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Transcriptional regulatory circuits controlling muscle fiber type switching

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Skeletal muscle fitness plays vital roles in human health and disease and is determined by developmental as well as physiological inputs. These inputs control and coordinate muscle fiber programs, including capacity for fuel burning, mitochondrial ATP production, and contraction. Recent studies have demonstrated crucial roles for nuclear receptors and their co-activators, and microRNAs (miRNAs) in the regulation of skeletal muscle energy metabolism and fiber type determination. In this review, we present recent progress in the study of nuclear receptor signaling and miRNA networks in muscle fiber type switching. We also discuss the therapeutic potential of nuclear receptors and miRNAs in disease states that are associated with loss of muscle fitness.

fiber type switching, gene regulation, muscle, nuclear receptor, microRNA

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Skeletal muscle is the biggest organ in the human body; it comprises ~40% of total body mass [1,2]. As one of the most metabolically demanding tissues that has very active lipid and glucose metabolism, skeletal muscle can account for ~80% of insulin-stimulated glucose disposal [3–5]. Skeletal muscle fitness plays important roles in human health and disease. Skeletal muscle fitness is determined by developmental as well as physiological inputs. These inputs control and coordinate muscle fiber programs, including fuel burning, mitochondrial ATP production, and contraction. Exercise is well known to be effective in improving muscle fitness by promoting favorable metabolic and structural adaptations [3–5]. Exercise training enhances muscle endurance and performance by augmenting the capacity of mitochondria to burn glucose and fatty acids and by increasing the proportion of slow oxidative fibers and blood supply [3–5]. Conversely, skeletal muscle dysfunction, including fiber type switching and decreased oxidative capacity, is a common consequence of physical inactivity, contributing to the progression of many chronic illness, including obesity, diabetes, muscular diseases and aging [2,3,5].

Skeletal muscle is a bundle of muscle fibers. Muscle fibers can be generally classified into oxidative slow-twitch and glycolytic fast-twitch myofibers. Based on myosin isoform expression, muscle in rodents can also be classified into Type I, Type IIa, Type IIx and Type IIb [1,2,5,6] (Figure 1). Type I and IIa fibers are characterized by high endurance and are mitochondria-rich (red), relying largely on mitochondrial oxidative metabolism for ATP production [2,5,6]. In contrast, Type IIb fibers generally have low endurance and less mitochondria, and are mainly reliant on glycolytic metabolism for energy production [2,5,6]. An intermediate IIx fiber type has also been defined in rodents, but not in human. How the slow twitch fiber is linked to high metabolic capacity is unclear.

Skeletal muscle exhibits remarkable plasticity, in response

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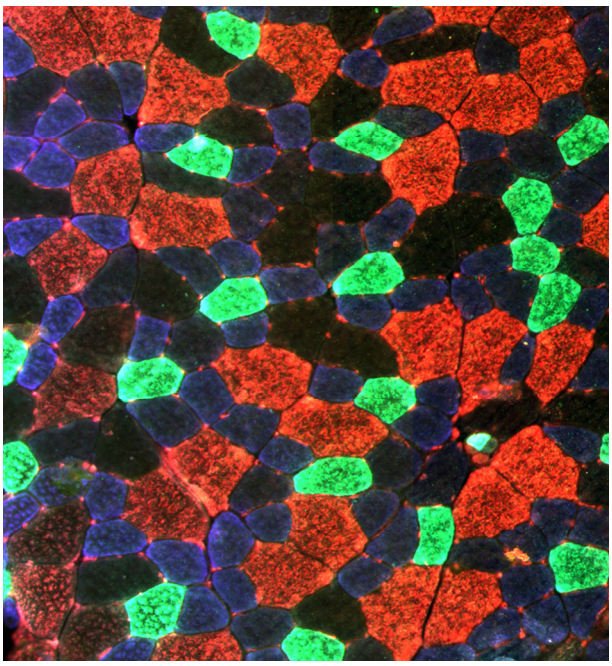

