Signaling pathways of adipose stem cell-derived exosomes promoting muscle regeneration

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Abstract

Severe muscle injury is still a challenging clinical problem. Exosomes derived from adipose stem cells (ASC-exos) may be a potential therapeutic tool, but their mechanism is not completely clear. This review aims to elaborate the possible mechanism of ASC-exos in muscle regeneration from the perspective of signal pathways and provide guidance for further study. Literature cited in this review was acquired through PubMed using keywords or medical subject headings, including adipose stem cells, exosomes, muscle regeneration, myogenic differentiation, myogenesis, wingless/integrated (Wnt), mitogen-activated protein kinases, phosphatidylinositol-4,5-bisphosphate 3-kinase/protein kinase B (PI3K/Akt), Janus kinase/signal transducers and activators of transcription, and their combinations. We obtained the related signal pathways from proteomics analysis of ASC-exos in the literature, and identified that ASC-exos make different contributions to multiple stages of skeletal muscle regeneration by those signal pathways.
Keywords: Adipose stem cells; Exosomes; Muscle regeneration; Signaling pathways

Introduction

Skeletal muscle accounts for approximately 30–40% of the human body mass,[1] is vital for activity, posture maintenance, and metabolism, and is essential for sustaining life and maintaining life quality. Skeletal muscle injury has a high incidence, which is usually due to traumatic events such as excessive exercise, trauma, traffic accidents, war, and tumor resection.[2] Muscular dystrophy, peripheral neuropathy, peripheral arterial disease, diabetes, uremia, and cirrhosis can also cause progressive muscle loss.[3] Mild muscle injury can recover endogenously with the help of physical therapy. However, the limited regenerative potential of skeletal muscle is insufficient to resolve severe injuries, such as complete muscle tears, high-energy impact injuries, and volumetric muscle loss, and often leads to fatty infiltration, fibrosis, and muscular atrophy.[4] Plastic surgeons must use autologous vascularized muscle tissue to repair muscle defects and improve appearance and function. Nevertheless, this practice causes additional damage to the donor site, and the recipient site may develop complications such as tendon adhesion, scarring, hematoma, infection, insufficient blood perfusion, and lack of innervation.[5] For severe skeletal muscle injury, to restore its structure and function is still a clinical challenge to be solved.

Recent preclinical data and clinical trials showed that cell therapy represented by mesenchymal stem cells (MSCs) significantly improves the regenerative potential of injured muscle.[6] According to the International Society for Cellular Therapy, MSCs refer to plastic-adherent cells under standard culture conditions, expressing cluster of differentiation (CD) 73, CD90, and CD105, lacking CD11b, CD14, CD19, CD34, CD45, CD79a, or human leukocyte antigen DR (HLA-DR), and with multi-potential to differentiate into adipocytes, chondroblasts, and osteoblasts *in vitro*.[7] MSCs can be isolated from bone marrow, adipose tissue, umbilical cord blood, sub-patellar fat pads, and dental tissue. The most commonly used are bone marrow-derived MSCs and adipose-derived MSCs (ASCs). ASCs, which have attracted attention recently, are plentiful, easy to collect, fast-growing, and keep highly expressed stem cell markers.[8] Secretion rather than differentiation of ASCs is thought to play a major role in helping with injured muscle regeneration.[9,10] As an essential part of cell secretion, exosomes derived from ASCs (ASC-exos) have advantages in production, storage, and delivery, and avoid some obstacles in applying cell therapy to the clinic, such as restricted cell survival, immunogenicity, and risk of abnormal tissue proliferation, so they are expected to replace cell therapy.[9,11] However, the unclear specific mechanism limits their further application. We hope to

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obtain a deeper understanding of how ASC-exos promote muscle regeneration. Thus, we write this review to summarize relevant studies and analyze possible mechanisms from the perspective of signaling pathways, and wish to provide direction for future research.

ASCs and Their Exosomes

ASCs can proliferate to maintain stem cell storage and differentiate into mesenchymal cell lines (e.g., fibroblasts, adipocytes, osteocytes, and chondrocytes) and non-mesenchymal cell lines (e.g., skeletal muscle cells, smooth muscle cells, cardiomyocytes, hepatocytes, and neurons). ASCs can secrete anti-inflammatory proteins (e.g., interleukin [IL] 4, IL-10, and IL-1 receptor antagonist) and growth factors (e.g., vascular endothelial growth factor [VEGF] and hepatocyte growth factor [HGF]) to promote immunomodulation, angiogenesis, and tissue regeneration.[12] Many studies consider that transplanted ASCs participate in muscle regeneration through differentiation and secretion. However, some studies have shown that implanted ASCs are not directly involved in forming new muscle fibers, arguing that ASCs promote muscle regeneration mainly by secreting extracellular vesicles and soluble substances.[9,10]

Exosomes (exos), a subtype of extracellular vesicles, are 50–150 nm in diameter, carrying CD63, CD9, and CD81. Late endosomes bud inward and become multivesicular bodies. Multivesicular bodies either fuse with lysosomes to degrade contents or fuse with the plasma membrane to release intraluminal vesicles.[13] These small vesicles, which are released from cells, are termed exosomes. They can act on target cells through direct ligand binding on the surface or delivering functional molecules by membrane fusion or endocytosis, mediating short-distance or long-distance cell communication and affecting target cells' behavior.[14] The bilayer lipid membrane structure is related to protecting bioactive goods and simplifying the processes of uptake and storage. Therefore, exosomes with the bilayer lipid membrane structure can also be used as carriers of targeted drugs and genes in regenerative medicine.

Exosomes are considered to be a miniature version of parent cells. The complex composition of ASC-exos allows them to participate in various cellular processes,[15] showing the possibility of treating complicated clinical diseases. In cardiovascular conditions, ASC-exos can enhance angiogenesis through the VEGF pathway,[16] downregulate the expression of miR-342-5p to protect vascular endothelial cells and prevent atherosclerosis,[17] and protect the myocardium from ischemia-reperfusion injury through the wingless/integrated (Wnt)/β-catenin pathway.[18] In neurological conditions, ASC-exos can lessen the extent of cerebral infarct after stroke and preserve neurological function.[19] ASC-exos can alleviate β-amyloid protein accumulation, decrease neuronal cell apoptosis in Alzheimer's disease, promote the recovery of damaged neurites,[20] and deliver neurotrophin-3 (NT-3) mRNA as a carrier to promote sciatic nerve regeneration and gastrocnemius function recovery.[21] In renal conditions, ASC-exos can protect the kidney from acute ischemia-reperfusion injury,[22] and transfer miR-486 to prevent podocytes injury through the small mothers against decapentaplegic 1 (Smad1)/mechanistic target of rapamycin (mTOR) pathway.[23] In addition, ASC-exos can improve ovarian function in premature ovarian failure,[24] accelerate the repair of bone defects,[25] and inhibit the formation of keloids.[26]

Several studies have confirmed the function of ASC-exos in promoting adult skeletal muscle regeneration. Zhang and Wang *et al*[27-29] demonstrated that ASC-exos can reduce muscle atrophy, degeneration, and fat infiltration, enhance healing, and improve regeneration and biomechanical characteristics in rotator cuff tears. El Baradie *et al*[30] proved that ASC-exos can reverse hypoxia-induced injury of muscle cells and promote skeletal muscle regeneration in mice after cardiotoxin (CTX)-induced acute injury. Ni *et al*[31] found that ASC-exos can improve functional and histological recovery after stress urinary incontinence by helping the growth of skeletal muscle and Schwann cells. Bonafede *et al*[32] verified that ASC-exos can protect muscles and slow down the clinical progress of amyotrophic lateral sclerosis.

Regeneration of Adult Skeletal Muscle

The regeneration of adult skeletal muscle is mainly mediated by muscle satellite cells. Satellite cells are located around muscle fibers between the cell membrane and the basement membrane, usually at rest in mature muscle tissue. Pax7, the marker of dormant satellite cells, is essential for satellite cells to maintain their function. When Pax7 mutates, the satellite cells will be gradually lost.[33] Pax7 can promote proliferation and inhibit differentiation as a pioneer transcription factor.[34] Myogenic regulatory factors (MRFs), markers during the myogenic differentiation, are a family of transcription factors containing basic helix-loop-helix motifs and include myogenic factor 5 (Myf5), myogenic differentiation (MyoD), myogenin (MyoG), and myogenic factor 6 (Myf6, also known as MRF4). The myocyte enhancer factor 2 (MEF2) protein family (MEF2A, MEF2B, MEF2C, and MEF2D) has no potential to promote myogenesis by itself, but it can upregulate MyoD, MyoG, and Myf6, and act synergistically to enhance myogenesis.[35] The multistep process of skeletal muscle regeneration is regulated by the regulatory network composed of Pax7, MRFs, and other common transcription factors.

