Muscle Dysfunction in COPD

Epigenetic regulation of muscle phenotype and adaptation: a potential role in COPD muscle dysfunction

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CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD) is projected to be the third leading cause of death worldwide in 2020. In COPD patients, skeletal muscle dysfunction is a common systemic manifestation that affects both respiratory and limb muscles and has a significant impact on their exercise tolerance and quality of life (38). Quadriceps muscle dysfunction, mainly characterized by reduced muscle force, appears in one-third of the patients, even at very early stages of the disease when severe airway obstruction has not yet developed (83). Importantly, quadriceps weakness and reduced muscle mass, as measured by midhigh cross-sectional area, were also shown to be reliable predictors of COPD mortality (60, 87).

Several pathophysiological mechanisms have been shown to participate in the multifactorial etiology of COPD muscle dysfunction. Deconditioning, muscle mass loss, systemic inflammation, oxidative stress, and structural abnormalities are among the most relevant factors and focus of research in the last decade (11–16, 18, 35, 58, 59, 68). Epigenetic control, defined as the process whereby gene expression is regulated by heritable mechanisms that do not affect DNA sequence, may also affect the susceptibility of COPD patients to develop skeletal muscle dysfunction (56). The precise contribution of most of these mechanisms to COPD muscle dysfunction has been extensively reviewed in other minireviews in this Highlighted Topic and will not be reconsidered here. In the present review, we discuss the epigenetic contribution to muscle dysfunction in COPD patients as it has been suggested to contribute to disease susceptibility, pathogenesis, and progression (56). Epigenetic mechanisms, defined as the process whereby gene expression is regulated by heritable mechanisms that do not affect DNA sequence, could be involved in the susceptibility to muscle dysfunction, pathogenesis, and progression. Herein, we review the role of epigenetic mechanisms in muscle development and adaptation to environmental factors such as immobilization and exercise, and their implications in the pathophysiology and susceptibility to muscle dysfunction in COPD. The epigenetic modifications identified so far include DNA methylation, histone acetylation and methylation, and non-coding RNAs such as microRNAs (miRNAs). In the present review, we describe the specific contribution of epigenetic mechanisms to the regulation of embryonic myogenesis, muscle structure and metabolism, immobilization, and exercise, and in muscles of COPD patients. Events related to muscle development and regeneration and the response to exercise and immobilization are tightly regulated by epigenetic mechanisms. These environmental factors play a key role in the outcome of muscle mass and function as well as in the susceptibility to muscle dysfunction in COPD. Future research remains to be done to shed light on the specific target pathways of miRNA function and other epigenetic mechanisms in the susceptibility, pathogenesis, and progression of COPD muscle dysfunction.

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initiation of muscle dysfunction in COPD. Conversely, endurance exercise has been shown to counterbalance the deleterious effects of disuse in muscles of COPD patients (Fig. 1). Specifically, the present minireview article focuses on the implications of epigenetic mechanisms in muscle development, structure and adaptation to environmental factors, as well as in the pathophysiology and susceptibility to muscle dysfunction in COPD patients (Fig. 1).

EPIGENETIC MECHANISMS IN CELLS

In eukaryote cells, genomic DNA is condensed in the form of chromatin within the nucleus. Chromatin is composed of nucleosomes and a core of histone proteins (H2A, H2B, H3, and H4) (Fig. 2). Chromatin packing, although absolutely required, can inhibit transcription as it reduces accessibility to DNA. As such, gene transcription may only occur when DNA-histone interaction is weaker, thus enabling access of transcription factors to DNA. Methylation of DNA and histone methylation and acetylation modulate the stability of the nucleosomes as well as protein-protein interactions that modify the transcriptional activity of the DNA (8). The epigenetic DNA modifications include DNA methylation, covalent histone modifications, noncovalent mechanisms such as incorporation of histone variants and nucleosome remodeling, and noncoding RNAs including microRNAs (miRNAs) (84). These different molecular mechanisms are briefly described below.