Figure 1 Skeletal muscle fiber types. Muscle fiber typing by immunofluorescence staining of gastrocnemius muscle from 3-month-old C57BL/6J mice. MHC1 (green), MHC2a (blue), MHC2b (red), and MHC2x (unstained).

to a variety of external stimulations; it undergoes extensive reprogramming to meet its energy and contraction demands. One of the best examples of this reprogramming is fiber type switching. Endurance exercise training results in an increased proportion of slow-twitch Type I and Type IIa fibers. Exercise training also increases the mitochondrial mass, which, in turn, increases capacity for oxidation of fat and glucose, and for exercise [3–5]. Conversely, reduced physical activity, such as occurs in chronic diseases states, results in a reduced proportion of Type I and Type IIa fibers [3–5]. Importantly, whole body insulin sensitivity and insulin-stimulated glucose uptake is significantly positively correlated with the proportion of slow-twitch muscle fibers [3,4,7]. The gene regulatory circuitry that controls muscle fiber type switching has been partially delineated over the past two decades. In addition to the widely accepted calcineurin/NFAT and the HDAC/MEF2 signaling pathways [8,9], it has recently been shown that nuclear receptor signaling and miRNAs also play crucial roles in the regulation of skeletal muscle energy metabolism and fiber type switching.

1 The PPAR subfamily of nuclear receptors

Nuclear receptors are a class of transcription factor that can sense hormones (also termed ligands) and that regulate gene expression in response to external stimuli [10–13]. The peroxisome proliferator-activated receptor (PPAR) subfamily of nuclear receptors comprises three members, PPARα, PPARβ/δ, and PPARγ [10–13]. PPARα is highly expressed in oxidative tissues such as skeletal muscle, liver and heart [14,15]. PPARβ/δ is ubiquitously expressed, with relatively high levels of expression in heart, skeletal muscle and brain [16], while PPARγ is adipose-specific and plays a vital role in adipocyte differentiation and fat storage [10,13]. PPARs form heterodimers with retinoid X receptors (RXRs) and bind to the PPAR response element (PPRE) in target gene promoters [10,17]. The activity of PPARs can be modulated by its expression levels, the availability of endogenous ligands, and its co-activators [10,13], as described below. Both gain of function and loss of function studies have demonstrated that PPARα and PPARβ/δ are key regulators of skeletal muscle energy metabolism and fiber type switching.

PPARα regulates the expression of genes involved in almost every step of fatty acid metabolism in skeletal muscle [14,15,18]. Transgenic mice, in which PPARα is over-expressed specifically in skeletal muscle (MCK-PPARα mice), have up-regulated expression of genes involved in fatty acid uptake, oxidation, storage, and mitochondrial oxidative phosphorylation, and the mice have high fatty acid oxidation (FAO) rates and are resistant to high-fat-diet induced obesity [18]. Interestingly, MCK-PPARα mice develop insulin resistance and glucose intolerance, and are able to run significant shorter distances than controls [18,19]. Gene expression profiling demonstrated that the expression of genes involved in glucose uptake and glycolysis was decreased in MCK-PPARα mice [18,19]. Even more surprisingly, fiber typing analysis demonstrated a dramatic reduction in Type I fibers in MCK-PPARα muscle compared to control littermates [20]. In contrast to the gain of function models, PPARα-deficient mice display decreased muscle FAO rates, and exhibit a mild age-associated cardiac fibrosis and an inability to compensate for increased cardiac workloads [21], indicating that PPARα is necessary for the heart's high capacity for burning fat. Moreover, PPARα-deficient muscle induces a shift in muscle fiber type proportion towards a slow oxidative type [20]. Taken together, although PPARα can potently activate mitochondrial FAO, it is also a repressor of slow-oxidative muscle fiber gene expression; therefore, its activity needs to be finely regulated in skeletal muscle.

