz A, Kain S, et al. (2008). Lack of association between the

- MEF2A gene and myocardial infarction. *Circulation*, 117(2): 185-191.
- [37] Elhawari S, Al-Boudari O, Muiya P, Khalak H, Andres E, Al-Shahid M, et al. (2010). A study of the role of the Myocyte-specific Enhancer Factor-2A gene in coronary artery disease. *Atherosclerosis*, 209(1): 152-154.
- [38] Gonzalez P, Garcia-Castro M, Reguero JR, Batalla A, Ordonez AG, Palop RL, et al. (2006). The Pro279Leu variant in the transcription factor MEF2A is associated with myocardial infarction. *J Med Genet*, 43(2): 167-169.
- [39] Coto E, Castro MG, Corao AI, Alonso-Montes C, Reguero JR, Moris C, et al. (2009). Mutation analysis of the myocyte enhancer factor 2A gene (MEF2A) in patients with left ventricular hypertrophy/hypertrophic cardiomyopathy. *Am J Med Genet A*, 149A(2): 286-289.
- [40] Foroughmand AM, Shahbazi Z, Galehdari H, Purmahdi Borujeni M, Dinarvand P, Golabgirkhademi K (2014). Association of MEF2A gene polymorphisms with coronary artery disease. *Iran Red Crescent Med J*, 16(8): e13533.
- [41] Han Y, Yang Y, Zhang X, Yan C, Xi S, Kang J (2007). Relationship of the CAG repeat polymorphism of the MEF2A gene and coronary artery disease in a Chinese population. *Clin Chem Lab Med*, 45(8): 987-992.
- [42] Yuan F, Qiu ZH, Wang XH, Sun YM, Wang J, Li RG, et al. (2018). MEF2C loss-of-function mutation associated with familial dilated cardiomyopathy. *Clin Chem Lab Med*, 56(3): 502-511.
- [43] Materna SC, Sinha T, Barnes RM, Lammerts van Bueren K, Black BL (2019). Cardiovascular development and survival require Mef2c function in the myocardial but not the endothelial lineage. *Dev Biol*, 445(2): 170-177.
- [44] Kula-Alwar D, Marber MS, Hughes SM, Hinits Y (2021). Mef2c factors are required for early but not late addition of cardiomyocytes to the ventricle. *Dev Biol*, 470: 95-107.
- [45] Lin Q, Lu J, Yanagisawa H, Webb R, Lyons GE, Richardson JA, et al. (1998). Requirement of the MADS-box transcription factor MEF2C for vascular development. *Development*, 125(22): 4565-4574.
- [46] Ramachandran B, Yu G, Gulick T (2008). Nuclear respiratory factor 1 controls myocyte enhancer factor 2A transcription to provide a mechanism for coordinate expression of respiratory chain subunits. *J Biol Chem*, 283(18): 11935-11946.
- [47] Schlesinger J, Schueler M, Grunert M, Fischer JJ, Zhang Q, Krueger T, et al. (2011). The cardiac transcription network modulated by Gata4, Mef2a, Nkx2.5, Srf, histone modifications, and microRNAs. *PLoS Genet*, 7(2): e1001313.
- [48] Karamboulas C, Dakubo GD, Liu J, De Repentigny Y, Yutzey K, Wallace VA, et al. (2006). Disruption of MEF2 activity in cardiomyoblasts inhibits cardiomyogenesis. *J Cell Sci*, 119(Pt 20): 4315-4321.
- [49] Ewen EP, Snyder CM, Wilson M, Desjardins D, Naya FJ (2011). The Mef2A transcription factor coordinately regulates a costamere gene program in cardiac muscle. *J Biol Chem*, 286(34): 29644-29653.
- [50] Xu J, Gong NL, Bodi I, Aronow BJ, Backx PH, Molkentin JD (2006). Myocyte enhancer factors 2A and 2C induce dilated cardiomyopathy in transgenic mice. *J Biol Chem*, 281(14): 9152-9162.
- [51] Lee KS, Park JH, Lim HJ, Park HY (2011). HB-EGF induces cardiomyocyte hypertrophy via an ERK5-MEF2A-COX2 signaling pathway. *Cell Signal*, 23(7): 1100-1109.
