Critical Reviews in Oral Biology & Medicine

Orofacial Muscles: Embryonic Development and Regeneration after Injury

Journal of Dental Research
2020, Vol. 99(2) 125–132

DOI: 10.1177/0022034519883673
journals.sagepub.com/home/jdr

D.H. Rosero Salazar[1], P.L. Carvajal Monroy[1,2], F.A.D.T.G. Wagener[1], and J.W. Von den Hoff[1]

Abstract

Orofacial congenital defects such as cleft lip and/or palate are associated with impaired muscle regeneration and fibrosis after surgery. Also, other orofacial reconstructions or trauma may end up in defective muscle regeneration and fibrosis. The aim of this review is to discuss current knowledge on the development and regeneration of orofacial muscles in comparison to trunk and limb muscles. The orofacial muscles include the tongue muscles and the branchiomeric muscles in the lower face. Their main functions are chewing, swallowing, and speech. All orofacial muscles originate from the mesoderm of the pharyngeal arches under the control of cranial neural crest cells. Research in vertebrate models indicates that the molecular regulation of orofacial muscle development is different from that of trunk and limb muscles. In addition, the regenerative ability of orofacial muscles is lower, and they develop more fibrosis than other skeletal muscles. Therefore, specific approaches need to be developed to stimulate orofacial muscle regeneration. Regeneration may be stimulated by growth factors such fibroblast growth factors and hepatocyte growth factor, while fibrosis may be reduced by targeting the transforming growth factor β1 (TGFβ1)/myofibroblast axis. New approaches that combine these 2 aspects will improve the surgical treatment of orofacial muscle defects.

Keywords: satellite cells, skeletal muscle, growth factors, cleft palate, tissue regeneration, fibrosis

Introduction

Orofacial clefts and orofacial trauma are clinical conditions that involve impaired orofacial muscle regeneration as well as fibrosis. Orofacial clefts are common birth defects that often include cleft palate and/or a cleft in the soft palate in about 46% of the cases (Andersson et al. 2010; Wehby and Cassell 2010). These disorders occur in 1:500 to 1:2,500 births and are surgically treated in the first or second year after birth. Prior to reconstructive surgery, these children show impaired speech development and swallowing. After surgery, speech problems persist in about 30% of the patients, which may lead to social and psychological problems. This is mainly caused by incomplete muscle regeneration and fibrosis (Carvajal Monroy et al. 2012).

The orofacial muscles include the muscles of mastication, the muscles of cheeks and lips, the soft palate muscles, the suprahyoid muscles, and the tongue muscles (Le Reverend et al. 2014). These muscles develop from the mesoderm of the pharyngeal arches and the occipital somites (Sugii et al. 2017; Chang and Kioussi 2018). The muscles of the trunk and limbs develop from the thoracic and lumbal somites, respectively (Chang and Kioussi 2018). The molecular pathways that regulate orofacial muscle development differ from those in trunk and limb muscles (Chang and Kioussi 2018). Embryonic myogenesis in skeletal muscles involves the differentiation of mesodermal cells into myogenic cells. These cells migrate to their specific body region and differentiate into myoblasts, proliferate, and fuse into myofibers (Buckingham 2017). Another population of precursor cells will later become the quiescent satellite cells (SCs) in the adult muscle that provide muscle growth and regeneration after injury, although their origin is still controversial (Ono et al. 2010). Upon injury, regulatory pathways are activated that also function during muscle development (Hernandez-Hernandez et al. 2017).

The functions of human orofacial muscles include chewing, swallowing, and speech. Chewing is carried out by movements of the lower jaw (Le Reverend et al. 2014) while swallowing requires the coordinated action of the tongue, soft palate, and the suprahyoid muscles (Le Reverend et al. 2014; Harandi et al. 2017). Speech is coordinated by contractions of the orofacial muscles and the larynx (Harandi et al. 2017). The functioning of muscle fibers is determined by specific myosin

[1]Department of Orthodontics and Craniofacial Biology, Radboud University Medical Centre, Nijmegen, the Netherlands
[2]Department of Oral and Maxillofacial Surgery, Special Dental Care and Orthodontics, Erasmus Medical Center, Rotterdam, the Netherlands