**DNA methylation.** DNA methylation is a biochemical process that involves the addition of a methyl group (CH3) to the 5 position of the cytosine that stands directly before a guanine molecule in the same chain (Fig. 3). It usually occurs in the CpG islands, a CG-rich region where C and G are connected by a phosphodiester bond. This reaction is mediated by methyltransferases and can be inherited through cell division. DNA methylation is the most stable modification of the chromatin structure and may vary at different time points in life such as during development and aging. DNA methylation at the 5 position of cytosine specifically reduces gene expression. Importantly, the pattern of DNA methylation, which leads to changes in gene expression, may also vary in response to environmental factors (54, 84).

**Histone acetylation.** Acetylation is a transient, enzymatically controlled biochemical process, and the commonest posttranslational modification of histones. The acetyl group from acetyl-CoA is transferred to a lysine residue, thus converting its basic side chain into a neutral residue. This modification results in a structural change of the histone tail, which in turn alters the interaction between histones and DNA together with the associations with the nucleosomes. These alterations result in a rather open chromatin (euchromatin) structure that is transcriptionally active (Fig. 3). Deacetylation reverses this process, leading to a closed chromatin structure (heterochromatin) that is transcriptionally blocked (Fig. 3). Acetylation also regulates protein-protein interactions. Hence, acetyl-lysine residues may recruit proteins to specific regions of the chromatin to further activate transcription.

Histone acetyltransferases (HTAs) and 18 known human histone deacetylases (HDACs), grouped into four classes (I, IIA, IIB, III or Sirtuins in mammals, and IV) modulate lysine acetylation in a dynamic fashion. Sirtuins serve as class III HDACs by removing acetylases coupled with nicotinamide adenine dinucleotide hydrolysis at histone tails and also play a relevant role in aging, stress, and apoptosis (54). HTAs are activated upon phosphorylation that leads to a shift in the balance from HDACs to HTAs within the nucleus, to the acetylation of specific histones, and to an increase in gene transcription. On this basis, acetylation is now being regarded as a biological process that is beyond chromatin remodeling and participates in metabolic control and the pathophysiology of chronic conditions and aging (1). Interestingly, HDACs, which do not directly bind DNA, also interact with chromatin through association with other histone-modifying proteins and transcription factors.
Histone methylation. Methylation of histones may take place at both lysine and arginine residues, which accept three and two methyl groups, respectively. Distinct from acetylation, methylation does not modify the charge of arginine and lysine and thus is unlikely to alter chromatin folding (Fig. 3). Methyllysine or methylarginine may activate or repress gene transcription depending upon the proteins that they recruit to the chromatin. For instance, methyllysine binds proteins that contain chromodomains (48) or plant homeodomains (PHD) (89), whereas both methyllysine and methylarginine are recognized by proteins containing Tudor domains, which are conserved protein structural motifs (26, 40).

miRNAs. miRNAs, encoded by eukaryotic nuclear DNA, are noncoding single-stranded RNA molecules (18–24 nucleotides) that function in the posttranscriptional regulation of gene expression. They exert their action via base-pairing with complementary sequences in mRNA molecules that results in gene silencing via translational repression or target degradation (Fig. 4). miRNAs may have different mRNA targets, and a given mRNA may also be targeted by multiple miRNAs in a similar fashion. miRNAs regulate many cellular processes and appear to have a role in the pathogenesis of lung diseases such as lung cancer, pulmonary fibrosis, asthma, and COPD (6). RNA polymerase II transcribes long transcripts known as primary miRNAs (pri-miRNAs) (Fig. 4). They are then processed by a nuclear multiprotein complex that contains Drosha and DGR8/Pasha, leading to the formation of precursor miRNAs (pre-miRNAs) with stem-loop structures (55, 77). These pre-miRNAs are then transported into the cytoplasm by the nucleocytoplasmic shuttle exporting-5, where they become substrates of RNase III enzyme Dicer to generate 22-nucleotide miRNA duplexes (53) (Fig. 4). Subsequently, miRNA assemble together with Argonaute proteins to form the RNA-induced silencing complex (RISC or miRISC), which contains Dicer and many associated proteins (43, 74) (Fig. 4). The mature miRNA is part of an active RNA-induced silencing complex (RISC) containing Dicer and many associated proteins, which with incorporated miRNA is known as miRISC. This complex silences specific target genes by base pairing with the 3′-untranslated region of their target mRNAs, which may result in mRNA degradation if the pairing is perfect or in inhibition of translation when the pairing is not complete (43, 74) (Fig. 4).