- [52] Phan NN, Wang CY, Lin YC (2014). The novel regulations of MEF2A, CAMKK2, CALM3, and TNNI3 in ventricular hypertrophy induced by arsenic exposure in rats. *Toxicology*, 324: 123-135.
- [53] Yu J, Yang Y, Xu Z, Lan C, Chen C, Li C, et al. (2020). Long Noncoding RNA Ahit Protects Against Cardiac Hypertrophy Through SUZ12 (Suppressor of Zeste 12 Protein Homolog)-Mediated Downregulation of MEF2A (Myocyte Enhancer Factor 2A). *Circ Heart Fail*, 13(1): e006525.
- [54] Lu YW, Martino N, Gerlach BD, Lamar JM, Vincent PA, Adam AP, et al. (2021). MEF2 (Myocyte Enhancer Factor 2) Is Essential for Endothelial Homeostasis and the Atheroprotective Gene Expression Program. *Arterioscler Thromb Vasc Biol*, 41(3): 1105-1123.
- [55] Sacilotto N, Chouliaras KM, Nikitenko LL, Lu YW, Fritzsche M, Wallace MD, et al. (2016). MEF2 transcription factors are key regulators of sprouting angiogenesis. *Genes Dev*, 30(20): 2297-2309.
- [56] Parmar KM, Larman HB, Dai G, Zhang Y, Wang ET, Moorthy SN, et al. (2006). Integration of flow-dependent endothelial phenotypes by Kruppel-like factor 2. *J Clin Invest*, 116(1): 49-58.
- [57] Sako K, Fukuhara S, Minami T, Hamakubo T, Song H, Kodama T, et al. (2009). Angiopoietin-1 induces Kruppel-like factor 2 expression through a phosphoinositide 3-kinase/AKT-dependent activation of myocyte enhancer factor 2. *J Biol Chem*, 284(9): 5592-5601.
- [58] Liu B, Wang L, Jiang W, Xiong Y, Pang L, Zhong Y, et al. (2019). Myocyte enhancer factor 2A delays vascular endothelial cell senescence by activating the PI3K/p-Akt/SIRT1 pathway. *Aging (Albany NY)*, 11(11): 3768-3784.
- [59] Liu B, Pang L, Ji Y, Fang L, Tian CW, Chen J, et al. (2022). MEF2A Is the Trigger of Resveratrol Exerting Protection on Vascular Endothelial Cell. *Front Cardiovasc Med*, 8: 775392.
- [60] Kim J, Hwangbo C, Hu X, Kang Y, Papangelis I, Mehrotra D, et al. (2015). Restoration of impaired endothelial myocyte enhancer factor 2 function rescues pulmonary arterial hypertension. *Circulation*, 131(2): 190-199.
- [61] Zhou WP, Zhang H, Zhao YX, Liu GQ, Zhang JY (2015). RNA interference of myocyte enhancer factor 2A accelerates atherosclerosis in apolipoprotein E-deficient mice. *PLoS One*, 10(3): e0121823.
- [62] Zhao C, Zong Z, Zhu Q, Wang Y, Li X, Zhang C, et al. (2021). The lncRNA MALAT1 participates in regulating coronary slow flow endothelial dysfunction through the miR-181b-5p-MEF2A-ET-1 axis. *Vascul Pharmacol*, 138: 106841.

- [63] Liu C, Yao MD, Li CP, Shan K, Yang H, Wang JJ, et al. (2017). Silencing Of Circular RNA-ZNF609 Ameliorates Vascular Endothelial Dysfunction. *Theranostics*, 7(11): 2863-2877.
- [64] Welten SMJ, de Vries MR, Peters EAB, Agrawal S, Quax PHA, Nossent AY (2017). Inhibition of Mef2a Enhances Neovascularization via Post-transcriptional Regulation of 14q32 MicroRNAs miR-329 and miR-494. *Mol Ther Nucleic Acids*, 7: 61-70.