Corresponding Author:
J.W. Von den Hoff, Department of Orthodontics and Craniofacial Biology, Radboud University Medical Centre, PO Box 9101, Nijmegen, 6500HB, the Netherlands.
Email: hans.vondenhoff@radboudumc.nl

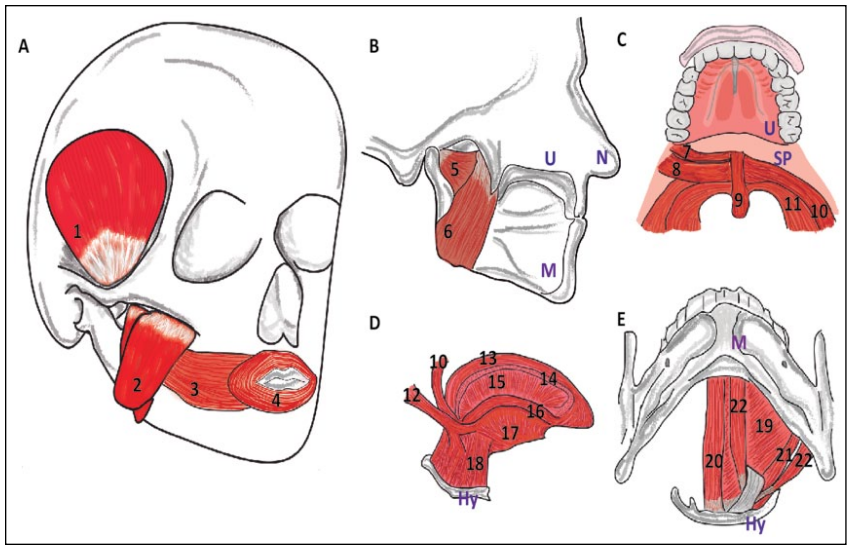

Figure 1. The orofacial muscles. (**A**) Schematic of the skull. 1. Temporalis. 2. Masseter. 3. Buccinator. 4. Orbicularis oris. (**B**) Sagittal view of the head. N, nose; M, mandible; U, upper jaw. 5. Lateral pterygoid. 6. Medial pterygoid. (**C**) Upper jaw and soft palate. SP, soft palate. 7. Tensor veli palatini. 8. Levator veli palatini. 9. Musculus uvulae. 10. Palatoglossus. 11. Palatopharyngeous. (**D**) Muscles of the tongue. Hy, hyoid bone. 12. Styloglossus. (**D**) 13. Superior longitudinalis. 14. Transverse muscle. 15. Vertical muscle. 16. Inferior longitudinalis. 17. Genioglossus. 18. Hyoglossus. (**E**) Suprahyoid muscles. Hy, hyoid bone. 19. Mylohyoid. 20. Geniohyoid. 21. Stylohyoid. 22. Digastricus (anterior and posterior belly).

heavy-chain (MyHC) isoforms (Schiaffino et al. 2015). During development, myofibers express embryonic MyHC, which is gradually replaced by other isoforms (Schiaffino et al. 2015). The exact regulation of expression of specific MyHC isoforms is still unclear.

In addition to the developmental differences, orofacial muscles seem to regenerate less and develop more fibrosis after injury than limb and trunk muscles (Pavlath et al. 1998; Carvajal Monroy et al. 2015; Cheng et al. 2018). Thus, specific approaches are required to improve orofacial muscle regeneration after surgical reconstruction. Here we discuss orofacial muscle development and regeneration to identify specific targets to improve the treatment of orofacial muscle defects.