Despite the fact that most of the miRNAs identified so far regulate different processes in different tissues, some miRNAs are tissue specific. For example, miR-1, miR-133, and miR-206 are abundantly expressed within skeletal muscles and are identified as muscle-specific miRNAs (myomiR, Table 1).

**EPIGENETIC REGULATION OF MUSCLE DEVELOPMENT**

Embryonic myogenesis. Myogenesis is the process of generating muscle, particularly during embryonic development.
Mesoderm-derived structures generate the first muscle fibers of the body that act as the template fibers into which additional fibers will be incorporated in subsequent waves during embryonic myogenesis (19). In the perinatal phase, muscle resident myogenic progenitors proliferate considerably to decrease as soon as both the number of myonuclei and myofibrillar protein synthesis reach a steady state. In mature muscles, myogenic progenitors will enter quiescence and will reside within the muscle fibers as satellite cells. These cells have the potential to mitotically proliferate and differentiate into new fibers, a process required to reestablish homeostasis in injured adult muscles. Common molecular mechanisms have been reported be-

Table 1. Regulation of muscle development by miRNAs: function and target molecules

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<tr>
<th>miRNAs</th>
<th>Action</th>
<th>Target Pathways</th>
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<tr>
<td>MyomiRs</td>
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<tr>
<td>miR-1</td>
<td>Promotes myotube formation</td>
<td>HDAC4 (23, 56)</td>
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<td>Innervation process</td>
<td>Connexin 43-dependent gap junctional communication (5)</td>
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<tr>
<td>miR-206</td>
<td>Promotes myotube formation</td>
<td>IGF-1 signal transduction cascade (33, 56)</td>
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<td>Commitment to terminal cell differentiation</td>
<td>Subunit p180 of DNA polymerase alpha (30, 71)</td>
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<td>miR-133</td>
<td>Induces myoblast proliferation</td>
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<td>Inhibition of myotube formation (23)</td>
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<td>Repression of SFR (23)</td>
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<td>Other miRNAs</td>
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<td>miR-27</td>
<td>Entry of satellite cells into myogenic differentiation program</td>
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<td>miR-181</td>
<td>MyoD induction</td>
<td>Repressor of myoblast terminal differentiation Hox-A11 (70)</td>
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<td>miR-29</td>
<td>Promotes myogenesis</td>
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MyomiRs, muscle-specific miRNAs; HDAC4, histone deacetylase 4; IGF-1, insulin-like growth factor; SFR, serum response factor.
between embryonic myogenesis and regeneration in the mature skeletal muscles (88).

The basic helix-loop-helix factor MyoD has the ability to transform several cell types including fibroblasts into myotubes (29). Furthermore, MyoD was also shown to activate muscle genes in a variety of already differentiated cell lines from several species (chicken, human, and rat), suggesting that MyoD is the only transcription factor required for terminal muscle differentiation (95). Other myogenic basic helix-loop-helix factors such as Myf5, myogenin, and Myf6, which are jointly expressed, also induce myoblast characteristics in non-muscle cell lines (20, 32). The basic domain of the myogenic regulatory factors (MRFs) mediates DNA binding, while heterodimerization with E proteins that mediate recognition of genomic E boxes, a motif found in the promoters of muscle-specific genes, requires the helix-loop-helix motifs.