Once the signal of muscle injury is received, satellite cells will be activated, re-enter the cell cycle, proliferate, and undergo asymmetric division. The subset expressing Pax7 without Myf5 continues proliferation and self-renewal, while the other subset expressing Pax7 and Myf5 starts myogenic differentiation.[36] As the expression of Pax7 and Myf5 drops from the peak, MyoD, a marker of myogenic commitment, gradually increases, and the cell reaches the myoblast stage. Later, MyoG and Myf6 are upregulated, and myoblasts enter terminal differentiation and exit the cell cycle.[37] In mature muscle fibers, MyoD and MyoG are downregulated, but Myf6 maintains a high expression level and becomes the main MRF [Figure 1].[38]

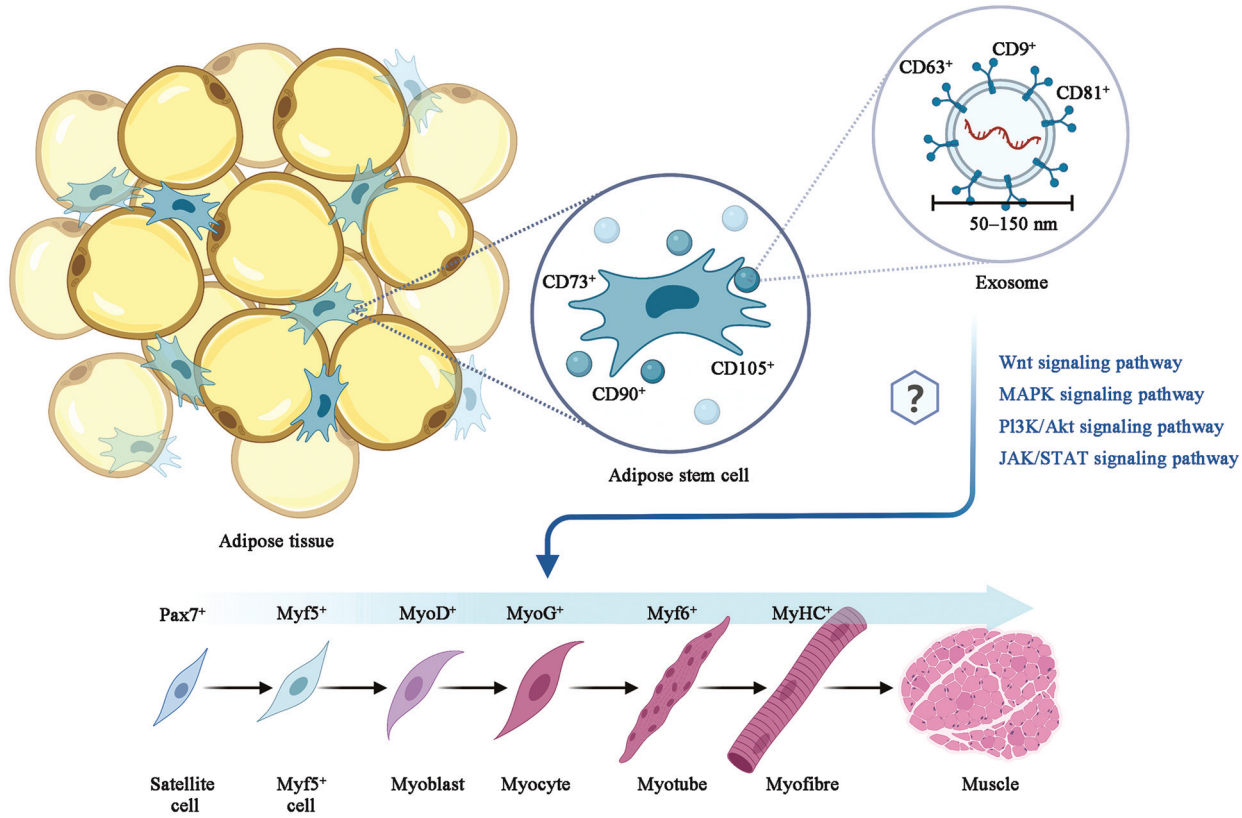

Figure 1: The schematic diagram for the process of muscle regeneration and the ASC-exos. ASC-exo: Exosomes derived from adipose stem cells; CD: Cluster of differentiation; JAK/STAT: Janus kinase/signal transducer and activator of transcription; MAPK: Mitogen-activated protein kinase; Myf: Myogenic factor; MyHC: Myosin heavy chain; MyoD: Myogenic differentiation; MyoG: Myogenin; Pax: Paired box; PI3K/Akt: Phosphatidylinositol 3-kinase/protein kinase B; Wnt: Wingless/integrated.

Signaling Pathways of ASC-exos Promoting Muscle Regeneration

ASC-exos may promote muscle regeneration through the wingless/integrated (Wnt) pathway

It has been proved that ASC-exos can activate the Wnt pathway to encourage tissue regeneration and repair, such as protecting the myocardium from ischemia-reperfusion injury,[18] inducing osteogenesis,[39,40] and promoting skin wound repair.[41-43] Presumably, ASC-exos may encourage muscle regeneration through the Wnt pathway [Figure 2]. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis demonstrated that the proteins relevant to the Wnt pathway are enriched in ASC-exos, such as β-catenin, low density lipoprotein receptor-related protein (LRP)1, α-catenin, rat sarcoma virus (ras)-related C3 botulinum toxin substrate (Rac)1, ras homolog family member A (RhoA), Rho-associated coiled-coil containing protein kinase (ROCK), phospholipase C (PLC) delta1, cell division cycle 42 (CDC42), and calmodulin-dependent protein kinase II (CaMKII) delta,[31,44-46] some of which may play a role in muscle regeneration promoted by ASC-exos.

The canonical Wnt pathway mediates the promoting effect of ASC-exos on muscle regeneration mainly by regulating the myogenic commitment of satellite cells.[47] Canonical Wnt signaling depends on β-catenin, which is concentrated in ASC-exos. When the Wnt ligand is missing, β-catenin is first phosphorylated by the destruction complex (including adenomatous polyposis coli protein [APC], glycogen synthase kinase 3β [GSK3β], creatine kinase 1α [CK1α], and scaffold protein Axin) and then degraded by ubiquitination. When the Wnt ligand binds to the frizzled class receptor (FZD) and LRP coreceptor which is identified in ASC-exos, LRP is phosphorylated and recruits dishevelled (Dvl) to the plasma membrane to form a Dvl polymer which inactivates the destruction complex.[48] Therefore, endogenous and ASC-exos-derived β-catenin can accumulate and transfer into the nucleus. In primary myoblasts, β-catenin is essential for the timely activation of myogenic programs through the inhibitory effect of miR-133b and miR-206 on Pax7 expression and alleviating the inhibitory effect of Pax7 on MRFs.[49] Subsequently, Wnt/β-catenin signaling increases the abundance and activity of MyoD and Barx2 in the myoblasts. With the help of MEF2, MyoD cooperates with Barx2 to control the expression of genes related to myoblast differentiation.[50] Besides, the process of muscle regeneration also involves that β-catenin cooperates with α-catenin transferred by ASC-exos, to control local filament assembly.[51]

However, persistent β-catenin overexpression in myoblast can cause premature differentiation, reduction in muscle fiber size, and fibrosis.[47,52] That is to say, failure to timely end Wnt/β-catenin signaling may also lead to poor adult muscle regeneration. To suppress inappropriate canonical Wnt signaling, β-catenin generates a negative feedback