PPARβ/δ can also potently activate the expression of genes involved in fatty acid metabolism in skeletal muscle [19,20,22–25]. Surprisingly, transgenic mice, in which PPARβ/δ is over-expressed specifically in skeletal muscle, have a phenotype that is remarkably different from that of the muscle-specific PPARα transgenic mice, despite the fact that both lines have increased capacity for mitochondrial FAO [19,20,23–25]. Three independent studies have demonstrated that over-expression of PPARβ/δ in skeletal muscle induces a slow-oxidative muscle fiber program [20,24,25]. Mice over-expressing a constitutively active

form of PPARβ/δ in skeletal muscle have increased endurance of more than 100% [25]. Consistently, the number of Type I muscle fibers is increased in the transgenic muscle and the mice are resistant to high-fat-diet induced obesity and have improved glucose tolerance [25]. Mice expressing a native PPARβ/δ protein also induce high FAO gene expression, and have more oxidative fibers than controls [20,24]. The MCK-PPARβ/δ mice exhibit many of the metabolic benefits of exercise, despite the absence of exercise training, including increased muscle glucose utilization [19,20]. For example, the MCK-PPARβ/δ mice have superb exercise capacity on a motorized treadmill; interestingly, this phenotype is more apparent with a high-intensity exercise protocol. Muscle glycogen levels are also significantly increased in the MCK-PPARβ/δ mice [19]. The most striking phenotype is that PPARβ/δ drives a complete shift towards a type I oxidative muscle fiber type program. Fiber type staining demonstrated a 100% increase in Type I fibers in MCK-PPARβ/δ soleus muscle [20]. Analysis of transcripts encoding the myosin heavy chain (MHC) isoforms revealed an increased expression of Type I MHC in MCK-PPARβ/δ muscle, whereas the levels of MHC Type IIa, IIx, and IIb mRNAs were markedly suppressed [20]. Moreover, several slow-twitch troponin genes are induced and fast twitch troponin genes suppressed in MCK-PPARβ/δ muscle [20]. In contrast to the muscle-specific PPARβ/δ transgenic mice, skeletal muscle-specific PPARβ/δ-null mice exhibit a shift towards a greater proportion of glycolytic-type muscle fibers. These mice have decreased expression of FAO genes. On a high-fat-diet, the mice gain more weight and are more prone than controls to developing insulin resistance and glucose intolerance [26]. Taken together, these data demonstrate that PPARβ/δ is necessary for both muscle FAO and the Type I fiber program, and that activation of PPARβ/δ in skeletal muscle can coordinately regulate muscle energy metabolism and fiber type switching to improve muscle fitness.

2 The PGC-1 co-activator

The discovery of PPARγ coactivator-1α (PGC-1α) as a coactivator of PPARγ in brown adipose tissue by the laboratory of Bruce Speigelman has provided significant insight into how PPARs and other nuclear receptors are regulated in response to the physiological and nutritional states of skeletal muscle [27–32]. PGC-1α is a member of a family of transcriptional coactivators that includes PGC-1α, PGC-1β, and the PGC-1-related coactivator (PRC) [29–32]. PGC-1β has significant homology with PGC-1α and shares many of its functions in skeletal muscle [33,34]. PGC-1α co-activates a number of transcription factors including the PPAR and estrogen-related receptor (ERR) nuclear receptors, the nuclear respiratory factors 1/2 (NRF1/NRF2) and MEF2 [29–32,35]. PGC-1α directly interacts with its target transcription factors, serving to recruit molecules that mediate chromatin remodeling via histone acetyltransferase (HAT) activity, and by recruiting components of the transcriptional machinery, such as RNA polymerase II to activate gene transcription [29–32]. PGC-1α expression can be induced by a variety of physiological stimuli, such as cold exposure, fasting, and exercise [27–32,36–38]. Thus, PGC-1α can orchestrate the activity of multiple transcription factors in response to a variety of external stimuli to control metabolic and structural adaptations in muscle.