The paired-homeobox transcription factors Pax3 and Pax7 genes contribute to early striated muscle development. Interestingly, it was demonstrated (42, 82) that ablation of Pax7-expressing cells only caused smaller muscles with fewer myofibers in the limbs at birth, while loss of the Pax3 lineage was embryonically lethal and prevented the emergence of Pax7-positive cells. These findings suggest that Pax3 is required for the formation of a template of initial fibers to which Pax7-positive fibers would be incorporated, thus establishing a satellite cell pool.

Epigenetic regulation of myogenesis. Mechanisms other than the classic transcription factors that control the myogenesis gene expression program are also involved in the regulation of such a complex process. In this regard, epigenetic regulation plays a crucial role in the maintenance of quiescence and proliferation states in muscle satellite cells, preventing their differentiation. DNA methylation appears to be a major repressive mechanism of muscle satellite cell differentiation (30, 71). Conversely, demethylation together with MyoD and myogenin are required for the initiation of the differentiation program in satellite cells (57, 75). Other repressive mechanisms acting on chromatin-associated histones have also been shown to regulate muscle satellite quiescence and proliferation. For instance, the histones of muscle differentiation-specific genes are hypoacetylated through the action of HDACs and histone lysine methyltransferases in quiescent and proliferating satellite cells (66, 75). It remains to be determined whether repressive epigenetic mechanisms affect all muscle-specific genes or have more specific/targeted effects on different sets of genes. In proliferating myoblasts, HDAC1, HDAC2, HDAC3, HDAC4, and HDAC5 and Sirtuins maintain transcription factors in a deacetylated state, especially in the absence of differentiation-promoting signals. Other epigenetic mechanisms of gene transcription regulation such as the replacement of canonical histones with histone variants or the expression of specific histone isoforms in a cell-state or cell-type manner may also regulate muscle satellite quiescence and proliferation (77).

The irreversible cell cycle withdrawal is required for the activation of the muscle differentiation program in satellite cells. Although still unclear, epigenetic mechanisms also seem to regulate the muscle differentiation gene program. The repressive scenario is quickly modified by the differentiation-promoting signals. In this regard, it has been shown that genes actively transcribing are marked by H3K4me3, whereas those ready to start transcription are tagged by H3K4me2 (39). For instance, Pax7 binds to H3K4me2 regulatory elements in target genes such as Myf5 in satellite cells (52, 66). This binding leads to the recruitment of TRXG histone methyltransferase complex, which in turn will induce strong H3K4 trimethylation on the transcription start site, thus establishing a transcriptionally active domain (52, 66). Eventually, the activation of transcription factors and nucleosomes via inactivation of HDACs and Sirtuins together with the concomitant activation of HTAs enables the muscle differentiation program to proceed.

Importantly, miRNAs also play a paramount role in myogenesis, especially during muscle development. For instance, inactivation of Dicer, which leads to the accumulation of unprocessed pre-miRNAs, results in perinatal lethality, reduced muscle mass, and abnormal myofiber structure (74). Importantly, miR-1 and miR-133, which are localized within the same chromosomal loci and transcribed together, become two independent mature miRNAs with completely different roles in the regulation of skeletal muscle proliferation and differentiation. While miR-1 promotes muscle cell differentiation by targeting HDAC4, miR-133 induces myoblast proliferation by repressing the serum response factor (SRF) and inhibiting myotube formation (23) (Table 1). In a similar fashion to miR-1, miR-206 promote myotube formation by targeting the p180 subunit of DNA polymerase alpha, which in turn leads to DNA synthesis inhibition and cell cycle withdrawal, and commitment to terminal cell differentiation (30, 71). Interestingly, miR-1 appears to target the insulin-like growth factor-1 (IGF-1) pathway and to exert a feedback loop between miR-1 expression and the IGF-1 signal transduction cascade (33) (Table 1). miR-1 and miR-206 also seem to participate in the innervation process of the muscle fibers through the downregulation of connexin 43-dependent gap junctional communication (5) (Table 1).