- [65] Tobin SW, Yang D, Girgis J, Farahzad A, Blais A, McDermott JC (2016). Regulation of Hspb7 by MEF2 and AP-1: implications for Hspb7 in muscle atrophy. *J Cell Sci*, 129(21): 4076-4090.
- [66] Wang YN, Yang WC, Li PW, Wang HB, Zhang YY, Zan LS (2018). Myocyte enhancer factor 2A promotes proliferation and its inhibition attenuates myogenic differentiation via myozenin 2 in bovine skeletal muscle myoblast. *PLoS One*, 13(4): e0196255.
- [67] Wu R, Wang J, Yao J, Dong Z, Liu Y, Liu M (2018). MEF2A regulates Calpain 3 expression in L6 myoblasts. *Gene*, 668: 204-210.
- [68] Durham JT, Brand OM, Arnold M, Reynolds JG, Muthukumar L, Weiler H, et al. (2006). Myospryn is a direct transcriptional target for MEF2A that encodes a striated muscle, alpha-actinin-interacting, costamere-localized protein. *J Biol Chem*, 281(10): 6841-6849.
- [69] Rauch C, Loughna PT (2005). Static stretch promotes MEF2A nuclear translocation and expression of neonatal myosin heavy chain in C2C12 myocytes in a calcineurin- and p38-dependent manner. *Am J Physiol Cell Physiol*, 288(3): C593-605.
- [70] Xiong Y, Wang L, Jiang W, Pang L, Liu W, Li A, et al. (2019). MEF2A alters the proliferation, inflammation-related gene expression profiles and its silencing induces cellular senescence in human coronary endothelial cells. *BMC Mol Biol*, 20(1): 8.
- [71] Zhao W, Zhao SP, Peng DQ (2012). The effects of myocyte enhancer factor 2A gene on the proliferation, migration and phenotype of vascular smooth muscle cells. *Cell Biochem Funct*, 30(2): 108-113.
- [72] Suzuki E, Satonaka H, Nishimatsu H, Oba S, Takeda R, Omata M, et al. (2004). Myocyte enhancer factor 2 mediates vascular inflammation via the p38-dependent pathway. *Circ Res*, 95(1): 42-49.
- [73] Firulli AB, Miano JM, Bi W, Johnson AD, Casscells W, Olson EN, et al. (1996). Myocyte enhancer binding factor-2 expression and activity in vascular smooth muscle cells. Association with the activated phenotype. *Circ Res*, 78(2): 196-204.
- [74] Lyons GE, Micales BK, Schwarz J, Martin JF, Olson EN (1995). Expression of mef2 genes in the mouse central nervous system suggests a role in neuronal maturation. *J Neurosci*, 15(8): 5727-5738.
- [75] Majidi SP, Reddy NC, Moore MJ, Chen H, Yamada T, Andzelm MM, et al. (2019). Chromatin Environment and Cellular Context Specify Compensatory Activity of Paralogous MEF2 Transcription Factors. *Cell Rep*, 29(7): 2001-2015 e2005.
- [76] Ma Q, Telese F (2015). Genome-wide epigenetic analysis of MEF2A and MEF2C transcription factors in mouse cortical neurons. *Commun Integr Biol*, 8(6): e1087624.
- [77] Gaudilliere B, Shi Y, Bonni A (2002). RNA interference reveals a requirement for myocyte enhancer factor 2A in activity-dependent neuronal survival. *J Biol Chem*, 277(48): 46442-46446.
- [78] Zhu B, Carmichael RE, Solabre Valois L, Wilkinson KA, Henley JM (2018). The transcription factor MEF2A plays a key role in the differentiation/maturation of rat neural stem cells into neurons. *Biochem Biophys Res Commun*, 500(3): 645-649.
- [79] Li M, Linseman DA, Allen MP, Meintzer MK, Wang X, Laessig T, et al. (2001). Myocyte enhancer factor 2A and 2D undergo phosphorylation and caspase-mediated degradation during apoptosis of rat cerebellar granule neurons. *J Neurosci*, 21(17): 6544-6552.