Orofacial Muscle Development

The orofacial muscles comprise the muscles of the lower face and the oral cavity. Their development takes place in 2 different regions of the embryo head (Chang and Kioussi 2018). The mesoderm of the pharyngeal arches gives rise to the branchiomeric muscles that include the masticatory muscles, the buccinators, the orbicularis oris muscles, the muscles of the soft palate, and the suprahyoid muscles (Fig. 1). The occipital somites give rise to the muscles of the tongue (Fig. 1D) (Michailovici et al. 2015; Chang and Kioussi 2018; Schubert et al. 2018). Studies in chicks, rodents, and zebrafish show that cranial neural crest cells (CNCCs) in the embryo head direct muscle development and differentiate into the intramuscular connective tissue (Tzahor et al. 2003; Grenier et al. 2009; McGurk et al. 2017). In zebrafish and other vertebrates, retinoic acid (RA) maintains the nondifferentiated state of precursor cells during proliferation (El Haddad et al. 2017; McGurk et al. 2017). Subsequently, the degradation of RA allows the precursor cells to form myofibers and tendons (McGurk et al. 2017). The main regulatory factors and pathways are discussed in the next sections.

Molecular Regulation of Branchiomeric Muscle Development

Cranial Neural Crest Cells and Myogenic Commitment. The branchiomeric muscles (Fig. 1) develop from the first, second, and fourth pharyngeal arches (Chang and Kioussi 2018). The third pharyngeal arch gives rise to the stylopharyngeous muscle, which is not considered an orofacial muscle (Frisdal and Trainor 2014). In the developing neural tube, embryonic cells in the margins of the neural folds undergo epithelial-mesenchymal transition (EMT) and form the neural crest (Scarpa and Mayor 2016; Szabo and Mayor 2018). Cells along the neural crest delaminate and migrate toward the different regions in the embryo (Scarpa and Mayor 2016; Szabo and Mayor 2018). Some CNCCs migrate to the pharyngeal arches, where they form the intramuscular connective tissue and regulate orofacial muscle development (Grenier et al. 2009; Scarpa and Mayor 2016). Their migration is stimulated by autocrine growth factors such as stromal-derived growth factor (SDF) and vascular endothelial growth factor (VEGF) (Scarpa and Mayor 2016). SDF and VEGF are also produced during muscle regeneration after injury, which requires the migration of myoblasts (Von den Hoff et al. 2019).

In the pharyngeal arches of zebrafish, the proliferation of CNCCs is regulated by platelet-derived growth factors and Talin 1, which connects the cytoplasmic tails of integrins to the actin cytoskeleton via vinculin (McCarthy et al. 2016; Ishii et al. 2018). After proliferation, the CNCCs induce the concentration of mesodermal cells in the core of the pharyngeal arches (Fig. 2). In mouse embryos, both mesodermal cells and CNCCs express paired-like homeodomain transcription factor 2 (*Pitx2*) (Shih et al. 2007). *Pitx2* knockout mouse embryos show reduced proliferation of both cell types, smaller muscles, and reduced expression of T-box transcription factor 1 (*Tbx1*) in mesodermal cells and CNCCs (Shih et al. 2007; Harel et al. 2012). In vitro, C2C12 cells overexpressing *Pitx2* express increased levels of *Tbx1* (Shih et al. 2007). These data indicate that PITX2 induces TBX1 to regulate the proliferation and concentration of mesodermal cells in the core of the pharyngeal arches surrounded by CNCCs (Grenier et al. 2009; Kong et al. 2014). Interestingly, *Tbx1* knockout mouse embryos lack the core mesodermal cells (Grenier et al. 2009; Kong et al. 2014).

The CNCCs then differentiate into connective tissue precursors, while the mesodermal cells commit to the myogenic lineage (Fig. 2). *Tbx1* knockout mouse embryos show reduced expressions of transcription factors such as scleraxis (SCX) in the CNCCs and capsulin (TCF21), myogenic repressor (MYOR), and myogenic factor 5 (MYF5) in the mesodermal cells (Shih et al. 2007; Grifone et al. 2008; Grenier et al. 2009; Moncaut et al. 2012; Kong et al. 2014). In these embryos, tendon and muscle formation is impaired. *Tcf21* and *MyoR* double-knockout mouse embryos show reduced MYF5 expression in the core mesoderm and impaired muscle formation (Moncaut et al. 2012). Thus, the connective tissue precursor cells express *Scx* while committed myogenic cells express *Myf5*. SCX-positive precursors proliferate and invade the mesodermal core to induce the differentiation of the committed cells into myoblasts. In *Tbx1* knockout mouse embryos, both the expression of *Scx* by precursor cells and the number of myoblasts are reduced (Grifone et al. 2008; Grenier et al. 2009; Kong et al. 2014). This also indicates that TBX1 is crucial for myogenesis in the branchiomeric muscles. To summarize, CNCCs induce the concentration of mesodermal cells in the core of the pharyngeal arches. CNCCs then differentiate into SCX-positive precursors and the mesodermal cells commit to myogenesis. Subsequently, the SCX-positive cells induce the early differentiation of the committed cells into myoblasts. This is a crucial interaction between the neuroectoderm and the mesoderm during orofacial myogenesis.