Extensive studies have supported a critical role for PGC-1 coactivators in skeletal muscle [39–48]. The first evidence came with observations in animal models and humans that PGC-1α gene expression is induced in skeletal muscle in physiological conditions that require increased energy expenditure, such as exercise [36,40,41]. Gain-of-function studies performed in cultured myocytes have shown that PGC-1α is capable of activating virtually all aspects of mitochondrial biogenesis in muscle cells, including increased expression of oxidative phosphorylation and FAO genes, increased number of mitochondria, and enhanced mitochondrial respiratory capacity [42]. Transgenic systems and gene knockout (KO) approaches have been used to define the physiological roles of PGC-1 coactivators in skeletal muscle. Two independent studies have shown that over-expression of PGC-1α in skeletal muscle induces an oxidative fiber-type transformation [43,44]. Forced over-expression of PGC-1α under a muscle specific MCK promoter increases muscle oxidative capacity and the proportions of Type I and IIa fibers [43]. A skeletal muscle-specific, tetracycline-regulated PGC-1α over-expression system has also been employed to mimic the induction of PGC-1α known to occur post-exercise. Induced expression of PGC-1α in adult skeletal muscle triggered mitochondrial biogenesis, increased "red" muscle, and expanded muscle glycogen stores, key features of exercise training [44]. Interestingly, a short form of PGC-1α (PGC-1α4) has been recently shown to induce muscle hypertrophy [49], while full length PGC-1α was shown to regulate kynurenine metabolism and to protect mice from stress-induced depression [50]. Taken together, the gain of function study results suggest that induction of PGC-1α, which is known to occur following exercise, drives a trained skeletal muscle phenotype.

Mice with targeted ablation of the genes encoding either PGC-1α or PGC-1β have a minimal phenotype until they are challenged with a physiological stress that increases demand for ATP production [33,34,45–48]. For example, PGC-1α KO mice have reduced exercise capacity on a treadmill compared to wild-type controls [47,48], while combined deficiency of PGC-1α and PGC-1β in skeletal muscle results in a dramatic exercise performance deficit

related to mitochondrial dysfunction [39]. The exercise phenotype of muscle-specific PGC-1α/β double-KO mice is associated with a marked decrease in muscle respiratory capacity and derangement of mitochondrial structure [39]. In addition, the conventional PGC-1α/β double-KO mice die soon after birth with heart failure [51]. Studies of PGC-1α/β double-KO heart sections by electron microscopy revealed that the normal perinatal mitochondrial biogenic response was completely absent [51]. Thus, these results of phenotypic analyses of the PGC-1α- and PGC-1β-deficient mice have shown that both coactivators are necessary for mitochondrial respiratory function in skeletal muscle and heart. However, despite severe mitochondrial abnormalities, the proportion of Type I and IIa fibers was not diminished in PGC-1α/β double-KO muscle [39], suggesting that PGC-1α/β deficiency triggers an adaptive reprogramming in skeletal muscle. These results support a surprising conclusion that the PGC-1 coactivators are dispensable for fundamental fiber type determination. Given that PGC-1α is induced by exercise training, it's still very likely that the induction of PGC-1α could have a booster function on muscle fiber type switching via its coactivating effects on downstream effecters such as PPARβ/δ.

3 Role of miRNAs in skeletal muscle fiber type determination

The recent breakthrough discovery of miRNAs as key players of gene regulation has unveiled a new dimension in the control of gene expression [52–57]. miRNAs are a family of small RNA molecules, usually 22 nucleotides long, that were first identified in *C. elegans* in the early 1990s [58,59]. Since then, miRNAs have been discovered in virtually all plants and mammals [60–62]. Many miRNAs are encoded in the introns of host genes, indicating that they are co-regulated with their host genes. miRNAs are transcribed by RNA polymerase II and after processing by the RNase III enzyme, Drosha, pre-miRNAs are exported to the cytoplasm to be further processed by Dicer [52,55]. Mature miRNAs are incorporated into the RNA-induced silencing complex (RISC), where they bind to their mRNA targets [52,55,63,64]. miRNAs can regulate gene expression either through translational repression or direct gene silencing [52,55,64]. Interestingly, miRNAs usually modulate a class of mRNA targets that share the same function, thereby controlling complex biological processes.