- [80] Tang X, Wang X, Gong X, Tong M, Park D, Xia Z, et al. (2005). Cyclin-dependent kinase 5 mediates neurotoxin-induced degradation of the transcription factor myocyte enhancer factor 2. *J Neurosci*, 25(19): 4823-4834.
- [81] Zhang L, Sun Y, Fei M, Tan C, Wu J, Zheng J, et al. (2014). Disruption of chaperone-mediated autophagy-dependent degradation of MEF2A by oxidative stress-induced lysosome destabilization. *Autophagy*, 10(6): 1015-1035.
- [82] She H, Yang Q, Mao Z (2012). Neurotoxin-induced selective ubiquitination and regulation of MEF2A isoform in neuronal stress response. *J Neurochem*, 122(6): 1203-1210.
- [83] Latchney SE, Jiang Y, Petrik DP, Eisch AJ, Hsieh J (2015). Inducible knockout of Mef2a, -c, and -d from nestin-expressing stem/progenitor cells and their progeny unexpectedly uncouples neurogenesis and dendritogenesis in vivo. *FASEB J*, 29(12): 5059-5071.
- [84] Shalizi A, Gaudilliere B, Yuan Z, Stegmuller J, Shirogane T, Ge Q, et al. (2006). A calcium-regulated MEF2 sumoylation switch controls postsynaptic differentiation. *Science*, 311(5763): 1012-1017.
- [85] Flavell SW, Cowan CW, Kim TK, Greer PL, Lin Y, Paradis S, et al. (2006). Activity-dependent regulation of MEF2 transcription factors suppresses excitatory synapse number. *Science*, 311(5763): 1008-1012.
- [86] Yamada T, Yang Y, Huang J, Coppola G, Geschwind DH, Bonni A (2013). Sumoylated MEF2A coordinately eliminates orphan presynaptic sites and promotes maturation of presynaptic boutons. *J Neurosci*, 33(11): 4726-4740.
- [87] Lu H, Liu B, You S, Chen L, Dongmei Q, Gu M, et al. (2013). SENP2 regulates MEF2A de-SUMOylation in an activity dependent manner. *Mol Biol Rep*, 40(3): 2485-2490.
- [88] Shalizi A, Bilimoria PM, Stegmuller J, Gaudilliere B, Yang Y, Shuai K, et al. (2007). PIASx is a MEF2 SUMO E3 ligase that promotes postsynaptic dendritic morphogenesis. *J Neurosci*, 27(37): 10037-10046.
- [89] Akhtar MW, Kim MS, Adachi M, Morris MJ, Qi X, Richardson JA, et al. (2012). In vivo analysis of MEF2 transcription factors in synapse regulation and neuronal

- survival. *PLoS One*, 7(4): e34863.
- [90] Chang CW, Wilkerson JR, Hale CF, Gibson JR, Huber KM (2017). Distinct stages of synapse elimination are induced by burst firing of CA1 neurons and differentially require MEF2A/D. *Elife*, 6.
- [91] Chen SX, Cherry A, Tari PK, Podgorski K, Kwong YK, Haas K (2012). The transcription factor MEF2 directs developmental visually driven functional and structural metaplasticity. *Cell*, 151(1): 41-55.
- [92] Pulipparacharuvil S, Renthal W, Hale CF, Taniguchi M, Xiao G, Kumar A, et al. (2008). Cocaine regulates MEF2 to control synaptic and behavioral plasticity. *Neuron*, 59(4): 621-633.
- [93] Cole CJ, Mercaldo V, Restivo L, Yiu AP, Sekeres MJ, Han JH, et al. (2012). MEF2 negatively regulates learning-induced structural plasticity and memory formation. *Nat Neurosci*, 15(9): 1255-1264.
- [94] Meyer M, Kuffner K, Winter J, Neumann ID, Wetzel CH, Jurek B (2020). Myocyte Enhancer Factor 2A (MEF2A) Defines Oxytocin-Induced Morphological Effects and Regulates Mitochondrial Function in Neurons. *Int J Mol Sci*, 21(6).
- [95] Meyer M, Berger I, Winter J, Jurek B (2018). Oxytocin alters the morphology of hypothalamic neurons via the transcription factor myocyte enhancer factor 2A (MEF2A). *Mol Cell Endocrinol*, 477: 156-162.