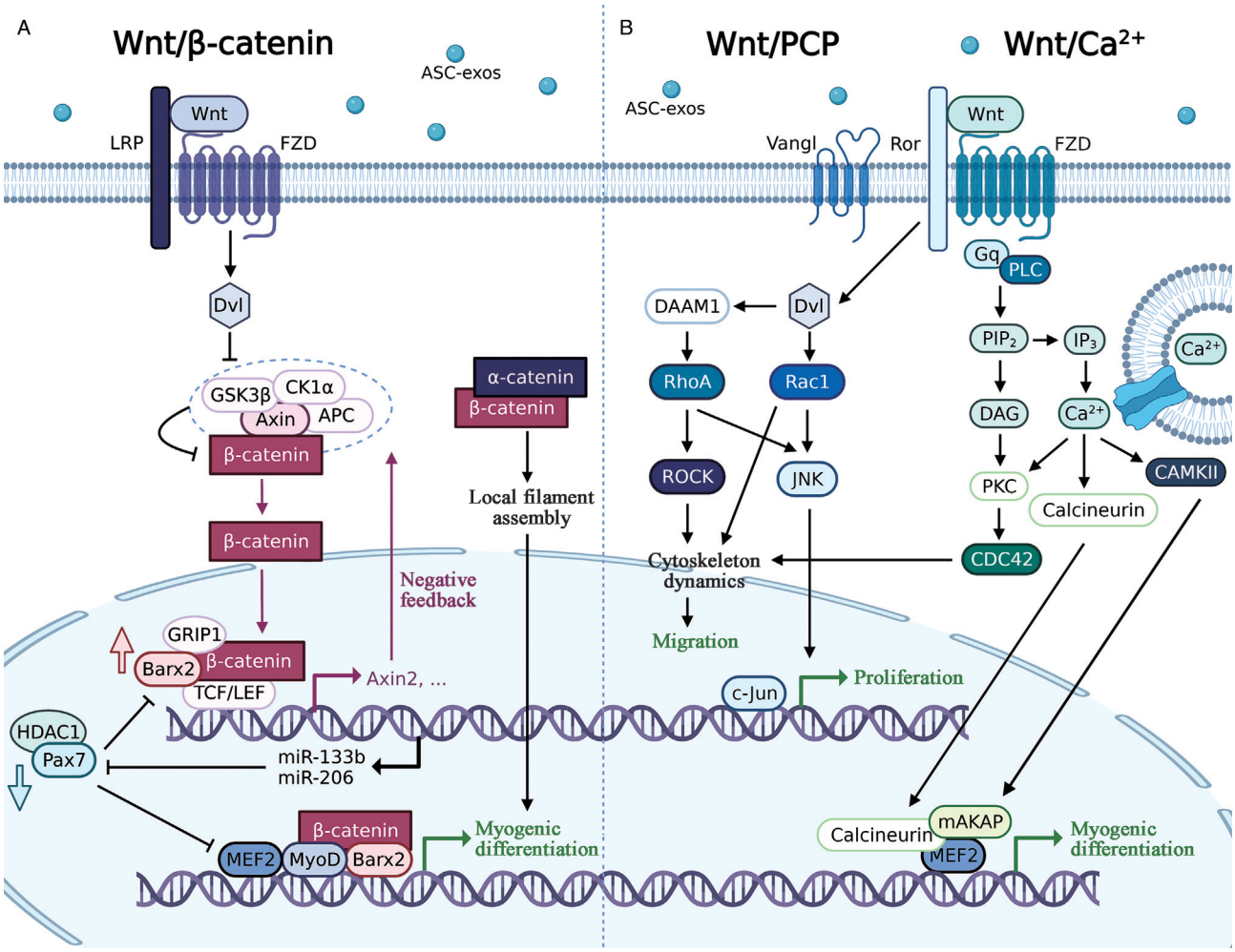

Figure 2: ASC-exos mediates the muscle regeneration by Wnt signaling pathway. (A) Canonical Wnt pathway. (B) Non-canonical Wnt pathway. APC: Adenomatous polyposis coli protein; ASC-exo: Exosomes derived from adipose stem cells; CAMKII: Calcium/calmodulin dependent protein kinase II; CDC42: Cell division cycle 42; CK1α: Casein kinase 1α; DAAM1: Dishevelled-associated activator of morphogenesis 1; DAG: Diacylglycerol; Dvl: Dishevelled; FZD: Frizzled class receptor; GRIP1: Glucocorticoid receptor interacting protein 1; GSK3β: Glycogen synthase kinase 3β; HDAC1: Histone deacetylase 1; IP3: Inositol-1,4,5-trisphosphate; JNK: c-Jun NH2-terminal kinase; LRP: Low density lipoprotein receptor related protein; mAKAP: Muscle-specific A-kinase anchoring protein; MEF2: Myocyte enhancer factor 2; miR: miRNA/microRNA; MyoD: Myogenic differentiation; Pax7: Paired box 7; PCP: Planar cell polarity; PIP_2: Phosphatidylinositol-4,5-bisphosphate; PKC: Protein kinase C; PLC: Phospholipase C; Rac1: Ras-related C3 botulinum toxin substrate 1; RhoA: Ras homolog gene family member A; ROCK: Rho associated coiled-coil containing protein kinase; Ror: Receptor tyrosine kinase-like orphan receptor; Ras: Rat sarcoma virus; TCF/LEF: T-cell factor/lymphoid enhance factor; Vangl: Van Gogh-like; Wnt: Wingless/integrated.

effect mediated by Barx2 and Axin2 during myogenic differentiation [Figure 2].[53] In the negative feedback, Barx2 is recruited to the *Axin2* gene via the T-cell factor/lymphoid enhancer factor (TCF/LEF) binding site, recruits β-catenin and the coactivator glucocorticoid receptor-interacting protein 1 (GRIP1), and then induces local histone H3 lysine acetylation to promote the expression of *Axin2* and form the destruction complex to suppress inappropriate canonical Wnt signaling.[54,55] Pax7 may retain histone deacetylase 1 (HDAC1) at the TCF/LEF site to function antagonistically toward Barx2.[56]

The non-canonical Wnt pathway mediates the promoting effect of ASC-exos on muscle regeneration by controlling the self-renewal and migration of satellite cells and impelling myogenic differentiation indirectly, and performs a different but complementary role to the canonical Wnt pathway [Figure 2]. The Wnt/Planar cell polarity (PCP) pathway is activated by the binding of WNT7a, FZD7, and receptor tyrosine kinase-like orphan receptor (Ror) in satellite cells. Then, Van Gogh-like (Vangl) is activated, and Dvl is recruited.[57] ASC-exos deliver Rac1, RhoA, and ROCK to satellite cells. Dvl can activate the small GTPases Rac1 and RhoA, trigger ROCK, c-Jun NH2-terminal kinase (JNK), and downstream cascade reactions, and results in directional migration and symmetrical expansion of satellite cells.[58] In the Wnt/Ca^{2+} pathway, Wnt/FZD activates PLC/inositol-1,4,5-trisphosphate (IP_3)/Ca^{2+}. PLC delta1 transported by ASC-exos may facilitate this process. Intracellular calcium release activates calcium-sensitive enzymes such as protein kinase C (PKC), CaMKII, and calcineurin.[59] CDC42 downstream of PKC is enriched in ASC-exos, and participates in directional migration of satellite cells after muscle injury via the cytoskeleton.[60] CaMKII delta is also enriched in ASC-exos, and CaMKII helps the activation of the transcriptional activity of MEF2.[61] The scaffold protein, muscle-specific A-kinase anchoring protein (mAKAP), organizes the calcineurin/MEF2 complex to regulate target gene transcription and promote myogenic differentiation.[62]

ASC-exos may promote muscle regeneration through the mitogen-activated protein kinase (MAPK) pathway

It has been proved that ASC-exos can activate the MAPK pathway to encourage tissue regeneration and repair, such as relieving neural injury caused by microglial activation,[63,64] inducing extracellular matrix remodeling, reducing scar formation, and promoting skin wound repair.[65] Presumably, ASC-exos may encourage muscle regeneration through the MAPK pathway [Figure 3]. The KEGG pathway analysis demonstrated that the proteins relevant to the MAPK pathway were enriched in ASC-exos, such as Rac1, RhoA, CDC42, extracellular signal-regulated kinase (ERK)1(also known as MAPK3), ERK2 (also known as MAPK1), MAPK/ERK kinase (MEK)1, MEK2, pleiotrophin (PTN), MAPK activated protein kinase 2 (MK2), integrin β1 (ITGβ1), and Rous sarcoma oncogene (Src),[31,44-46] some of which may play a role in muscle regeneration promoted by ASC-exos.

MAPKs contain four subfamilies: ERK1/2, JNK, p38, and ERK5, which can phosphorylate specific serine and threonine residues of target substrates. MAPK signaling is triggered by environmental stress, growth factors, and inflammatory cytokines and transmits the signal down through Ras, Rac, CDC42, Rho, etc.[66] ASC-exos may deliver Rac1, RhoA, and CDC42 to get involved in the process.

At the early stage of muscle regeneration, ERK1/2 signaling prevents premature differentiation of myoblasts. MEK1, MEK2, ERK1, and ERK2 in ASC-exos may accelerate this process. Phosphorylated MEK1 interacts with the MyoD transcription complex to inhibit transactivation and promote proliferation.[67] ERK1/2 regulates cell cycle progression, which is necessary for proliferation.[68] JNK signaling also promotes myoblast proliferation and prevents premature differentiation. Active c-Jun enters the nucleus and forms the activating protein-1 (AP-1) complex to inhibit MyoD transcription. At the later stage of