Other miRNAs ubiquitously expressed in tissues may also participate in the regulation of skeletal muscle development and phenotype (Table 1). For instance, miR-27, which targets Pax3 mRNA, is expressed in satellite cells of the adult muscle and in embryonic myotomes (27). This association between miR-27 and Pax3 results in the entry of the satellite cells into the myogenic differentiation program (27) (Table 1). Interestingly, miR-181 targets the repressor of myoblast terminal differentiation Hox-A11, resulting in MyoD induction (70) (Table 1). miR-29 is another miRNA that promotes myogenesis through the feedback inhibition of the transcriptional regulator Ying Yang 1, which in conjunction with Ezh2 acts as a repressor of muscle-specific gene expression (92) (Table 1). Eventually, myomiR genes may also be regulated by muscle-specific transcription factors such as MyoD, myogenin, myocyte-enhancing factor (MEF)2, and the SRF (101), as were shown to activate the expression of miR-1, miR-133, and miR-206 (79).

Epigenetic regulation of muscle structure. The different types of skeletal muscle fibers are established during development independently of neural control. However, postnatal innervation is a major regulator of the fiber-type characteristics in adult muscles (8, 77). Muscle fiber contraction velocity relies upon the expression of different myosin heavy chain (MyHC) isoforms: type I slow-twitch fibers and type IIa, IIx, and IIb fast-twitch fibers. Evoepigenetics also seem to regulate muscle fiber structure and function, and adaptation to different environmental conditions in adult muscles. For instance, Pandorff et al. (76) showed that the pattern of acetylation and methylation sites of histone H3 correlated with the expression of MyHC, I,
The metabolic profile of the different muscle fiber types has mostly either oxidative or glycolytic properties. Differentiated myofibers are likely to consume greater amounts of ATP, thus requiring the biogenesis of more mitochondria. Nevertheless, while overload can lead to more slow myofibers, mitochondrial enzyme activity levels apparently did not seem to change (9, 44, 45). Other reports (91, 96) have suggested a differential control of mitochondrial activity to modulate slow myofiber formation. In those studies (91, 96), high-capacity runner rats indeed exhibited greater mitochondrial enzyme activity than the low-capacity runners, while showing no differences in muscle fiber types between the groups. In this regard, the duration of the endurance exercise could be proposed as a key factor accounting for the uncoupled epigenetic modulation of mitochondrial biogenesis and muscle fiber types (50). As such, prolonged activity appears to be key to overcome the epigenetic regulation of fiber type shifting.

**EPIGENETIC REGULATION OF MUSCLE ADAPTATION TO ENVIRONMENTAL FACTORS: IMMOBILIZATION, EXERCISE, AND MUSCLE MASS**

**Acetylation/deacetylation balance.** In skeletal muscle adaptation to environmental factors, the balance between acetylation/deacetylation seems to be relevant. Myocyte enhancer factor (MEF)-2, whose transcriptional activity is repressed by class II HDACs, plays a significant role in muscle differentiation, development, and in the determination of muscle fiber type (67). MEF2 is selectively activated in slow-twitch myofibers and responds to calcium-dependent signaling pathways that are involved in the fiber type shift toward a more fatigue-resistant phenotype. Potthoff et al. (78) suggested that in order for MEF2 to activate the slow-twitch fiber gene program, class II HDACs were degraded by the proteasome in oxidative fibers. Interestingly, proteasome inhibitors blocked fast-toslow myofiber transformation in the same study (78). Additionally, expression of a hyperactive form of MEF2 protein in HDACs conditional knockout mice induced a more fatigue-resistant myofiber phenotype, which in turn enabled the animals to enhance their exercise capacity (78).