- [96] Thygesen JH, Zambach SK, Ingason A, Lundin P, Hansen T, Bertalan M, et al. (2015). Linkage and whole genome sequencing identify a locus on 6q25-26 for formal thought disorder and implicate MEF2A regulation. *Schizophr Res*, 169(1-3): 441-446.
- [97] Barker SJ, Raju RM, Milman NEP, Wang J, Davila-Velderrain J, Gunter-Rahman F, et al. (2021). MEF2 is a key regulator of cognitive potential and confers resilience to neurodegeneration. *Sci Transl Med*, 13(618): eabd7695.
- [98] Carmichael RE, Wilkinson KA, Craig TJ, Ashby MC, Henley JM (2018). MEF2A regulates mGluR-dependent AMPA receptor trafficking independently of Arc/Arg3.1. *Sci Rep*, 8(1): 5263.
- [99] Wang IF, Wang Y, Yang YH, Huang GJ, Tsai KJ, Shen CJ (2021). Activation of a hippocampal CREB-pCREB-miRNA-MEF2 axis modulates individual variation of spatial learning and memory capability. *Cell Rep*, 36(5): 109477.
- [100] Yin JW, Li J, Ren YM, Li Y, Wang RX, Wang S, et al. (2021). Dexmedetomidine and Netrin-1 Combination Therapy Inhibits Endoplasmic Reticulum Stress by Regulating the ERK5/MEF2A Pathway to Attenuate Cerebral Ischemia Injury. *Front Neurosci*, 15: 641345.
- [101] Bachinski LL, Sirito M, Bohme M, Baggerly KA, Udd B, Krahe R (2010). Altered MEF2 isoforms in myotonic dystrophy and other neuromuscular disorders. *Muscle Nerve*, 42(6): 856-863.
- [102] Di Giorgio E, Franforte E, Cefalu S, Rossi S, Dei Tos AP, Brenca M, et al. (2017). The co-existence of transcriptional activator and transcriptional repressor MEF2 complexes influences tumor aggressiveness. *PLoS Genet*, 13(4): e1006752.
- [103] Ishikawa F, Miyoshi H, Nose K, Shibnuma M (2010). Transcriptional induction of MMP-10 by TGF-beta, mediated by activation of MEF2A and downregulation of class IIa HDACs. *Oncogene*, 29(6): 909-919.
- [104] Tian F, Wang H, Ma H, Zhong Y, Liao A (2020). miR1443p inhibits the proliferation, migration and angiogenesis of multiple myeloma cells by targeting myocyte enhancer factor 2A. *Int J Mol Med*, 46(3): 1155-1165.
- [105] Xiao Q, Gan Y, Li Y, Fan L, Liu J, Lu P, et al. (2021). MEF2A transcriptionally upregulates the expression of ZEB2 and CTNNB1 in colorectal cancer to promote tumor progression. *Oncogene*, 40(19): 3364-3377.
- [106] Liu J, Wen D, Fang X, Wang X, Liu T, Zhu J (2015). p38MAPK Signaling Enhances Glycolysis Through the Up-Regulation of the Glucose Transporter GLUT-4 in Gastric Cancer Cells. *Cell Physiol Biochem*, 36(1): 155-165.
- [107] Ma Y, Shi Y, Li W, Sun A, Zang P, Zhang P (2014). Epigallocatechin-3-gallate regulates the expression of Kruppel-like factor 4 through myocyte enhancer factor 2A. *Cell Stress Chaperones*, 19(2): 217-226.
- [108] Chen W, Zhang K, Yang Y, Guo Z, Wang X, Teng B, et al. (2021). MEF2A-mediated lncRNA HCP5 Inhibits Gastric Cancer Progression via MiR-106b-5p/p21 Axis. *Int J Biol Sci*, 17(2): 623-634.
- [109] Kim JM, Hwang IH, Jang IS, Kim M, Bang IS, Park SJ, et al. (2017). *Houttuynia cordata* Thunb Promotes Activation of HIF-1A-FOXO3 and MEF2A Pathways to Induce Apoptosis in Human HepG2 Hepatocellular Carcinoma Cells. *Integr Cancer Ther*, 16(3): 360-372.