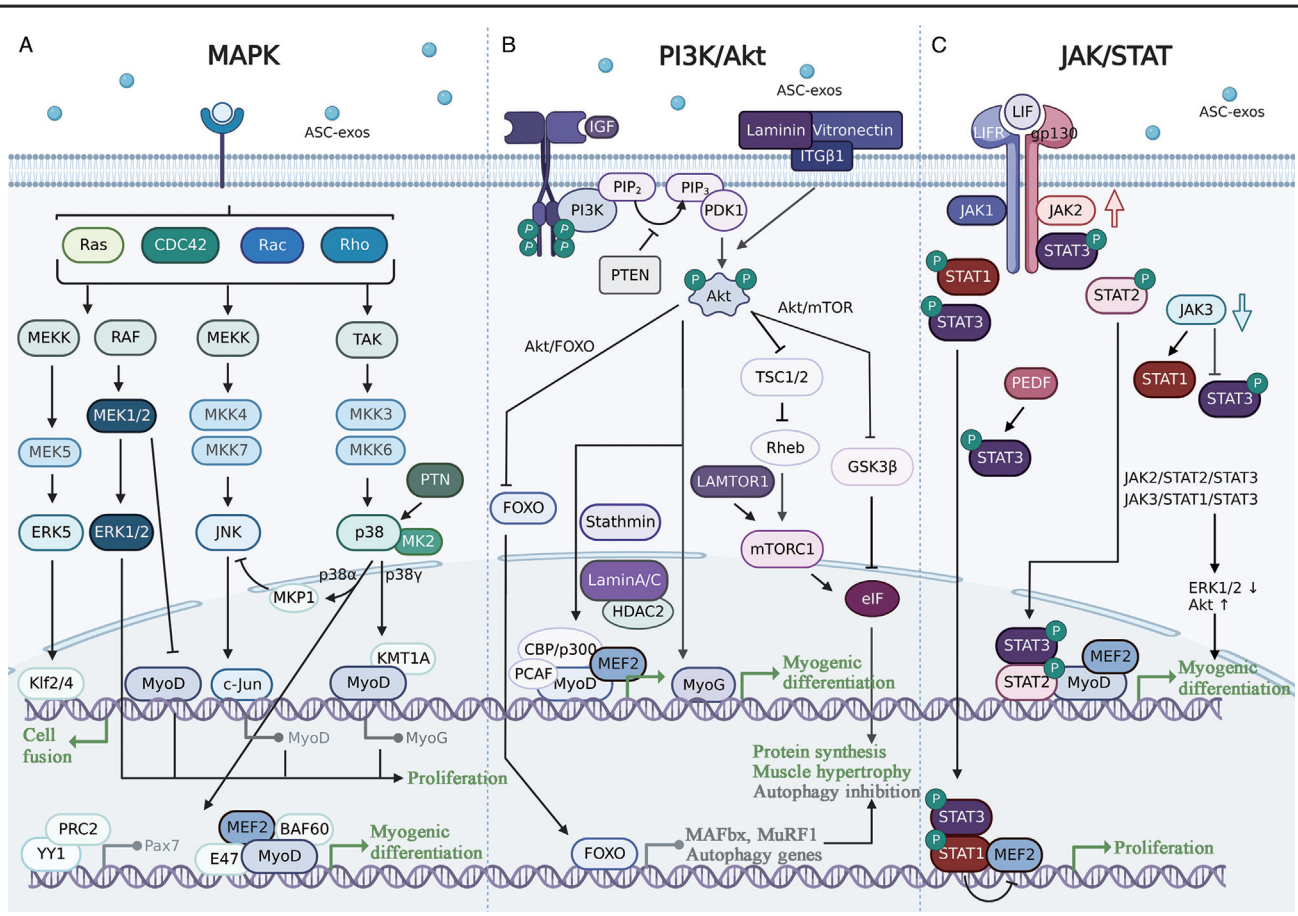

Figure 3: ASC-exos mediates the muscle regeneration by MAPK pathway, PI3K/Akt pathway and JAK/STAT pathway. (A) MAPK pathway. (B) PI3K/Akt pathway. (C) JAK/STAT pathway. ASC-exo: Exosomes derived from adipose stem cells; Akt: Protein kinase B; BAF60: Brahma-related gene 1 associated factor 60; CBP: cAMP-response element binding protein binding protein; CDC42: Cell division control protein 42; eIF: Eukaryotic translation initiation factor; ERK: Extracellular signal regulated kinase; FOXO: Forkhead box transcription factor; gp130: Interleukin 6 cytokine family signal transducer; GSK3β: Glycogen synthase kinase 3β; HDAC2: Histone deacetylase 2; IGF: Insulin-like growth factor; ITGβ1: Integrin β1; JAK: Janus kinase; JNK: c-Jun NH2-terminal kinase; Klf: Kruppel-like factor; KMT1A: Lysine methyltransferase 1A; LAMTOR1: Late endosomal/lysosomal adaptor, MAPK and mTOR activator 1; LIF: Leukemia inhibitory factor; LIFR: Leukemia inhibitory factor receptor; MAFbx: Muscle atrophy F-box protein; MAPK: Mitogen-activated protein kinase; MEF2: Myocyte enhancer factor 2; MEK: MAPK ERK kinase; MEKK: MEK kinase; MK2: MAPK activated protein kinase 2; MKK: MAPK kinase; MKP1: MAPK phosphatase 1; mTOR: Mechanistic target of rapamycin; mTORC1: Mechanistic target of rapamycin complex 1; MuRF1: Muscle-specific RING finger protein 1; MyoD: Myogenic differentiation; MyoG: Myogenin; Pax7: Paired box 7; PCAF: p300/CBP-associated factor; PDK1: 3-phosphoinositide dependent protein kinase 1; PEDF: Pigment-epithelium derived factor; PI3K: Phosphatidylinositol 3-kinase; PIP_2: Phosphatidylinositol-4,5-bisphosphate; PIP_3: Phosphatidylinositol-3,4,5-trisphosphate; PRC2: Polycomb repressive complex 2; PTEN: Phosphatase and tensin homolog; PTN: Pleiotrophin; Rac: Ras-related C3 botulinum toxin substrate; RAF: Rapidly accelerated fibrosarcoma; Ras: Rat sarcoma virus; Rheb: Ras homologue enriched in brain; Rho: Ras homologous GTPase; STATs: Signal transducer and activator of transcription; Src: Rous sarcoma oncogene; TAK: Transforming growth factor beta activated kinase; TSC: Tuberous sclerosis complex; YY1: Yin Yang 1.

myogenesis, active p38α upregulates the JNK phosphatase MAPK phosphatase 1 (MKP1) and inhibits JNK signaling, leading to cell cycle exit and myogenic differentiation.[69] PTN transferred by ASC-exos helps with p38 signaling activation.[70] MK2 transferred by ASC-exos is directly phosphorylated by p38 and regulates myoblast differentiation in conjunction with p38.[71] Chromatin-wide and transcriptome profiling integration shows that p38α binds to a large set of active promoters to globally regulate muscle regeneration during the transition of myoblasts from proliferation to differentiation stages.[72] For instance, p38α promotes the interaction of polycomb repressive complex 2 (PRC2) and Yin Yang 1(YY1) via threonine 372 phosphorylation of enhancer of zeste 2 (EZH2) at threonine 372 to inhibit Pax7 expression, directing myogenic commitment of satellite cells.[73] Then, during the differentiation, p38α can directly phosphorylate MEF2C and E47 to assist the transcriptional activity of MyoD.[74] p38α can also phosphorylate the SWItch/Sucrose Nonfermentable (SWI/SNF) subunit BRG1 associated factor 60 (BAF60). BAF60 facilitates MyoD binding to target genes and marks the chromatin for recruitment of the SWI/SNF chromatin remodeling complex to improve accessibility.[75] As for p38γ, it helps assemble MyoD/histone lysine methyltransferase 1A (KMT1A) inhibitory transcription complex to prevent MyoG expression, restricting myoblasts from prematurely entering further differentiation and promoting proliferation relatively.[76] Additionally, p38α plays an essential role in forming multinucleated myotubes by upregulating CD53.[77] However, p38β MAPK phosphorylates p300 to stimulate acetylation of CCAAT/enhancer binding protein β (C/EBPβ), which may cause cancer-induced muscle wasting. Research found that nilotinib can selectively bind p38β to avoid cancer-induced protein loss without suppressing p38α-dependent myogenesis, and it was considered a promising treatment.[78]

ERK5 plays a crucial role in muscle cell fusion. Inhibition of the ERK5 pathway blocks the formation of multinucleated myotubes. During myogenic differentiation, the expression of Kruppel-like factor (Klf)2 and Klf4 is upregulated in an ERK5-dependent manner. Nephronectin, a target of Klf, is also significantly upregulated. The interaction of nephronectin with ITGβ1 enriched in ASC-exos is thought to be essential for muscle cell fusion.[15,79] Interestingly, increasing the activity of ERK5 signaling alone cannot induce cell fusion. ERK5 promotes this process only in cooperation with myogenic factors, such as MyoD and MEF2.[79] In addition, Yes-associated protein (YAP)/Abelson murine leukemia viral oncogene homolog 1 (Abl)/Src can activate the MEK kinase 3 (MEKK3)/MEK5/ERK5 kinase cascade to encourage myogenic differentiation, and Src is also concentrated in ASC-exos.[80]

ASC-exos may promote muscle regeneration through the phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) pathway

It has been proved that ASC-exos can activate the PI3K/Akt pathway to encourage tissue regeneration and repair, such as inhibiting inflammation,[81] reducing scar formation, promoting skin wound repair,[82] and inducing angiogenesis.[83] Presumably, ASC-exos may encourage muscle regeneration through the PI3K/Akt pathway [Figure 3]. The KEGG pathway analysis demonstrated that the proteins relevant to the PI3K/Akt pathway were enriched in ASC-exos, such as insulin-like growth factor (IGF)1, IGF2 receptor (IGF2R), laminin γ1, laminin α4, laminin β2, laminin β1, ITGβ1, vitronectin, lamin A/C, late endosomal/lysosomal adaptor, MAPK and mTOR activator 1 (LAMTOR1), eukaryotic translation initiation factor (eIF)2B3, eIF2B4, eIF4A1, and eIF4A3,[31,44-46] some of which may play a role in muscle regeneration promoted by ASC-exos.