MEF2 and its regulation by class II HDACs also have a role in the metabolic adaptation to endurance exercise in muscles. For example, class II HDACs are removed from the nucleus upon phosphorylation, and become activated in response to endurance exercise (8). Furthermore, in order for peroxisome proliferator-activated receptor gamma coactivator (PGC)-1α, a regulator of mitochondrial biogenesis, and the GLUT4 glucose transporter to be transcribed by MEF2, HDACs must also be removed (63, 65). Collectively, these data point toward a prominent role of MEF2 and its HDACs regulators in muscle adaptation to endurance exercise both from structural and metabolic standpoints. In this regard, it was reported that the regulation of muscle differentiation and that of mitochondrial biogenesis share common mechanisms (51). Whether genes regulating mitochondrial biogenesis (PGC-1α) and fiber type shift toward a more fatigue-resistant phenotype are commonly regulated by epigenetic mechanisms remains to be elucidated.

Importantly, the balance between acetylation and deacetylation also plays a major role in the regulation of muscle mass (1, 2, 98, 99). The nuclear cofactor p300 has HTA activity and interacts with different transcription factors and regulatory proteins in tissues (94, 100). Besides, p300 has also been shown to regulate muscle differentiation and mass maintenance through the acetylation of histones and nonhistone proteins such as transcription factors specifically involved in those biological processes (1).

Remarkably, hyperacetylation may also lead to enhanced protein breakdown as a result of the following mechanisms: 1) activation of transcription factors and regulatory proteins involved in muscle wasting (24), 2) ubiquitin ligase and polyubiquitination activities that several HTAs may have in addition to their acetyltransferase activity or through the formation of complexes with enzymes regulating protein ubiquitination (22, 81), and 3) acetylation of the chaperone protein HSP90, which reduces its protective effect, thus leading to enhanced degradation of the HSP90-interacting proteins (10). In line with this, a few investigations have clearly demonstrated that p300 and HTA activity were increased in muscles of septic rats (2) and in dexamethasone-treated myotubes (98, 99), while expression levels of HDAC3, HDAC6, and SIRT1 were reduced in the septic rat muscles (2).

Nonetheless, other studies have shown contradictory results regarding the effects of hyperacetylation on muscle mass. For instance, the transcription factor Smad7 was ubiquitinated and degraded through specific mechanisms involving deacetylation by HDAC1 and SIRT1 (85). Moreover, other proteins such as p53 (47) and Runx3 (49) were also shown to be stabilized by lysine acetylation. Interestingly, in models of denervation-induced muscle atrophy, the expression and activity of HDAC4 and HDAC5 were shown to be increased, rather than decreased (25, 69), whereas exercise-induced muscle hypertrophy was associated with reduced activity of both HDAC4 and HDAC5 (64). Taken together, it is reasonable to propose that in the regulation of protein stability and muscle mass, acetylation and deacetylation balance may be protein- and condition-specific (1). Future research remains to help us elucidate to what extent protein acetylation contributes to muscle mass loss and atrophy in models of disuse and denervation and in those characterized by a rather catabolic state such as sepsis, cancer, and other chronic conditions including COPD.

**DNA methylation.** As abovementioned, epigenetic modulation through DNA methylation is involved in the determination of cell fate during development. In line with this, in a seminal study (90), it was shown that demethylation of DNA, using 5-azacytidine, led to the development of myocytes, adipocytes, or chondrocyte lineages from mesenchymal cells. Recently, it has also been reported (41) that DNA demethylation using 5-azacytidine favored myogenesis of C2C12 myotubes, which exhibited a better defined sarcomere organization than the untreated myotubes. However, further work is still needed to determine whether the balance between DNA methylation and demethylation plays a significant role in skeletal muscle adaptation to environmental factors in adult skeletal muscles.

