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Emerging role of extracellular vesicles in musculoskeletal diseases

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ABSTRACT

Research into the biology of extracellular vesicles (EVs), including exosomes and microvesicles, has expanded significantly with advances in EV isolation techniques, a better understanding of the surface markers that characterize exosomes and microvesicles, and greater information derived from −omics approaches on the proteins, lipids, mRNAs, and microRNAs (miRNAs) transported by EVs. We have recently discovered a role for exosome-derived miRNAs in age-related bone loss and osteoarthritis, two conditions that impose a significant public health burden on the aging global population. Previous work has also revealed multiple roles for EVs and their miRNAs in muscle regeneration and congenital myopathies. Thus, EVs appear to be involved in a number of degenerative conditions that impact the musculoskeletal system, indicating that the musculoskeletal system is an excellent model for investigating the role of EVs in tissue maintenance and repair. This review highlights the role of EVs in bone, skeletal muscle, and joint health, including both normal tissue metabolism as well as tissue injury repair and regeneration. A consistent theme that emerges from study of musculoskeletal EVs is that various miRNAs appear to mediate a number of key pathological processes. These findings point to a potential therapeutic opportunity to target EV-derived miRNAs as a strategy for improving musculoskeletal function.

1. Introduction

Research on extracellular vesicles (EVs), including exosomes and microvesicles, has expanded significantly in recent years. This expanded knowledge is due in part to advances in EV isolation techniques, a better understanding of the surface markers that characterize exosomes and microvesicles, and greater information derived from −omics approaches on the proteins, lipids, mRNAs, and microRNAs (miRNAs) transported by EVs (Helwa et al., 2017; Lobb et al., 2015; Rider et al., 2016; Haraszti et al., 2016). Exosomes and microvesicles differ in size, with exosomes being smaller (40–150 nm) in diameter and microvesicles being larger (>150 nm). These EVs also differ in their biogenesis, where exosomes develop by inward budding of the plasma membrane, formation of a multivesicular body (MVB), and then MVB fusion with the plasma membrane to release the exosome particles. In contrast, microvesicles form by direct outward budding of the plasma membrane. EVs can be isolated by sequential ultracentrifugation, precipitation using polyethylene glycol-based reagents, or size exclusion chromatography and are now characterized by well-established markers (http://exocarta.org/exosome_markers). Together, this work has established EV-mediated transport of signaling factors as a novel mode of intercellular communication and epigenetic regulation (Collino et al., 2010). EVs are thought to contribute to a number of important physiological functions such as immune responses, tissue repair, and neural communication via their role(s) in intercellular transport (Yáñez-Mó et al., 2015). In addition, EVs appear to play key roles in cancer progression and metastasis (Hoshino et al., 2015). Because EVs have been shown to play important roles in both normal and abnormal physiology, they are also likely to be involved in the normal maintenance and degeneration of musculoskeletal tissues. Indeed, we have recently highlighted a role for exosome-derived miRNAs in age-related bone loss and osteoarthritis (Davis et al., 2017; Kolhe et al., 2017), two conditions that impose a significant public health burden on the aging global population. This review highlights the role of EVs in bone, skeletal muscle, and joint health, including normal tissue metabolism as well as tissue injury repair and regeneration.

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2. Extracellular vesicles in muscle cell differentiation, muscle regeneration, and congenital myopathies

Skeletal muscle comprises approximately 30–40% of body weight. Myofibers are prone to injury because they are regularly exposed to high contractile forces that can damage the sarcolemma, especially during eccentric muscle contraction (Demonbreun and McNally, 2017). The response of muscle fibers to injury and mechanical stress ranges from plasma membrane repair to a regenerative process involving the recruitment of satellite cells. The congenital absence of dystrophin elevates the incidence of muscle injury, ultimately leading to long-term skeletal muscle dysfunction. Muscle regeneration requires the coordinate, sequential expression of various factors including secreted proteins, inflammatory cytokines, miRNAs, and membrane lipids (Demonbreun and McNally, 2017; Wang and Wang, 2016; Tidball, 2005). EVs are actively secreted by differentiating myoblasts throughout this process (Le Bihan et al., 2012), these EVs can enhance muscle regeneration, and circulating muscle-derived EVs may serve as biomarkers of disease progression in congenital myopathies.