ASC-exos participates in the upstream activation of PI3K/Akt in myoblasts by sending IGF1, IGF2R, laminin, ITGβ1, and vitronectin. PI3K converts phosphatidylinositol-4,5-bisphosphate (PIP2) to phosphatidylinositol-3,4,5-trisphosphate (PIP3). Akt, activated by 3-phosphoinositide dependent protein kinase 1 (PDK1), is the most critical downstream effector of IGF1 and IGF2 in skeletal muscle.[84] Laminin interacting with ITGβ1 participates in activating PI3K/Akt to regulate muscle regeneration.[85] Vitronectin-mediated Akt activation may also be involved in this process.[86]

The PI3K/Akt pathway mediates the promoting effect of ASC-exos on muscle regeneration partly by accelerating myogenic differentiation. PI3K/Akt stimulates upregulation of stathmin, which is essential for MyoD expression.[87] Lamin A/C identified in ASC-exos combines with HDAC2 to promote the interaction between p300/CBP-associated factor (PCAF) and MyoD.[88] Akt1/2 directly phosphorylates p300 to allow the binding of MyoD to p300 and PCAF and promote transcription.[89] PI3K/Akt can induce the phosphorylation and transcriptional activity of MEF2 to assist MRFs.[90] The PI3K/Akt pathway also affects the expression and transcriptional activity of MyoG.[91]

Moreover, the PI3K/Akt pathway can mediate the promoting effect of ASC-exos on muscle regeneration by inducing protein synthesis and skeletal muscle hypertrophy.[92] Akt can inhibit tuberous sclerosis complex (TSC)1/2 and activate mechanistic target of rapamycin complex 1 (mTORC1).[93] LAMTOR1 transferred by ASC-exos is a component of the Ragulator complex required for mTORC1 activation.[94] ASC-exos also deliver eIF2B3, eIF2B4, eIF4A1, and eIF4A3 to myoblasts. Akt may activate these translation initiation factors via mTORC1 signaling or phosphorylating GSK3β to promote protein synthesis.[95,96] Besides, Akt phosphorylates and inhibits the FOXO transcription factor family and reduces the expression of E3 ubiquitin ligases muscle atrophy F-box protein (MAFbx) and muscle atrophy F-box protein (MuRF1), substrates of which include MyoD, calcineurin, myosin heavy chain (MyHC), myosin light chain kinase (MLCK) and myosin binding protein C (MyBP-C). Therefore, Akt/FOXO reduces protein degradation to inhibit muscle atrophy.[97] However, the effect of PI3K/Akt on muscle regeneration may not be all favorable. Autophagy is crucial for muscle regeneration,[98] but is likely inhibited by Akt via mTOR and FOXO.[94,99]

ASC-exos may promote muscle regeneration through the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway

It has been proved that ASC-exos can activate the JAK/STAT pathway to encourage tissue regeneration and repair, such as inducing the anti-inflammatory M2 phenotype,[100] and reducing transforming growth factor (TGF)-β1 induced liver fibrosis.[101] Presumably, ASC-exos may encourage muscle regeneration through the JAK/STAT pathway [Figure 3]. The KEGG pathway analysis demonstrated that the proteins relevant to the JAK/STAT pathway were enriched in ASC-exos, such as JAK1, STAT1, STAT3, leukemia inhibitory factor (LIF) receptor (LIFR), and pigment-epithelium derived factor (PEDF),[31,44-46] some of which may play a role in ASC-exos promoting muscle regeneration.

JAK1/STAT1/STAT3, which are all enriched in ASC-exos, plays a crucial role in muscle regeneration by promoting myoblast proliferation and preventing premature differentiation.[102] At the early stage of muscle regeneration, active STAT1 binds to MEF2 and inhibits its transcriptional activity to prevent myoblasts from differentiating prematurely.[103] As muscle regeneration progresses, STAT1 activity decreases naturally.[104] ASC-exos increase the number of LIFR in myoblasts. LIF/LIFR stimulates proliferation and inhibits differentiation through phosphorylating STAT1/STAT3 and activating ERK.[105,106] PEDF in ASC-exos also stimulates myoblast proliferation, probably by inducing phosphorylation of STAT3.[107]

JAK2/STAT2/STAT3 mediates the promoting effect of ASC-exos on muscle regeneration mainly by promoting myogenic differentiation. ASC-exos deliver STAT3 to get involved in this process. STAT3 activates and interacts with MyoD-MEF2 to improve the transcription of myogenic-specific genes.[108,109] JAK2/STAT2/STAT3 also regulates the expression of HGF and IGF2, inhibits HGF/ERK at the early stage, and enhances IGF2/PI3K/Akt at the late stage, indirectly regulating myogenic differentiation.[110] Besides, JAK3 inhibition promotes myogenic differentiation. When JAK3 is inhibited, STAT1 is suppressed and STAT3 is activated, which results in ERK downregulation and Akt upregulation and plays an essential role in terminal differentiation.[104]

Consequently, STAT3 concentrated in ASC-exos may play a positive role in myoblast proliferation and differentiation, and the specified JAK type may determine the direction of proliferation or differentiation.

Conclusions

ASC-exos have shown great potential in tissue regeneration and the possibility of preventing and combating various clinical diseases, and also attract attention in the field of treating skeletal muscle injury. This review started with the results of ASC-exos proteomics, and analyzed the possible mechanism of ASC-exos in muscle regeneration. ASC-exos encourage the canonical Wnt pathway to dominate the myogenic commitment of satellite cells, and exert functions in the non-canonical Wnt pathway to coordinate their self-renewal and migration. Activated by ASC-exos, the ERK1/2 and JNK MAPK pathways stimulate myoblast proliferation at the early stage, the p38 MAPK pathway mainly promotes myogenic differentiation, and the ERK5 MAPK pathway is crucial for forming multinucleated myotubes. In addition to acting on MRFs and MEF2 to upregulate myogenic differentiation, ASC-exos also assist the PI3K/Akt pathway in promoting protein synthesis and muscle fiber hypertrophy, but inhibit autophagy via mTOR and FOXO. With the participation of ASC-exos, the JAK1/STAT1/STAT3 pathway prevents premature differentiation, and the JAK2/STAT2/STAT3 and JAK3/STAT1/STAT3 pathways push forward the process of myogenic differentiation.

Of course, the specific molecular mechanism of ASC-exos regulating muscle regeneration remains to be confirmed by experiments. In order to popularize the use of exosomes in clinical practice, a more standardized production system is required for quality control, and more clinical trials are necessary to confirm the safety and effectiveness. Simple cell-free therapy with exosomes to treat severe muscle defects may be difficult to make quick profits, so further integration with tissue engineering is needed. This review may provide a theoretical basis for these research interests.

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Conflicts of interest

None.

References

1. Lee RC, Wang Z, Heo M, Ross R, Janssen I, Heymsfield SB. Total-body skeletal muscle mass: development and cross-validation of anthropometric prediction models. Am J Clin Nutr 2000;72:796–803. doi: 10.1093/ajcn/72.3.796.
2. Grasman JM, Zayas MJ, Page RL, Pins GD. Biomimetic scaffolds for regeneration of volumetric muscle loss in skeletal muscle injuries. Acta Biomater 2015;25:2–15. doi: 10.1016/j.actbio.2015.07.038.
3. Kalyani RR, Corriere M, Ferrucci L. Age-related and disease-related muscle loss: the effect of diabetes, obesity, and other diseases. Lancet Diabetes Endocrinol 2014;2:819–829. doi: 10.1016/S2213-8587(14)70034-8.
4. Corona BT, Wenke JC, Ward CL. Pathophysiology of volumetric muscle loss injury. Cells Tissues Organs 2016;202:180–188. doi: 10.1159/000443925.
5. Mihaly E, Altamirano DE, Tuffaha S, Grayson W. Engineering skeletal muscle: building complexity to achieve functionality. Semin Cell Dev Biol 2021;119:61–69. doi: 10.1016/j.semcdb.2021.04.016.
6. Qazi TH, Duda GN, Ort MJ, Perka C, Geissler S, Winkler T. Cell therapy to improve regeneration of skeletal muscle injuries. J Cachexia Sarcopenia Muscle 2019;10:501–516. doi: 10.1002/jcsm.12416.
7. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, *et al*. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006;8:315–317. doi: 10.1080/14653240600855905.
8. Meligy FY, Shigemura K, Behnsawy HM, Fujisawa M, Kawabata M, Shirakawa T. The efficiency of in vitro isolation and myogenic differentiation of MSCs derived from adipose connective tissue, bone marrow, and skeletal muscle tissue. In Vitro Cell Dev Biol Anim 2012;48:203–215. doi: 10.1007/s11626-012-9488-x.