**miRNAs.** An epigenetic control through the action of miRNAs also seems to regulate muscle adaptation to environmental factors such as disuse and exercise. For instance, prolonged immobilization was shown to induce changes in the expression of genes involved in muscle growth and fiber type in mice (3).
Interestingly, spaceflight and hindlimb suspension resulted in decreased miR-206 levels in the limb muscles of mice (3). In another study, PGC-1α and miR-696 were downregulated in limb muscles of mice during prolonged immobilization (7). A variety of myomiRs were also shown to be downregulated in slow- and fast-twitch muscles during hindlimb suspension in another investigation (62). The same authors demonstrated that in response to muscle overload, the upregulation of miR-206 was associated with slow fiber type formation, while leading to a downregulation of miR-1 and miR-133 (61). It would be possible to conclude from these findings that a network of myomiR seems to modulate the expression of MyHC during muscle atrophy and immobilization. Their specific target pathways in muscles remain to be fully elucidated.

Research is warranted to shed light on the specific contribution of the modifications in the expression of the different myomiR to immobilization/disuse muscle atrophy in humans with highly prevalent chronic conditions such as prolonged immobilization of any cause, critically ill patients, COPD, and aging. Nevertheless, recent reports have partially addressed such a question. For instance, upregulation of pri-mRNAs was detected in the vastus lateralis of elderly men compared with young controls at rest (31). In the same study, levels of miR-1 were decreased after an anabolic stimulus only in young subjects (31). Also, insulin resistance in human muscles was associated with changes in miRNAs that target important cellular signaling pathways involved in muscle fiber metabolism (37). In critically ill patients with severe sepsis, mitochondrial loss and dysfunction together with alterations in miRNA processing were also demonstrated in muscles of the lower limbs (36).

Whether the expression of miRNAs may also be modulated in response to exercise in humans has recently been investigated. For instance, the vastus lateralis muscle of healthy men exhibited a differential expression profile in miR-378, miR-29a, miR-26a, and miR-451 between low and high responders to resistance exercise training (28). In that study, gene ontology analysis of the differentially regulated miRNA genes indicated that miRNA changes in the low responders could reflect a failure of the individuals to activate muscle growth and remodeling genes (28). In another report, muscle (vastus lateralis) miR-1 and miR-133a expression were upregulated in response to an acute bout of exercise in healthy young males (73). Importantly, in the same study, it was also demonstrated that endurance training for 12 wk resulted in a decrease in miR-1, miR-133a, miR-133b, and miR-206 in the peripheral muscles of the trained individuals (73). The specific targets of these miRNAs in adult muscles remain to be explored. These results have potential clinical implications in the management of patients with chronic lung diseases and muscle dysfunction including COPD, especially in the design of specific exercise training programs. Indeed, it has been clearly demonstrated that both the intensity and length of the training programs are of paramount importance to improve exercise capacity, muscle function, biology, and structure in those patients (17, 21, 58, 68, 80).

**EPIGENETIC REGULATION OF MUSCLES IN COPD**

Recently, a potential role of miRNAs in the pathophysiology of muscle dysfunction has also been suggested in patients with COPD (56). Deconditioning is a major contributor to the loss of muscle function and mass in COPD. As described earlier, several epigenetic mechanisms are definitely involved in the disuse muscle atrophy observed in several models. It remains to be elucidated, however, which are the specific epigenetic alterations regulating the biochemical and structural alterations that lead to functional modifications of the muscle fiber properties in patients with COPD. Future research is clearly needed to answer such a relevant question.

A pioneering study in this field has recently reported that the vastus lateralis of severe COPD patients exhibited a significant decrease in the expression of miR-1 together with an increase in HDAC4 protein levels (56). Expression of the myocardin-related transcription factors (MRTF) A and B was also reduced in limb muscles of COPD patients compared with control subjects (56). As MRTF/SRF activity is relevant in the regulation of MyHC-I expression (4), the reduction in both MRTF/SRF and miR-1 expression could explain the significant decrease in type I fibers observed in the severe COPD patients (56).