Myofiber Formation. The final steps in branchiomeric muscle formation are the differentiation of myoblasts into myofibers and the formation of the intramuscular connective tissue and the tendons (Fig. 2). In chick embryos, SCX-positive precursors also express bone morphogenic protein 4 and 7 (*BMP4* and *BMP7*), *WNT1, WNT3a*, noggin, gremlin, and frizzled-related protein 3 (*FRZB*) during proliferation (Tzahor et al. 2003). In vitro, BMP and WNT inhibit the proliferation and differentiation of myoblasts, which can be rescued by the addition of noggin, gremlin, or FRZB (Tzahor et al. 2003). Simultaneously, *Dlx5* is expressed by SCX-positive precursors (Sugii et al. 2017). *Dlx5* knockout mouse embryos in this study show reduced fibroblast growth factor 10 (FGF10) expression and impaired branchiomeric muscle formation. In cultured myogenic cells from these knockouts, myofiber formation is rescued by FGF10 (Sugii et al. 2017). This suggests that FGF10 produced by SCX-positive precursors regulates the differentiation of myofibers by inducing myogenic regulatory factors (MRFs; see Table). In zebrafish, Scx-positive precursor cells express cytochrome P450-26B1 (*cyp26b1*), which degrades RA and thus promotes the differentiation of *Scx*-positive precursors in the pharyngeal arches (McGurk et al. 2017). The precursors differentiate to form the tendons and the intramuscular connective tissue (McGurk et al. 2017). This indicates a neuroectodermal origin of the muscular connective tissue in branchiomeric muscles. This aspect will be discussed in the regeneration section of this review.

During myofiber formation, the MYF5-positive myoblasts express paired homeobox factor 7 (*Pax7*) and myogenic differentiation factor (*MyoD*) (Grifone et al. 2008; Moncaut et al. 2012). *Myf5* and *MyoD* double-knockout embryos lack differentiated branchiomeric muscles (Kablar et al. 2003). Normally, MYOD-positive cells start to express myogenin (*MyoG*), fuse, and form myofibers (Hernandez-Hernandez et al. 2017; Chang and Kioussi 2018) (Fig. 2). Myoblasts also express *Notch* during myofiber formation, while *Notch* knockout embryos show small or absent branchiomeric muscles (Czajkowski et al. 2014). In vitro, PAX7- and MYOD-positive myoblasts overexpressing *Notch* do not form myotubes but remain in the proliferation phase (Mourikis et al. 2012). Within myofibers, satellite cells (SCs) reside between the sarcolemma and the basal lamina in a quiescent state (Mourikis et al. 2012; Czajkowski et al. 2014). In vivo and in vitro studies show that NOTCH maintains the pool of SCs throughout life (Mourikis et al. 2012; Czajkowski et al. 2014). As in trunk and limb muscles, the SCs can regenerate orofacial muscle tissue, which will be discussed later.