9. Mitchell R, Mellows B, Sheard J, Antonioli M, Kretz O, Chambers D, *et al*. Secretome of adipose-derived mesenchymal stem cells promotes skeletal muscle regeneration through synergistic action of extracellular vesicle cargo and soluble proteins. Stem Cell Res Ther 2019;10:116. doi: 10.1186/s13287-019-1213-1.
10. Gorecka A, Salemi S, Haralampieva D, Moalli F, Stroka D, Candinas D, *et al*. Autologous transplantation of adipose-derived stem cells improves functional recovery of skeletal muscle without direct participation in new myofiber formation. Stem Cell Res Ther 2018;9:195. doi: 10.1186/s13287-018-0922-1.
11. Xiong M, Zhang Q, Hu W, Zhao C, Lv W, Yi Y, *et al*. Exosomes From Adipose-Derived Stem Cells: The Emerging Roles and Applications in Tissue Regeneration of Plastic and Cosmetic Surgery. Front Cell Dev Biol 2020;8:574223. doi: 10.3389/fcell.2020.574223.
12. Torres-Torrillas M, Rubio M, Damia E, Cuervo B, Del Romero A, Peláez P, *et al*. Adipose-derived mesenchymal stem cells: a promising tool in the treatment of musculoskeletal diseases. Int J Mol Sci 2019;20:3105. doi: 10.3390/ijms20123105.
13. Hessvik NP, Llorente A. Current knowledge on exosome biogenesis and release. Cell Mol Life Sci 2018;75:193–208. doi: 10.1007/s00018-017-2595-9.
14. Mathieu M, Martin-Jaular L, Lavieu G, Théry C. Specificities of secretion and uptake of exosomes and other extracellular vesicles for cell-to-cell communication. Nat Cell Biol 2019;21:9–17. doi: 10.1038/s41556-018-0250-9.
15. Keerthikumar S, Chisanga D, Ariyaratne D, Al Saffar H, Anand S, Zhao K, *et al*. ExoCarta: a web-based compendium of exosomal cargo. J Mol Biol 2016;428:688–692. doi: 10.1016/j.jmb.2015.09.019.
16. Han Y, Ren J, Bai Y, Pei X, Han Y. Exosomes from hypoxia-treated human adipose-derived mesenchymal stem cells enhance angiogenesis through VEGF/VEGF-R. Int J Biochem Cell Biol 2019;109:59–68. doi: 10.1016/j.biocel.2019.01.017.
17. Xing X, Li Z, Yang X, Li M, Liu C, Pang Y, *et al*. Adipose-derived mesenchymal stem cells-derived exosome-mediated microRNA-342-5p protects endothelial cells against atherosclerosis. Aging (Albany NY) 2020;12:3880–3898. doi: 10.18632/aging.102857.
18. Cui X, He Z, Liang Z, Chen Z, Wang H, Zhang J. Exosomes from adipose-derived mesenchymal stem cells protect the myocardium against ischemia/reperfusion injury through Wnt/β-Catenin Signaling Pathway. J Cardiovasc Pharmacol 2017;70:225–231. doi: 10.1097/FJC.0000000000000507.
19. Chen KH, Chen CH, Wallace CG, Yuen CM, Kao GS, Chen YL, *et al*. Intravenous administration of xenogenic adipose-derived mesenchymal stem cells (ADMSC) and ADMSC-derived exosomes markedly reduced brain infarct volume and preserved neurological function in rat after acute ischemic stroke. Oncotarget 2016;7:74537–74556. doi: 10.18632/oncotarget.12902.
20. Lee M, Ban JJ, Yang S, Im W, Kim M. The exosome of adipose-derived stem cells reduces β-amyloid pathology and apoptosis of neuronal cells derived from the transgenic mouse model of Alzheimer's disease. Brain Res 2018;1691:87–93. doi: 10.1016/j.brainres.2018.03.034.
21. Yang Z, Yang Y, Xu Y, Jiang W, Shao Y, Xing J, *et al*. Biomimetic nerve guidance conduit containing engineered exosomes of adipose-derived stem cells promotes peripheral nerve regeneration. Stem Cell Res Ther 2021;12:442. doi: 10.1186/s13287-021-02528-x.
22. Lin KC, Yip HK, Shao PL, Wu SC, Chen KH, Chen YT, *et al*. Combination of adipose-derived mesenchymal stem cells (ADMSC) and ADMSC-derived exosomes for protecting kidney from acute ischemia-reperfusion injury. Int J Cardiol 2016;216:173–185. doi: 10.1016/j.ijcard.2016.04.061.
23. Jin J, Shi Y, Gong J, Zhao L, Li Y, He Q, *et al*. Exosome secreted from adipose-derived stem cells attenuates diabetic nephropathy by promoting autophagy flux and inhibiting apoptosis in podocyte. Stem Cell Res Ther 2019;10:95. doi: 10.1186/s13287-019-1177-1.
24. Huang B, Lu J, Ding C, Zou Q, Wang W, Li H. Exosomes derived from human adipose mesenchymal stem cells improve ovary function of premature ovarian insufficiency by targeting SMAD. Stem Cell Res Ther 2018;9:216. doi: 10.1186/s13287-018-0953-7.
25. Li W, Liu Y, Zhang P, Tang Y, Zhou M, Jiang W, *et al*. Tissue-engineered bone immobilized with human adipose stem cells-derived exosomes promotes bone regeneration. ACS Appl Mater Interfaces 2018;10:5240–5254. doi: 10.1021/acsami.7b17620.
26. Wu ZY, Zhang HJ, Zhou ZH, Li ZP, Liao SM, Wu ZY, *et al*. The effect of inhibiting exosomes derived from adipose-derived stem cells via the TGF-β1/Smad pathway on the fibrosis of keloid fibroblasts. Gland Surg 2021;10:1046–1056. doi: 10.21037/gs-21-4.
27. Zhang X, Cai Z, Wu M, Huangfu X, Li J, Liu X. Adipose stem cell-derived exosomes recover impaired matrix metabolism of torn human rotator cuff tendons by maintaining tissue homeostasis. Am J Sports Med 2021;49:899–908. doi: 10.1177/0363546521992469.
28. Wang C, Hu Q, Song W, Yu W, He Y. Adipose stem cell-derived exosomes decrease fatty infiltration and enhance rotator cuff healing in a rabbit model of chronic tears. Am J Sports Med 2020;48:1456–1464. doi: 10.1177/0363546520908847.
29. Wang C, Song W, Chen B, Liu X, He Y. Exosomes isolated from adipose-derived stem cells: a new cell-free approach to prevent the muscle degeneration associated with torn rotator cuffs. Am J Sports Med 2019;47:3247–3255. doi: 10.1177/0363546519876323.
30. El Baradie K, Nouh M, O'Brien Iii F, Liu Y, Fulzele S, Eroglu A, *et al*. Freeze-dried extracellular vesicles from adipose-derived stem cells prevent hypoxia-induced muscle cell injury. Front Cell Dev Biol 2020;8:181. doi: 10.3389/fcell.2020.00181.
31. Ni J, Li H, Zhou Y, Gu B, Xu Y, Fu Q, *et al*. Therapeutic potential of human adipose-derived stem cell exosomes in stress urinary incontinence - an in vitro and in vivo study. Cell Physiol Biochem 2018;48:1710–1722. doi: 10.1159/000492298.
32. Bonafede R, Turano E, Scambi I, Busato A, Bontempi P, Virla F, *et al*. ASC-exosomes ameliorate the disease progression in SOD1 (G93A) murine model underlining their potential therapeutic use in human ALS. Int J Mol Sci 2020;21:3651. doi: 10.3390/ijms21103651.
33. Relaix F, Montarras D, Zaffran S, Gayraud-Morel B, Rocancourt D, Tajbakhsh S, *et al*. Pax3 and Pax7 have distinct and overlapping functions in adult muscle progenitor cells. J Cell Biol 2006;172:91–102. doi: 10.1083/jcb.200508044.
34. von Maltzahn J, Jones AE, Parks RJ, Rudnicki MA. Pax7 is critical for the normal function of satellite cells in adult skeletal muscle. Proc Natl Acad Sci U S A 2013;110:16474–16479. doi: 10.1073/pnas.1307680110.
35. Asfour HA, Allouh MZ, Said RS. Myogenic regulatory factors: the orchestrators of myogenesis after 30 years of discovery. Exp Biol Med (Maywood) 2018;243:118–128. doi: 10.1177/1535370217749494.
36. Relaix F, Bencze M, Borok MJ, Der Vartanian A, Gattazzo F, Mademtzoglou D, *et al*. Perspectives on skeletal muscle stem cells. Nat Commun 2021;12:692. doi: 10.1038/s41467-020-20760-6.
37. Zammit PS. Function of the myogenic regulatory factors Myf5, MyoD, Myogenin and MRF4 in skeletal muscle, satellite cells and regenerative myogenesis. Semin Cell Dev Biol 2017;72:19–32. doi: 10.1016/j.semcdb.2017.11.011.
38. Hinterberger TJ, Sassoon DA, Rhodes SJ, Konieczny SF. Expression of the muscle regulatory factor MRF4 during somite and skeletal myofiber development. Dev Biol 1991;147:144–156. doi: 10.1016/s0012-1606(05)80014-4.
39. Lu Z, Chen Y, Dunstan C, Roohani-Esfahani S, Zreiqat H. Priming adipose stem cells with tumor necrosis factor-alpha preconditioning potentiates their exosome efficacy for bone regeneration. Tissue Eng Part A 2017;23:1212–1220. doi: 10.1089/ten.tea.2016.0548.
40. Yang S, Guo S, Tong S, Sun X. Promoting osteogenic differentiation of human adipose-derived stem cells by altering the expression of exosomal miRNA. Stem Cells Int 2019;2019:1351860. doi: 10.1155/2019/1351860.
41. Qian L, Pi L, Fang BR, Meng XX. Adipose mesenchymal stem cell-derived exosomes accelerate skin wound healing via the lncRNA H19/miR-19b/SOX9 axis. Lab Invest 2021;101:1254–1266. doi: 10.1038/s41374-021-00611-8.
42. He L, Zhu C, Jia J, Hao XY, Yu XY, Liu XY, *et al*. ADSC-Exos containing MALAT1 promotes wound healing by targeting miR-124 through activating Wnt/β-catenin pathway. Biosci Rep 2020;40:BSR20192549. doi: 10.1042/BSR20192549.
43. Ma T, Fu B, Yang X, Xiao Y, Pan M. Adipose mesenchymal stem cell-derived exosomes promote cell proliferation, migration, and inhibit cell apoptosis via Wnt/β-catenin signaling in cutaneous wound healing. J Cell Biochem 2019;120:10847–10854. doi: 10.1002/jcb.28376.