- [110] Blixt N, Norton A, Zhang A, Aparicio C, Prasad H, Gopalakrishnan R, et al. (2020). Loss of myocyte enhancer factor 2 expression in osteoclasts leads to opposing skeletal phenotypes. *Bone*, 138: 115466.
- [111] Zhang W, Ping J, Zhou Y, Chen G, Xu L (2019). Salvianolic Acid B Inhibits Activation of Human Primary Hepatic Stellate Cells Through Downregulation of the Myocyte Enhancer Factor 2 Signaling Pathway. *Front Pharmacol*, 10: 322.
- [112] Li L, Rubin LP, Gong X (2018). MEF2 transcription factors in human placenta and involvement in cytotrophoblast invasion and differentiation. *Physiol Genomics*, 50(1): 10-19.
- [113] Ornatsky OI, Cox DM, Tangirala P, Andreucci JJ, Quinn ZA, Wrana JL, et al. (1999). Post-translational control of the MEF2A transcriptional regulatory protein. *Nucleic Acids Res*, 27(13): 2646-2654.
- [114] Cox DM, Du M, Marback M, Yang EC, Chan J, Siu KW, et al. (2003). Phosphorylation motifs regulating the stability and function of myocyte enhancer factor 2A. *J Biol Chem*, 278(17): 15297-15303.
- [115] Barsyte-Lovejoy D, Galanis A, Clancy A, Sharrocks AD (2004). ERK5 is targeted to myocyte enhancer factor 2A (MEF2A) through a MAPK docking motif. *Biochem J*, 381(Pt 3): 693-699.
- [116] Dionysiou MG, Nowacki NB, Hashemi S, Zhao J, Kerr A, Tsushima RG, et al. (2013). Cross-talk between glycogen synthase kinase 3beta (GSK3beta) and p38MAPK regulates myocyte enhancer factor 2 (MEF2) activity in skeletal and cardiac muscle. *J Mol Cell*

- Cardiol, 54: 35-44.
- [117] Kato Y, Zhao M, Morikawa A, Sugiyama T, Chakravorty D, Koide N, et al. (2000). Big mitogen-activated kinase regulates multiple members of the MEF2 protein family. *J Biol Chem*, 275(24): 18534-18540.
- [118] Satoh K, Ohnishi J, Sato A, Takeyama M, Iemura S, Natsume T, et al. (2007). Nemo-like kinase-myocyte enhancer factor 2A signaling regulates anterior formation in *Xenopus* development. *Mol Cell Biol*, 27(21): 7623-7630.
- [119] Angelelli C, Magli A, Ferrari D, Ganassi M, Matafora V, Parise F, et al. (2008). Differentiation-dependent lysine 4 acetylation enhances MEF2C binding to DNA in skeletal muscle cells. *Nucleic Acids Res*, 36(3): 915-928.
- [120] Gregoire S, Xiao L, Nie J, Zhang X, Xu M, Li J, et al. (2007). Histone deacetylase 3 interacts with and deacetylates myocyte enhancer factor 2. *Mol Cell Biol*, 27(4): 1280-1295.
- [121] Yuan H, Niu Y, Liu X, Fu L (2014). Exercise increases the binding of MEF2A to the Cpt1b promoter in mouse skeletal muscle. *Acta Physiol (Oxf)*, 212(4): 283-292.
- [122] Riquelme C, Barthel KK, Liu X (2006). SUMO-1 modification of MEF2A regulates its transcriptional activity. *J Cell Mol Med*, 10(1): 132-144.
- [123] Toro R, Saadi I, Kuburas A, Nemer M, Russo AF (2004). Cell-specific activation of the atrial natriuretic factor promoter by PITX2 and MEF2A. *J Biol Chem*, 279(50): 52087-52094.
- [124] Lemerrier C, Verdel A, Galloo B, Curtet S, Brocard MP, Khochbin S (2000). mHDA1/HDAC5 histone deacetylase interacts with and represses MEF2A transcriptional activity. *J Biol Chem*, 275(20): 15594-15599.