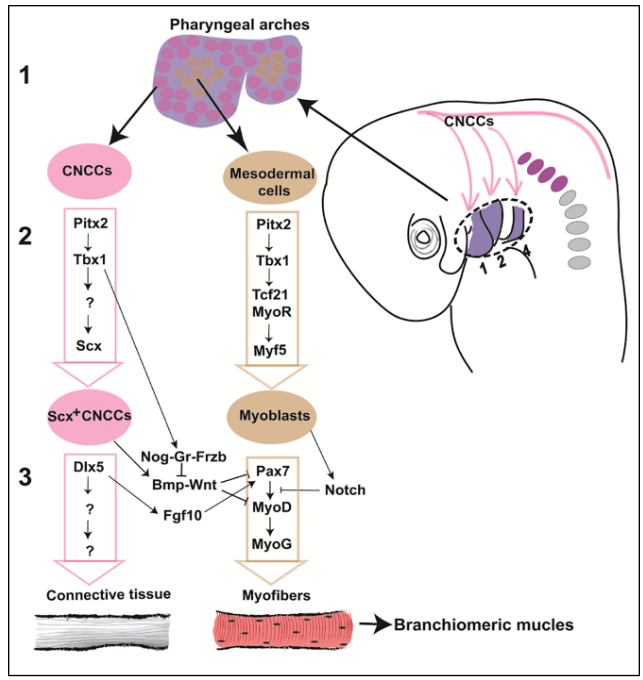

Figure 2. Branchiomeric muscle development. Cranial neural crest cells (CNCCs, pink) migrate toward the first, second, and fourth (1, 2, 4) pharyngeal arches (purple). PITX2 stimulates *Tbx1* expression in CNCCs and mesodermal cells (brown color). The mesodermal cells concentrate in the core of the pharyngeal arches and become surrounded by CNCCs (step 1). Noggin (Nog), Gr (gremlin), and FRZB secreted by CNCCs prevent the downregulation of *Pax7* and *MyoD* by Bmp and Wnt. FGF10 produced by CNCCs expressing *Dlx5* stimulates the expression of *Mrfs*. The CNCCs finally express *Scx* and differentiate into the intramuscular connective tissue and the tendons, while the mesodermal cells differentiate into myofibers (steps 2 and 3). Notch expressed by proliferating myoblasts limits their differentiation into myofibers.

Molecular Regulation of Tongue Muscle Development

Occipital Somites, Cell Migration, and Myogenic Commitment. The development of the tongue muscles occurs in the occipital somites and the mesoderm of the tongue bud (Fig. 3).

Table. Key Factors Involved in Orofacial Muscle Development and the Development of Trunk and Limb Muscles.

	Function in Orofacial Muscles	Function in Trunk and Limb Muscles	References
Transcription factors			
Pitx2	Myoblast proliferation Myogenic differentiation ↑ Tbx1 and MyoR	↑ MyoD and Myf5 Myogenic differentiation	Shih et al. (2007)
Tbx1	Myogenesis first and second arches ↑ Myf-5, MyoD, FGF10	No known function	Tzahor et al. (2003); Grifone et al. (2008); Kong et al. (2014)
Msc (MyoR), Tcf21 (capsulin)	Myogenesis of the first arch ↑ Tbx1, Myf-5, MyoD	No known function	Shih et al. (2007); Kong et al. (2014)
Pax3	↑ Migration and tongue muscle development	↑ Induce myogenic pathways	Bismuth and Relaix (2010); Nassari et al. (2017)
Dlx5	↑ Myogenesis	Not essential for myogenesis	Sugii et al. (2017)
Scx	Tendon development	Neural crest cells induce Scx-positive precursor cells Tendon development	Bismuth and Relaix (2010); Nassari et al. (2017)
Six1, Six4	No known function	↑ Pax3	Bismuth and Relaix (2010); Chang and Kioussi (2018)
Mrfs (Myf5, Pax7, MyoD, MyoG)	↑ Proliferation and differentiation	Idem	
Growth factor signaling[a]			
Noggin, gremlin, Frzb	↓ Wnt, Shh, Bmp ↑ Myogenesis	↓ Myogenesis	Han et al. (2014); Chang and Kioussi (2018)
Shh, Wnt1, Wnt3, Wnt3a, Bmp4, Bmp7, Tgfβ1	↓ Myogenesis in branchiomeric muscles ↑ Tongue myogenesis ↓ Muscle regeneration	↑ Myogenesis ↑ Mrfs Tgfβ1 ↓ muscle regeneration	Han et al. (2014); Chang and Kioussi (2018)
Fgf1, Fgf2, Fgf5, Fgf6	↑ Mrfs in the tongue bud	↓ Myogenesis during development ↑ Muscle regeneration	Chang and Kioussi (2018); Pawlikowski et al. (2017)
Fgf10	Interaction between cranial neural crest cells and mesodermal cells	Somitogenesis Outgrowth of the limb buds	Sugii et al. (2017)
Notch	↓ MyoD (myogenic differentiation) Maintains the pool of satellite cells postnatally	Idem	Czajkowski et al. (2014)

[a]Growth factor signaling includes growth factors, receptors, and inhibitors.