44. Xing X, Han S, Cheng G, Ni Y, Li Z, Li Z. Proteomic analysis of exosomes from adipose-derived mesenchymal stem cells: a novel therapeutic strategy for tissue injury. Biomed Res Int 2020;2020:6094562. doi: 10.1155/2020/6094562.
45. Bonafede R, Brandi J, Manfredi M, Scambi I, Schiaffino L, Merigo F, *et al*. The anti-apoptotic effect of ASC-exosomes in an in vitro ALS model and their proteomic analysis. Cells 2019;8:1087. doi: 10.3390/cells8091087.
46. Wang ZG, He ZY, Liang S, Yang Q, Cheng P, Chen AM. Comprehensive proteomic analysis of exosomes derived from human bone marrow, adipose tissue, and umbilical cord mesenchymal stem cells. Stem Cell Res Ther 2020;11:511. doi: 10.1186/s13287-020-02032-8.
47. Rudolf A, Schirwis E, Giordani L, Parisi A, Lepper C, Taketo MM, *et al*. Catenin activation in muscle progenitor cells regulates tissue repair. Cell Rep 2016;15:1277–1290. doi: 10.1016/j.celrep.2016.04.022.
48. Metcalfe C, Mendoza-Topaz C, Mieszczanek J, Bienz M. Stability elements in the LRP6 cytoplasmic tail confer efficient signalling upon DIX-dependent polymerization. J Cell Sci 2010;123:1588–1599. doi: 10.1242/jcs.067546.
49. Cui S, Li L, Mubarokah SN, Meech R. Wnt/β-catenin signaling induces the myomiRs miR-133b and miR-206 to suppress Pax7 and induce the myogenic differentiation program. J Cell Biochem 2019;120:12740–12751. doi: 10.1002/jcb.28542.
50. Hulin JA, Nguyen TD, Cui S, Marri S, Yu RT, Downes M, *et al*. Barx2 and Pax7 regulate Axin2 expression in myoblasts by interaction with β-Catenin and chromatin remodelling. Stem Cells 2016;34:2169–2182. doi: 10.1002/stem.2396.
51. Cui S, Li L, Yu RT, Downes M, Evans RM, Hulin JA, *et al*. β-Catenin is essential for differentiation of primary myoblasts via cooperation with MyoD and α-catenin. Development 2019;146:dev167080. doi: 10.1242/dev.167080.
52. Murphy MM, Keefe AC, Lawson JA, Flygare SD, Yandell M, Kardon G. Transiently active Wnt/β-catenin signaling is not required but must be silenced for stem cell function during muscle regeneration. Stem Cell Reports 2014;3:475–488. doi: 10.1016/j.stemcr.2014.06.019.
53. Figeac N, Zammit PS. Coordinated action of Axin1 and Axin2 suppresses β-catenin to regulate muscle stem cell function. Cell Signal 2015;27:1652–1665. doi: 10.1016/j.cellsig.2015.03.025.
54. MacDonald BT, Tamai K, He X. Wnt/beta-catenin signaling: components, mechanisms, and diseases. Dev Cell 2009;17:9–26. doi: 10.1016/j.devcel.2009.06.016.
55. Shao JS, Cheng SL, Pingsterhaus JM, Charlton-Kachigian N, Loewy AP, Towler DA. Msx2 promotes cardiovascular calcification by activating paracrine Wnt signals. J Clin Invest 2005;115:1210–1220. doi: 10.1172/JCI24140.
56. Zhuang L, Hulin JA, Gromova A, Tran Nguyen TD, Yu RT, Liddle C, *et al*. Barx2 and Pax7 have antagonistic functions in regulation of wnt signaling and satellite cell differentiation. Stem Cells 2014;32:1661–1673. doi: 10.1002/stem.1674.
57. Le Grand F, Jones AE, Seale V, Scimè A, Rudnicki MA. Wnt7a activates the planar cell polarity pathway to drive the symmetric expansion of satellite stem cells. Cell Stem Cell 2009;4:535–547. doi: 10.1016/j.stem.2009.03.013.
58. Bentzinger CF, von Maltzahn J, Dumont NA, Stark DA, Wang YX, Nhan K, *et al*. Wnt7a stimulates myogenic stem cell motility and engraftment resulting in improved muscle strength. J Cell Biol 2014;205:97–111. doi: 10.1083/jcb.201310035.
59. Gao C, Chen YG. Dishevelled: the hub of Wnt signaling. Cell Signal 2010;22:717–727. doi: 10.1016/j.cellsig.2009.11.021.
60. Roman W, Pinheiro H, Pimentel MR, Segalés J, Oliveira LM, García-Domínguez E, *et al*. Muscle repair after physiological damage relies on nuclear migration for cellular reconstruction. Science 2021;374:355–359. doi: 10.1126/science.abe5620.
61. Boubali S, Liopeta K, Virgilio L, Thyphronitis G, Mavrothalassitis G, Dimitracopoulos G, *et al*. Calcium/calmodulin-dependent protein kinase II regulates IL-10 production by human T lymphocytes: a distinct target in the calcium dependent pathway. Mol Immunol 2012;52:51–60. doi: 10.1016/j.molimm.2012.04.008.
62. Li J, Vargas MA, Kapiloff MS, Dodge-Kafka KL. Regulation of MEF2 transcriptional activity by calcineurin/mAKAP complexes. Exp Cell Res 2013;319:447–454. doi: 10.1016/j.yexcr.2012.12.016.
63. Feng N, Jia Y, Huang X. Exosomes from adipose-derived stem cells alleviate neural injury caused by microglia activation via suppressing NF-kB and MAPK pathway. J Neuroimmunol 2019;334:576996. doi: 10.1016/j.jneuroim.2019.576996.
64. Chen Y, Li J, Ma B, Li N, Wang S, Sun Z, *et al*. MSC-derived exosomes promote recovery from traumatic brain injury via microglia/macrophages in rat. Aging (Albany NY) 2020;12:18274–18296. doi: 10.18632/aging.103692.
65. Wang L, Hu L, Zhou X, Xiong Z, Zhang C, Shehada H, *et al*. Exosomes secreted by human adipose mesenchymal stem cells promote scarless cutaneous repair by regulating extracellular matrix remodelling. Sci Rep 2017;7:13321. doi: 10.1038/s41598-017-12919-x.
66. Kassouf T, Sumara G. Impact of conventional and atypical MAPKs on the development of metabolic diseases. Biomolecules 2020;10:1256. doi: 10.3390/biom10091256.
67. Perry RL, Parker MH, Rudnicki MA. Activated MEK1 binds the nuclear MyoD transcriptional complex to repress transactivation. Mol Cell 2001;8:291–301. doi: 10.1016/s1097-2765(01)00302-1.
68. Meloche S, Pouysségur J. The ERK1/2 mitogen-activated protein kinase pathway as a master regulator of the G1- to S-phase transition. Oncogene 2007;26:3227–3239. doi: 10.1038/sj.onc.1210414.
69. Perdiguero E, Ruiz-Bonilla V, Serrano AL, Muñoz-Cánoves P. Genetic deficiency of p38alpha reveals its critical role in myoblast cell cycle exit: the p38alpha-JNK connection. Cell Cycle 2007;6:1298–1303. doi: 10.4161/cc.6.11.4315.
70. Zhang L, Xia D, Wang C, Gao F, Hu L, Li J, *et al*. Pleiotrophin attenuates the senescence of dental pulp stem cells. Oral Dis 2021. doi: 10.1111/odi.13929.
71. Fan W, Gao XK, Rao XS, Shi YP, Liu XC, Wang FY, *et al*. Hsp70 Interacts with mitogen-activated protein kinase (MAPK)-activated protein kinase 2 to regulate p38MAPK stability and myoblast differentiation during skeletal muscle regeneration. Mol Cell Biol 2018;38:e00211-18. doi: 10.1128/MCB.00211-18.
72. Segalés J, Islam AB, Kumar R, Liu QC, Sousa-Victor P, Dilworth FJ, *et al*. Chromatin-wide and transcriptome profiling integration uncovers p38α MAPK as a global regulator of skeletal muscle differentiation. Skelet Muscle 2016;6:9. doi: 10.1186/s13395-016-0074-x.
73. Palacios D, Mozzetta C, Consalvi S, Caretti G, Saccone V, Proserpio V, *et al*. TNF/p38α/polycomb signaling to Pax7 locus in satellite cells links inflammation to the epigenetic control of muscle regeneration. Cell Stem Cell 2010;7:455–469. doi: 10.1016/j.stem.2010.08.013.
74. Lluís F, Ballestar E, Suelves M, Esteller M, Muñoz-Cánoves P. E47 phosphorylation by p38 MAPK promotes MyoD/E47 association and muscle-specific gene transcription. EMBO J 2005;24:974–984. doi: 10.1038/sj.emboj.7600528.
75. Forcales SV, Albini S, Giordani L, Malecova B, Cignolo L, Chernov A, *et al*. Signal-dependent incorporation of MyoD-BAF60c into Brg1-based SWI/SNF chromatin-remodelling complex. EMBO J 2012;31:301–316. doi: 10.1038/emboj.2011.391.
76. Gillespie MA, Le Grand F, Scimè A, Kuang S, von Maltzahn J, Seale V, *et al*. p38-{gamma}-dependent gene silencing restricts entry into the myogenic differentiation program. J Cell Biol 2009;187:991–1005. doi: 10.1083/jcb.200907037.
77. Liu QC, Zha XH, Faralli H, Yin H, Louis-Jeune C, Perdiguero E, *et al*. Comparative expression profiling identifies differential roles for Myogenin and p38α MAPK signaling in myogenesis. J Mol Cell Biol 2012;4:386–397. doi: 10.1093/jmcb/mjs045.
78. Sin TK, Zhang G, Zhang Z, Zhu JZ, Zuo Y, Frost JA, *et al*. Cancer-induced muscle wasting requires p38α MAPK Activation of p300. Cancer Res 2021;81:885–897. doi: 10.1158/0008-5472.CAN-19-3219.
79. Sunadome K, Yamamoto T, Ebisuya M, Kondoh K, Sehara-Fujisawa A, Nishida E. ERK5 regulates muscle cell fusion through Klf transcription factors. Dev Cell 2011;20:192–205. doi: 10.1016/j.devcel.2010.12.005.
80. Chen TH, Chen CY, Wen HC, Chang CC, Wang HD, Chuu CP, *et al*. YAP promotes myogenic differentiation via the MEK5-ERK5 pathway. FASEB J 2017;31:2963–2972. doi: 10.1096/fj.201601090R.
81. Wang J, Wu H, Peng Y, Zhao Y, Qin Y, Zhang Y, *et al*. Hypoxia adipose stem cell-derived exosomes promote high-quality healing of diabetic wound involves activation of PI3K/Akt pathways. J Nanobiotechnology 2021;19:202. doi: 10.1186/s12951-021-00942-0.