An attempt to account for the findings in this study (56) is that HDAC4 inhibits MEF2 and SRF activities, which are important regulators of MyHC-I expression probably via the expression of miR-1. As earlier mentioned, miR-1 also targets IGFI-expression, which was significantly increased together with protein levels of HDAC4 in the limb muscles of the COPD patients (56). The fact that miR-1 was also shown to inhibit calcineurin pathway (46) and that this pathway mediates MEF2 activation (97) could be another contributing mechanism to the characteristic fiber type shift toward a less fatigue-resistant phenotype and muscle wasting observed in patients with advanced COPD (56). Eventually, another likely explanation is that HDAC4 inhibits the expression of follistatin, which antagonizes the action of myostatin, thereby inducing muscle mass loss (86). Taken together, these biological mechanisms could, in part, account for the lesser fatigue-resistant phenotype encountered in the peripheral muscles of patients with severe COPD and muscle atrophy (13, 16, 18, 35, 56). In keeping with, Lewis et al. (56) also reported that muscle miR-1 expression correlated with the amount of type I fibers as well as with several clinical variables such as smoking history, lung function, body composition, and exercise capacity. In the same muscles, the expression of miR-133 and miR-206, whose levels did not differ between patients and healthy controls, inversely correlated with daily physical activity among the severe patients (56).

Ying Yang 1 (YY1) is a transcription factor that has been implicated in histone modification, since it may direct histone deacetylases and histone acetyltransferases to a promoter to activate or repress its function, and it may also inhibit the binding of the transcription activator SRF (34). YY1 expression has also been shown to inhibit muscle regeneration through transcriptional silencing of myofibrillar genes (93). Importantly, the localization of YY1 also regulates its activity and expression: it remains inactive in the cytoplasm, whereas it becomes active upon translocation to the nucleus in response to several stimuli such as depolymerized actin (34). Interestingly, in a previous investigation (72), protein levels of YY1 were assessed in the peripheral muscles of both severe COPD patients and healthy controls. Although muscle protein levels did not differ between patients and control subjects, inverse correlations were found between YY1 levels and the size of type I and type IIx fibers, the latter being significantly smaller in the patients (72). In the same study (72),

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localization of YY1 within the myofibers differed between patients and healthy controls. It could be suggested that this transcription factor may participate in the pathophysiology of the fiber type shift and muscle atrophy events observed in peripheral muscles of patients with advanced COPD. Future research is clearly warranted to further elucidate the precise mechanisms whereby epigenetic mechanisms and their target molecules may regulate muscle mass and function in patients with chronic conditions including COPD.

CONCLUSIONS

Collectively, the different epigenetic mechanisms and miRNAs are likely to play a role in the pathophysiology of muscle dysfunction in chronic lung diseases such as COPD. Molecular and cellular events related to muscle development and repair, and muscle structure and metabolism, which in turn, are key determinants of muscle mass and function, are tightly regulated by epigenetic mechanisms. Environmental factors such as exercise and immobilization modify the epigenetic regulation in skeletal muscles, thereby inducing further modifications in their biology and structure. In stable COPD patients, the resulting muscle phenotype and performance is the consequence of the continuous interaction between intrinsic elements, including epigenetic regulation, and environmental factors. Epigenetic mechanisms are likely to play a significant role in the orchestration of the relationships between these two types of factors in COPD muscle dysfunction (Fig. 1). Additionally, muscle metabolic profile and fiber type outcome may also share common epigenetic mechanisms depending upon the intensity and duration of the exercise stimulus.

More research is warranted to shed light on the signaling pathways and molecular targets of miRNA function and other epigenetic mechanisms in the muscle dysfunction of patients with COPD and other lung diseases. Another relevant question will be to establish whether epigenetics may explain the differential susceptibility observed in patients with COPD to systemic manifestations of the disease such as muscle mass loss and dysfunction. Finally, it will also be of interest to assess the potential differential response profile to exercise training in patients with different stages of disease severity and body composition.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

Author contributions: E.B. prepared figures; E.B. and J.I.S. drafted manuscript; E.B. and J.I.S. edited and revised manuscript; E.B. and J.I.S. approved final version of manuscript.

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