Mesodermal cells commit to myogenesis in the occipital somites, which is induced by CNCCs (Fig. 3–1). These CNCCs have migrated from the neural crest region toward the occipital somites (Noden et al. 1999; Szabo and Mayor 2018). There, the early myogenic differentiation of mesodermal cells occurs. Cultured somites of chick embryo heads express *MRFs* in the mesodermal cells upon addition of sonic hedgehog (SHH), WNT1, or WNT3 (Munsterberg et al. 1995; Noden et al. 1999). In the occipital somites of mouse embryos, *Bmp4, Wnt3a*, and *Shh* are expressed by CNCCs, while *Mrfs* are expressed by the mesodermal cells (Tzahor et al. 2003). The factors that stimulate the expression of these proteins are not known.

After commitment, the myogenic cells from the occipital somites migrate toward the forming tongue and differentiate into myoblasts (Fig. 3–2). Chick embryo studies indicate that PAX3- and PAX7-positive myogenic cells migrate along the hypoglossal nerve (Huang et al. 1999; Buckingham 2017). The tongue bud is growing by the proliferation of mesodermal cells and the invading CNCCs in the midline of the first pharyngeal arch (Han et al. 2014; Du et al. 2016; Szabo and Mayor 2018). In the developing tongue bud of mouse embryos, CNCCs start to express *Scx* and turn into connective tissue precursors (Han et al. 2014). *Scx* expression by CNCC-derived precursors is induced by transforming growth factor β1 (TGFβ1) and WNT1, as shown in studies on double-knockout mouse embryos (Hosokawa et al. 2010). These embryos exhibit microglossia and disorganized myofibers. *Wnt3a, Fgf1, Fgf2, Fgf5*, and *Fgf6* are expressed in the tongue mesoderm of mouse embryos and seem to induce *Myf5, Pax7*, and *MyoD* (Han et al. 2014; Du et al. 2016). However, *Myf5* knockout mouse embryos show reduced levels of FGF6 and an atrophic tongue (Han et al. 2012). In vitro, myogenesis in the tongue bud of these knockouts can be rescued by FGF6 (Han et al. 2012). These data indicate that FGFs induce myogenesis in the forming tongue, while there seems to be a positive feedback by MRFs.

Myofiber Formation. Finally, myofibers and connective tissue are formed in the tongue bud (Fig. 3–3). Organ cultures of mouse embryo tongues show that *Mrf* expression and myofiber formation require BMP- and SMAD-dependent *Tgfb1* expression by SCX-positive precursors (Han et al. 2012). *Myf5-Smad* double-knockout mouse embryos show continued myoblast proliferation, reduced *Myog* expression, and low myofiber formation, leading to an atrophic tongue. In wild-type tongue buds, SCX-positive precursors surround the myogenic cells and induce their differentiation (Han et al. 2012). As in branchiomeric muscles, NOTCH represses myofiber formation and maintains the pool of postnatal SCs (Czajkowski et al. 2014). Also, the connective tissue induces the differentiation of myogenic cells into myoblasts. Later, the association between the

myofibers and the connective tissue allows complex tongue movements in speaking and deglutition. The interaction between the neuroectodermal connective tissue and the pharyngeal mesoderm is also crucial during tongue myogenesis. Similar interactions might influence muscle regeneration after injury. This will be discussed in the next sections of this review.