82. Zhang W, Bai X, Zhao B, Li Y, Zhang Y, Li Z, *et al*. Cell-free therapy based on adipose tissue stem cell-derived exosomes promotes wound healing via the PI3K/Akt signaling pathway. Exp Cell Res 2018;370:333–342. doi: 10.1016/j.yexcr.2018.06.035.
83. Pi L, Yang L, Fang BR, Meng XX, Qian L. Exosomal microRNA-125a-3p from human adipose-derived mesenchymal stem cells promotes angiogenesis of wound healing through inhibiting PTEN. Mol Cell Biochem 2022;477:115–127. doi: 10.1007/s11010-021-04251-w.
84. Feng L, Li B, Xi Y, Cai M, Tian Z. Aerobic exercise and resistance exercise alleviate skeletal muscle atrophy through IGF-1/IGF-1R-PI3K/Akt pathway in mice with myocardial infarction. Am J Physiol Cell Physiol 2022;322:C164–C176. doi: 10.1152/ajpcell.00344.2021.
85. Luo W, Lin Z, Chen J, Chen G, Zhang S, Liu M, *et al*. TMEM182 interacts with integrin beta 1 and regulates myoblast differentiation and muscle regeneration. J Cachexia Sarcopenia Muscle 2021;12:1704–1723. doi: 10.1002/jcsm.12767.
86. Dallabrida SM, Ismail N, Oberle JR, Himes BE, Rupnick MA. Angiopoietin-1 promotes cardiac and skeletal myocyte survival through integrins. Circ Res 2005;96:e8–24. doi: 10.1161/01.RES.0000158285.57191.60.
87. Lin YH, Chou LY, Chou HC, Chen CH, Kang L, Cheng TL, *et al*. The Essential Role of Stathmin in Myoblast C2C12 for Vertical Vibration-Induced Myotube Formation. Biomolecules 2021;11:1583. doi: 10.3390/biom11111583.
88. Santi S, Cenni V, Capanni C, Lattanzi G, Mattioli E. PCAF involvement in Lamin A/C-HDAC2 interplay during the early phase of muscle differentiation. Cells 2020;9:1735. doi: 10.3390/cells9071735.
89. Serra C, Palacios D, Mozzetta C, Forcales SV, Morantte I, Ripani M, *et al*. Functional interdependence at the chromatin level between the MKK6/p38 and IGF1/PI3K/AKT pathways during muscle differentiation. Mol Cell 2007;28:200–213. doi: 10.1016/j.molcel.2007.08.021.
90. Tamir Y, Bengal E. Phosphoinositide 3-kinase induces the transcriptional activity of MEF2 proteins during muscle differentiation. J Biol Chem 2000;275:34424–34432. doi: 10.1074/jbc.M005815200.
91. Ling M, Quan L, Lai X, Lang L, Li F, Yang X, *et al*. VEGFB promotes myoblasts proliferation and differentiation through VEGFR1-PI3K/Akt signaling pathway. Int J Mol Sci 2021;22:13352. doi: 10.3390/ijms222413352.
92. Glass DJ. PI3 kinase regulation of skeletal muscle hypertrophy and atrophy. Curr Top Microbiol Immunol 2010;346:267–278. doi: 10.1007/82_2010_78.
93. Liu R, Chen Y, Liu G, Li C, Song Y, Cao Z, *et al*. PI3K/AKT pathway as a key link modulates the multidrug resistance of cancers. Cell Death Dis 2020;11:797. doi: 10.1038/s41419-020-02998-6.
94. Nowosad A, Jeannot P, Callot C, Creff J, Perchey RT, Joffre C, *et al*. p27 controls Ragulator and mTOR activity in amino acid-deprived cells to regulate the autophagy-lysosomal pathway and coordinate cell cycle and cell growth. Nat Cell Biol 2020;22:1076–1090. doi: 10.1038/s41556-020-0554-4.
95. Kalista S, Schakman O, Gilson H, Lause P, Demeulder B, Bertrand L, *et al*. The type 1 insulin-like growth factor receptor (IGF-IR) pathway is mandatory for the follistatin-induced skeletal muscle hypertrophy. Endocrinology 2012;153:241–253. doi: 10.1210/en.2011-1687.
96. Léger B, Cartoni R, Praz M, Lamon S, Dériaz O, Crettenand A, *et al*. Akt signalling through GSK-3beta, mTOR and Foxo1 is involved in human skeletal muscle hypertrophy and atrophy. J Physiol 2006;576:923–933. doi: 10.1113/jphysiol.2006.116715.
97. Egerman MA, Glass DJ. Signaling pathways controlling skeletal muscle mass. Crit Rev Biochem Mol Biol 2014;49:59–68. doi: 10.3109/10409238.2013.857291.
98. Call JA, Wilson RJ, Laker RC, Zhang M, Kundu M, Yan Z. Ulk1-mediated autophagy plays an essential role in mitochondrial remodeling and functional regeneration of skeletal muscle. Am J Physiol Cell Physiol 2017;312:C724–C732. doi: 10.1152/ajpcell.00348.2016.
99. Kim J, Kundu M, Viollet B, Guan KL. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. Nat Cell Biol 2011;13:132–141. doi: 10.1038/ncb2152.
100. Zhao H, Shang Q, Pan Z, Bai Y, Li Z, Zhang H, *et al*. Exosomes from adipose-derived stem cells attenuate adipose inflammation and obesity through polarizing M2 macrophages and beiging in white adipose tissue. Diabetes 2018;67:235–247. doi: 10.2337/db17-0356.
101. Qu Y, Zhang Q, Cai X, Li F, Ma Z, Xu M, *et al*. Exosomes derived from miR-181-5p-modified adipose-derived mesenchymal stem cells prevent liver fibrosis via autophagy activation. J Cell Mol Med 2017;21:2491–2502. doi: 10.1111/jcmm.13170.
102. Sun L, Ma K, Wang H, Xiao F, Gao Y, Zhang W, *et al*. JAK1-STAT1-STAT3, a key pathway promoting proliferation and preventing premature differentiation of myoblasts. J Cell Biol 2007;179:129–138. doi: 10.1083/jcb.200703184.
103. Xiao F, Wang H, Fu X, Li Y, Ma K, Sun L, *et al*. Oncostatin M inhibits myoblast differentiation and regulates muscle regeneration. Cell Res 2011;21:350–364. doi: 10.1038/cr.2010.144.
104. Jang YN, Lee IJ, Park MC, Baik EJ. Role of JAK3 in myogenic differentiation. Cell Signal 2012;24:742–749. doi: 10.1016/j.cellsig.2011.11.009.
105. Jo C, Kim H, Jo I, Choi I, Jung SC, Kim J, *et al*. Leukemia inhibitory factor blocks early differentiation of skeletal muscle cells by activating ERK. Biochim Biophys Acta 2005;1743:187–197. doi: 10.1016/j.bbamcr.2004.11.002.
106. Broholm C, Brandt C, Schultz NS, Nielsen AR, Pedersen BK, Scheele C. Deficient leukemia inhibitory factor signaling in muscle precursor cells from patients with type 2 diabetes. Am J Physiol Endocrinol Metab 2012;303:E283–292. doi: 10.1152/ajpendo.00586.2011.
107. Ho TC, Chiang YP, Chuang CK, Chen SL, Hsieh JW, Lan YW, *et al*. PEDF-derived peptide promotes skeletal muscle regeneration through its mitogenic effect on muscle progenitor cells. Am J Physiol Cell Physiol 2015;309:C159–168. doi: 10.1152/ajpcell.00344.2014.
108. Yang Y, Xu Y, Li W, Wang G, Song Y, Yang G, *et al*. STAT3 induces muscle stem cell differentiation by interaction with myoD. Cytokine 2009;46:137–141. doi: 10.1016/j.cyto.2008.12.015.
109. Steyn PJ, Dzobo K, Smith RI, Myburgh KH. Interleukin-6 induces myogenic differentiation via JAK2-STAT3 signaling in mouse C2C12 myoblast cell line and primary human myoblasts. Int J Mol Sci 2019;20:5273. doi: 10.3390/ijms20215273.
110. Wang K, Wang C, Xiao F, Wang H, Wu Z. JAK2/STAT2/STAT3 are required for myogenic differentiation. J Biol Chem 2008;283:34029–34036. doi: 10.1074/jbc.M803012200.

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