Evidence from several *in vitro* studies reveals that the secretion of EVs increases during muscle differentiation (Fig. 1). This has been demonstrated using mouse C2C12 cells (Romancino et al., 2017) as well as primary human myoblasts (Choi et al., 2016). EVs derived from myoblasts contain growth factors that act as potential regulators of development, function, and repair such as basic fibroblast growth factor (bFGF), insulin-like growth factor-1 (IGF-1), transforming growth factor-beta1 (TGF-B1), and vascular endothelial growth factor (VEGF), among others (VEGF R3, PDGF) (Do et al., 2017; Yao-Hua Song et al., 2013; Hoier et al., 2013). Importantly, these paracrine growth factors play a significant role in muscle satellite cell chemotaxis and lineage commitment. Additional studies have sought to define the biological activity of EVs derived from differentiating myoblasts and fully differentiated myotubes. Fluorescent labeling approaches have been employed to detect EVs in the cytoplasm of treated myoblasts (Braun and Gautel, 2011; Quattrocelli and Sampaolesi, 2015). Thus, muscle cells not only secrete EVs but muscle cells also readily endocytose these vesicles. In vitro studies also provide insights into the potential functions of these EVs. Myotube-derived EVs can promote the differentiation of myoblasts by altering expression of cyclin-D1 and myogenin (Braun and Gautel, 2011; Forterre et al., 2014a). It is important to note here that while EVs are secreted by both myoblasts and myotubes (Fig. 1), telocytes may also represent another source of EVs in muscle. These stromal cells have projections called telopods that extend into skeletal muscle interstitium (Popescu et al., 2011). Importantly, these projects are found to be associated in situ with exosomes and other shed vesicles, suggesting a potential role for telocyte-derived EVs in cellular signaling during muscle regeneration (Popescu et al., 2011).

The *in vitro* studies referenced above suggest that muscle cells secrete EVs, which may in turn be endocytosed by neighboring myoblasts, impacting their potential for proliferation and differentiation. These observations point to a role for muscle-derived EVs in mediating muscle regeneration. This potential role for EVs is supported by work indicating that treatment of wounded muscle with muscle-derived EVs reduces fibrosis and increases the number of regenerating myofibers (Choi et al., 2016). A set of muscle-specific miRNAs termed myoMirs, which includes miR-1, -133a,b, and −206, have been identified in skeletal muscle (Wang and Wang, 2016; Braun and Gautel, 2011; Aoi, 2014; Matsuzaka et al., 2016; Forterre et al., 2014b). Not surprisingly, EVs released from skeletal muscle are enriched in these miRNAs, and circulating muscle-derived EVs transport these miRNAs (Guescin et al., 2015). Moreover, muscle injury and calcium influx stimulate EV release (Matsuzaka et al., 2016), further implicating EVs as signaling molecules involved in communication during injury and repair. MiR-206 is induced by MyoD, and miR-206 can in turn promote myoblast differentiation through the downregulation of Twist-1 (Koutalianos et al., 2015). EVs released following muscle injury may therefore promote repair in neighboring fibers by transporting myoMirs such as miR-206 (Table 1) (Fig. 1).

Serum levels of miR-1, -133a, and −206 are increased in patients with Duchenne muscular dystrophy as well as in dystrophin-deficient mdx mice (Table 1) (Aoi and Sakuma, 2014). These miRNAs are in some cases associated with the CD63 antigen, which is located on the surface on EVs, consistent with an exosomal origin for these circulating miRNAs (Matsuzaka et al., 2016). Exposure of myoblasts to EVs from serum of mdx mice, or overexpression of myoMirs themselves, promotes cell survival and reduces cell death (Aoi, 2014). EVs may also play a role in myotonic dystrophy (DM1), the most common form adult-onset muscular dystrophy (Table 1) (Koutsoulidou et al., 2015). In the case of DM1 the myoMirs miR-1, -133a,b, and miR-206 are all elevated in DM1 patients that had progressive disease; however, these same markers are not elevated in those who did not demonstrate disease progression (Koutsoulidou et al., 2015). It is important to note here that these miRNAs were not directly isolated from exosomes, and so they could be circulating bound to other proteins or lipids. Nevertheless, these studies and those from healthy human subjects (Guescin et al., 2015) suggest that monitoring EV-derived miRNAs may serve as one approach for tracking disease progression or non-invasively assessing muscle injury following exercise.

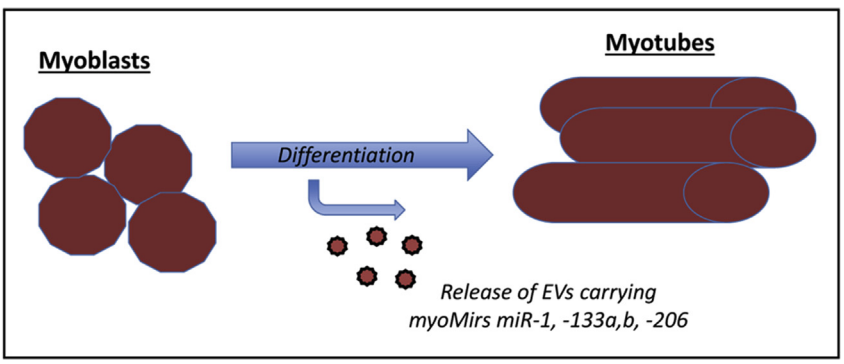

Fig. 1. Muscle regeneration requires the commitment of satellite cells to the myoblast lineage, and their further maturation into myotubes. Specific muscle-derived microRNAs termed myoMirs, including miR-1, 133a,b, and −206 show increased expression during myoblast differentiation. These microRNAs are also secreted in EVs during myogenic differentiation, presumably favoring the differentiation and maturation of neighboring myoblasts (Matsuzaka et al., 2016).

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