Genetic Factors in Orofacial Clefts and Muscle Development

Orofacial clefts (OFCs) are classified into syndromic and nonsyndromic cases. The nonsyndromic cases represent about 70% of all OFC cases (Beaty et al. 2016; Adeyemo and Butali 2017). Genes such as *PAX7, GRHL3, FGF, FGFR2, IRF6, BMP4, TGFA, TGFB1*, and *VAX1* are reported as causal genes for OFC (Beaty et al. 2016; Li et al. 2016; Adeyemo and Butali 2017). Of these genes, *PAX7, BMP4, FGF2*, and *FGF5* are also related to muscle development. In vertebrates, *PAX7* is involved in the regulation of CNCC migration, and at later stages, it is also expressed by myogenic cells and myoblasts (Leslie et al. 2015; Buckingham 2017). Postnatally, PAX7 in SCs regulates muscle regeneration after injury (Leslie et al. 2015; Buckingham 2017). In mouse embryos, BMP4 induces the proliferation of myogenic cells during tongue, lip, and soft palate development but not their differentiation (Han et al. 2014; Leslie et al. 2015; Saket et al. 2016). In contrast, FGF2 and FGF5 only stimulate the differentiation of myogenic cells into myoblasts and their fusion into myofibers (Han et al. 2012; Li et al. 2016). Although there is no direct evidence, these genes might be related to impaired muscle development and regeneration in OFC patients.

Differences with Trunk and Limb Muscles during Development

In trunk and limb muscles, the regulation of myogenesis is quite different. Several excellent reviews discuss this in detail, but we will give a short overview here (Bismuth and Relaix 2010; Michailovici et al. 2015; Hernandez-Torres et al. 2017; Nassari et al. 2017; Chang and Kioussi 2018; Schubert et al. 2018). In the thoracic and lumbal somites, the committed myogenic cells migrate under the control of WNT1, WNT3, WNT7a, and sonic hedgehog (SHH) expressed in the notochord and the neural tube (Chang and Kioussi 2018). In contrast to the branchiomeric muscles, FRZB, gremlin, and noggin do not seem to play a role in myogenesis. In trunk and limbs, the mesoderm differentiates into the tendons and the intramuscular connective tissue while the migrated committed cells differentiate into myoblasts (Nassari et al. 2017). In trunk and limb myogenesis, SIX1 and SIX4 induce PAX3, which stimulates the expression of MRFs by myoblasts (Bismuth and Relaix 2010). The MRFs induce the proliferation and differentiation of myoblasts into myofibers. PAX3 also stimulates the expression of PITX2, which induces PAX7 and MYOD to regulate myofiber formation (Bismuth and Relaix 2010; Hernandez-Torres et al. 2017). Thus, orofacial myogenesis differs from that in other skeletal muscles by the molecular interactions between CNCCs and mesodermal cells.