- [125] Kang Y, Kim J, Anderson JP, Wu J, Gleim SR, Kundu RK, et al. (2013). Apelin-APJ signaling is a critical regulator of endothelial MEF2 activation in cardiovascular development. *Circ Res*, 113(1): 22-31.
- [126] Aude-Garcia C, Collin-Faure V, Bausinger H, Hanau D, Rabilloud T, Lemerrier C (2010). Dual roles for MEF2A and MEF2D during human macrophage terminal differentiation and c-Jun expression. *Biochem J*, 430(2): 237-244.
- [127] Mao Z, Nadal-Ginard B (1996). Functional and physical interactions between mammalian achaete-scute homolog 1 and myocyte enhancer factor 2A. *J Biol Chem*, 271(24): 14371-14375.
- [128] Di-Luoffo M, Brousseau C, Tremblay JJ (2016). MEF2 and NR2F2 cooperate to regulate Akr1c14 gene expression in mouse MA-10 Leydig cells. *Andrology*, 4(2): 335-344.
- [129] Feng Y, Desjardins CA, Cooper O, Kontor A, Nocco SE, Naya FJ (2015). EGR1 Functions as a Potent Repressor of MEF2 Transcriptional Activity. *PLoS One*, 10(5): e0127641.
- [130] Chen D, Chen F, Xu Y, Zhang Y, Li Z, Zhang H, et al. (2017). AKT2 deficiency induces retardation of myocyte development through EndoG-MEF2A signaling in mouse heart. *Biochem Biophys Res Commun*, 493(4): 1410-1417.
- [131] Ghosh-Choudhury N, Abboud SL, Mahimainathan L, Chandrasekar B, Choudhury GG (2003). Phosphatidylinositol 3-kinase regulates bone morphogenetic protein-2 (BMP-2)-induced myocyte enhancer factor 2A-dependent transcription of BMP-2 gene in cardiomyocyte precursor cells. *J Biol Chem*, 278(24): 21998-22005.
- [132] Ramachandran B, Yu G, Li S, Zhu B, Gulick T (2008). Myocyte enhancer factor 2A is transcriptionally autoregulated. *J Biol Chem*, 283(16): 10318-10329.
- [133] Joseph JS, Ayeleso AO, Mukwevho E (2017). Exercise increases hyper-acetylation of histones on the Cis-element of NRF-1 binding to the Mef2a promoter: Implications on type 2 diabetes. *Biochem Biophys Res Commun*, 486(1): 83-87.
- [134] Black BL, Lu J, Olson EN (1997). The MEF2A 3' untranslated region functions as a cis-acting translational repressor. *Mol Cell Biol*, 17(5): 2756-2763.
- [135] Wang Q, Lu G, Chen Z (2019). MALAT1 promoted cell proliferation and migration via MALAT1/miR-155/MEF2A pathway in hypoxia of cardiac stem cells. *J Cell Biochem*, 120(4): 6384-6394.
- [136] Zhang C, Li J, Li H, Wang G, Wang Q, Zhang X, et al. (2021). lncRNA MIR155HG Accelerates the Progression of Sepsis via Upregulating MEF2A by Sponging miR-194-5p. *DNA Cell Biol*, 40(6): 811-820.
- [137] Mora S, Pessin JE (2000). The MEF2A isoform is required for striated muscle-specific expression of the insulin-responsive GLUT4 glucose transporter. *J Biol Chem*, 275(21): 16323-16328.
- [138] Chen L, Wang F, Sun X, Zhou J, Gao L, Jiao Y, et al. (2010). Chronic ethanol feeding impairs AMPK and MEF2 expression and is associated with GLUT4 decrease in rat myocardium. *Exp Mol Med*, 42(3): 205-215.
- [139] Thi Do D, Phan NN, Wang CY, Sun Z, Lin YC (2016). Novel regulations of MEF2-A, MEF2-D, and CACNA1S in the functional incompetence of adipose-derived mesenchymal stem cells by induced indoxyl sulfate in chronic kidney disease. *Cytotechnology*, 68(6): 2589-2604.