Evidence is emerging that gene regulatory programs involved in skeletal muscle biology involve miRNAs [53,54,56,65–67]. Several miRNAs, named "MyomiRs", have been shown by Eric Olson's laboratory, to be involved in muscle fiber type programs [65]. Specifically, miR-208b, which is encoded within a type I MHC gene (Myh7), and miR-499, which is encoded within another type I MHC gene (Myh7b), have been shown to drive a slow-twitch fiber program by down-regulating transcriptional repressors that suppress slow-twitch contractile protein gene expression [65,68]. The 3′-UTRs of Sox6 and PurB contain conserved target sites for both miR-499 and miR-208b, each of which has been reported to repress Type I marker expression [65,68,69]. In addition, gain-of-function and loss-of-function studies in mice have identified an important role for Sox6 in muscle development, fiber type specification, and muscle performance [70–72].

Interestingly, muscle-specific miR-499 transgenic mice run over 50% longer than wild-type littermates when subjected to a regimen of forced treadmill running [65], indicating an endurance exercise training phenotype in these mice. Moreover, the miR-499 transgenic background completely reverses the exercise performance deficit of MCK-PPARα mice (the "couch potato" mouse) [20], further supporting a dominant role of miR-499 in muscle physiology. Importantly, miR-499 is relevant in humans. The expression levels of miR-499 are significantly increased in human muscle biopsies from trained athletes compared to sedentary controls [20]. In addition, there is a significant positive correlation between the expression of miR-499 and Type I fiber %, ATPmax, and VO_2max in humans [20]. Taken together, these results demonstrate that miR-499 is linked to both the Type I muscle program and to metabolic capacity in humans, providing an important translational relevance.

4 Cross talk between nuclear receptor signaling and miRNA networks

Accumulating evidence suggests that there is a cross talk between nuclear receptor signaling and miRNA networks. An interesting connection between PPARs and miRNAs has been discovered in a miRNA expression profiling study with RNA isolated from MCK-PPARs mice [20]. Consistent with the strikingly different phenotypes between the MCK-PPARα and MCK-PPARβ mice, the miRNA expression patterns were remarkably different among the MCK-PPARs lines and a subset of miRNAs exhibited reciprocal regulation [20]. For example, levels of miR-208b and miR-499 are significantly increased in MCK-PPARβ/δ muscle but dramatically reduced in MCK-PPARα muscle [20]. Gain- and loss-of-function studies in primary skeletal muscle cells have demonstrated that PPARα and PPARβ/δ have opposing roles in the regulation of the Type I fiber program through this miR-499/208b circuit [20]. These results support a model in which PPAR signaling is upstream of miR-208b/miR-499 and that PPARβ/δ activates, while PPARα suppresses, the miRNA circuit in muscle.

Interestingly, no consensus PPAR binding sites have been identified in the miR-499/208b promoters. However,

highly conserved sites for another nuclear receptor, ERR, were identified in both miRNA promoters [20]. ERRγ has been shown to promote the Type I fiber program in skeletal muscle [73]. ERRγ is suggested to bridge the gap between PPARβ/δ and the increase in miR-499/208b expression given that levels of ERRγ protein were increased in MCK-PPARβ/δ muscle [20]. Moreover, chromatin immunoprecipitation (ChIP) of the miR-499/208b promoters demonstrated that ERRγ occupies the region containing the proposed ERR response elements [20]. As expected, levels of miR-499 and miR-208b are dramatically reduced in the ERRβ/γ double KO muscle [20]. Therefore, ERRγ serves a critical role in the Type I fiber program by directly activating transcription of miRNA genes via a highly conserved ERR response element. It is very likely that PGC-1 could have a booster function on this mechanism via its coactivating effects on ERRγ and PPARβ/δ.