- [140] Han TH, Prywes R (1995). Regulatory role of MEF2D in serum induction of the c-jun promoter. *Mol Cell Biol*, 15(6): 2907-2915.
- [141] Ornatsky OI, McDermott JC (1996). MEF2 protein expression, DNA binding specificity and complex composition, and transcriptional activity in muscle and non-muscle cells. *J Biol Chem*, 271(40): 24927-24933.
- [142] Medrano JL, Naya FJ (2017). The transcription factor MEF2A fine-tunes gene expression in the atrial and ventricular chambers of the adult heart. *J Biol Chem*, 292(51): 20975-20988.
- [143] Snyder CM, Rice AL, Estrella NL, Held A, Kandarian SC, Naya FJ (2013). MEF2A regulates the Gtl2-Dio3 microRNA mega-cluster to modulate WNT signaling in skeletal muscle regeneration. *Development*, 140(1): 31-42.
- [144] Wang Y, Mei C, Su X, Wang H, Yang W, Zan L (2019). MEF2A Regulates the MEG3-DIO3 miRNA Mega

- Cluster-Targeted PP2A Signaling in Bovine Skeletal Myoblast Differentiation. *Int J Mol Sci*, 20(11).
- [145] Mukwevho E, Kohn TA, Lang D, Nyatia E, Smith J, Ojuka EO (2008). Caffeine induces hyperacetylation of histones at the MEF2 site on the Glut4 promoter and increases MEF2A binding to the site via a CaMK-dependent mechanism. *Am J Physiol Endocrinol Metab*, 294(3): E582-588.
- [146] Smith JA, Kohn TA, Chetty AK, Ojuka EO (2008). CaMK activation during exercise is required for histone hyperacetylation and MEF2A binding at the MEF2 site on the Glut4 gene. *Am J Physiol Endocrinol Metab*, 295(3): E698-704.
- [147] Gong H, Xie J, Zhang N, Yao L, Zhang Y (2011). MEF2A binding to the Glut4 promoter occurs via an AMPKalpha2-dependent mechanism. *Med Sci Sports Exerc*, 43(8): 1441-1450.
- [148] Silva JL, Giannocco G, Furuya DT, Lima GA, Moraes PA, Nachev S, et al. (2005). NF-kappaB, MEF2A, MEF2D and HIF1-a involvement on insulin- and contraction-induced regulation of GLUT4 gene expression in soleus muscle. *Mol Cell Endocrinol*, 240(1-2): 82-93.
- [149] Sparling DP, Griesel BA, Weems J, Olson AL (2008). GLUT4 enhancer factor (GEF) interacts with MEF2A and HDAC5 to regulate the GLUT4 promoter in adipocytes. *J Biol Chem*, 283(12): 7429-7437.
- [150] Holmes BF, Sparling DP, Olson AL, Winder WW, Dohm GL (2005). Regulation of muscle GLUT4 enhancer factor and myocyte enhancer factor 2 by AMP-activated protein kinase. *Am J Physiol Endocrinol Metab*, 289(6): E1071-1076.
- [151] Sparling DP, Griesel BA, Olson AL (2007). Hyperphosphorylation of MEF2A in primary adipocytes correlates with downregulation of human GLUT4 gene promoter activity. *Am J Physiol Endocrinol Metab*, 292(4): E1149-1156.
- [152] Mora S, Yang C, Ryder JW, Boeglin D, Pessin JE (2001). The MEF2A and MEF2D isoforms are differentially regulated in muscle and adipose tissue during states of insulin deficiency. *Endocrinology*, 142(5): 1999-2004.
- [153] Erck C, Seidl K (1998). MyoD and MEF2A mediate activation and repression of the p75NGFR gene during muscle development. *Biochem Biophys Res Commun*, 245(3): 871-877.
- [154] Wales S, Hashemi S, Blais A, McDermott JC (2014). Global MEF2 target gene analysis in cardiac and skeletal muscle reveals novel regulation of DUSP6 by p38MAPK-MEF2 signaling. *Nucleic Acids Res*, 42(18): 11349-11362.