In contrast to PPARβ/δ and ERRγ, PPARα exerts a repression effect on miR-499 and miR-208b [20]. Interestingly, this effect seams independent of exogenously added PPARα ligand and promoter mapping indicates that PPARα suppresses miR-208b promoter activity through the proximal promoter region [20].

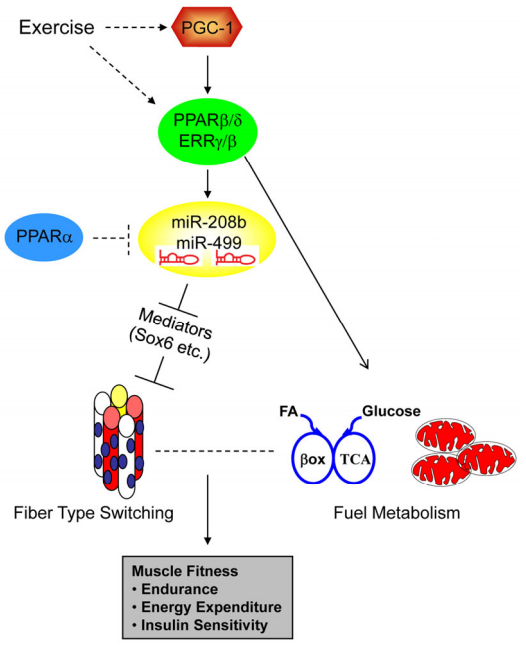

Figure 2 (color online) Model of nuclear receptor signaling and a miRNA network in the control of muscle fiber type switching. The schematic depicts a proposed model for a nuclear receptor-miRNA circuit that controls the muscle Type I fiber program. PPARβ/δ, ERRγ and probably ERRβ activate miR-208b and miR-499 triggering a Type I fiber program while PPARα suppresses the program. The effect of exercise could stimulate this program via the transcriptional coactivator PGC-1α.

5 Conclusion and perspectives

It is clear that a sedentary life style is a major risk factor for many chronic diseases and that chronic diseases, such as obesity, diabetes, and muscular diseases, are associated with decreased muscle fitness [2,3,5]. Exercise is well known to enhance muscle fitness. Genetically modified mouse models have demonstrated that there is a nuclear receptor-miRNA circuit that orchestrates programs controlling muscle energy metabolism and fiber type (Figure 2): (i) PGC-1α/PPARβ/δ/ERRγ signaling can drive a trained muscle fiber program; (ii) surprisingly, the closely-related nuclear receptors, PPARβ/δ and PPARα exert opposing actions upon fiber type programs through a muscle miRNA network; (iii) PPARβ/δ activates ERRγ which drives the miR-208b/499 circuit and, thus, the Type I fiber program; (iv) studies of human muscle confirmed that this circuit links control of muscle fiber type with metabolic energy capacity. This new information has provided important insight into the role of nuclear receptor signaling and miRNAs networks in muscle fiber type switching.

Given the recent exciting evidence that the expression of ERRγ and miR-499 are linked to human fitness [20], the nuclear receptor/miRNA regulatory circuit shows promise as a therapeutic target aimed at enhancing muscle fitness in a variety of chronic disease states that are associated with loss of muscle fitness, including but not limited to, obesity, diabetes, muscular disease, and aging [74]. It will be important to fully define the downstream targets of the nuclear receptor-miRNA networks involved in the coordinated regulation of metabolic and structural programs in muscle. Future studies aimed at targeting the PPARβ/δ, ERRγ and miR-499 components of the circuit will be of interest. Of note, nuclear receptors form the second largest class of drug targets, with an estimated share of 10%–15% of the global pharmaceutical market. In addition, direct manipulation of miR-499 levels in muscle through biological approaches is also warranted. We are optimistic that one day a trained effect can be attained without exercise training by taking an "exercise pill".

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