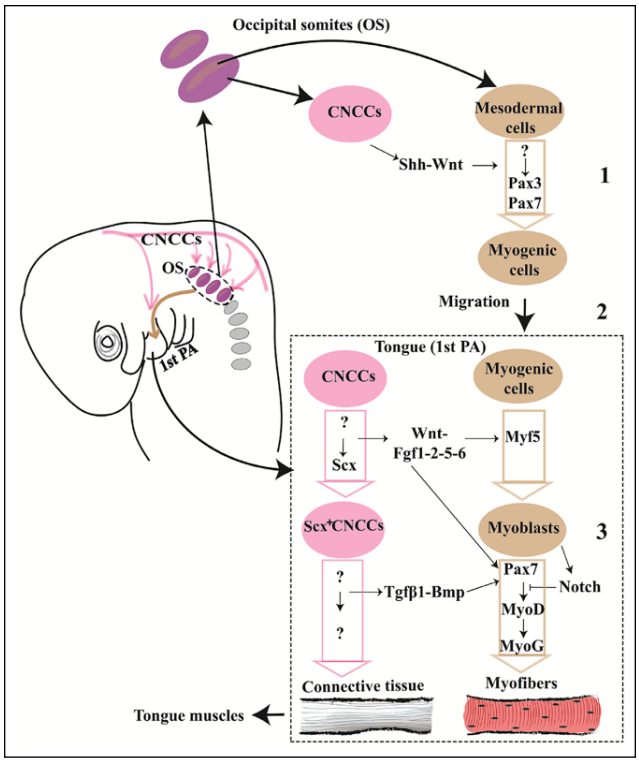

Figure 3. Tongue muscle development. Cranial neural crest cells (CNCCs; pink) and mesodermal cells (brown) regulate tongue myogenesis in the first pharyngeal arch (PA). CNCCs migrate directly from the neural crest to the first pharyngeal arch but also to the occipital somites (OSs). In the OSs, the CNCCs induce the commitment of mesodermal cells to the myogenic lineage by expressing Shh-Wnt. The committed cells migrate (brown arrow) toward the first pharyngeal arch (steps 1 and 2). The CNCCs in the tongue bud start to express *Scx* and will form the intramuscular connective tissue and the tendons. These SCX-positive precursors secrete TGFB1 and BMP to induce the differentiation of the committed cells. The committed cells then differentiate into myoblasts under control of MRFs and form the myofibers (step 3). Notch expressed by proliferating myoblasts controls their differentiation into myofibers. This figure is available in color online.

The Origins of Satellite Cells

In the branchiomeric muscles of chick embryos, SCs are present in the core of the pharyngeal arches together with myogenic cells (Harel et al. 2009). The SCs remain quiescent during myofiber formation. This suggests that branchiomeric SCs and myogenic cells share a common mesodermal origin. In mouse embryos, SCs are also present in the occipital somites (Harel et al. 2009). These SCs migrate to the first pharyngeal arch together with myogenic cells (Harel et al. 2009). In trunk and limb muscles, mesodermal cells in the somites give rise to both the myogenic cells and the SCs (Aziz et al. 2012; Yin et al. 2013). Both cell types migrate toward specific body regions where the myogenic cells differentiate into myofibers (Aziz et al. 2012; Yin et al. 2013). The SCs reside at the outer

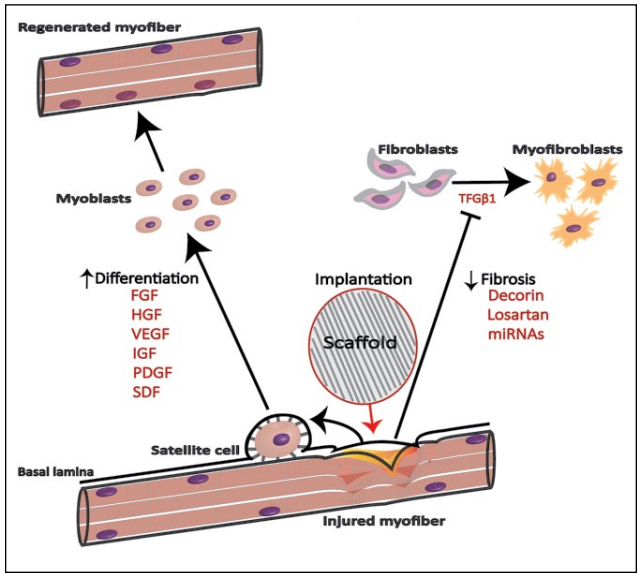

Figure 4. Promoting orofacial muscle regeneration. Enhancement of satellite cell (SC) differentiation is key to improve the regeneration of orofacial muscles. Growth factors that can stimulate SC differentiation are fibroblast growth factor (FGF), hepatocyte growth factor (HGF), insulin-like growth factor (IGF), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and stromal cell–derived factor (SDF). Targeting transforming growth factor β1 (TGFβ1) is the main strategy to reduce fibrosis. Aligned scaffolds (gray lines) are required to deliver growth factors and antifibrotic factors into the wound site and to guide myofiber orientation.

side of the myofibers (Fig. 4). Alternative origins of SCs in the somites and the developing muscles are endothelial cells and neural crest cells (NCCs) (Aziz et al. 2012; Yin et al. 2013). Intriguingly, SCs from orofacial muscles have a mesodermal origin, while SCs from trunk and limb muscles can have a mesodermal or an ectodermal origin (Harel et al. 2009). In contrast, the connective tissue in orofacial muscles originates from the ectoderm, while in trunk and limb muscles, it originates from the mesoderm (Aziz et al. 2012; Yin et al. 2013). This suggests that the difference in behavior of SCs in terms of proliferation and differentiation is related to their origin. Similarly, this might explain the differences in regeneration and fibrosis between these muscle groups. In the next section, the regeneration of orofacial muscles is discussed.

Orofacial Muscle Regeneration

In all skeletal muscles, regeneration starts with inflammation followed by tissue formation and remodeling, which may end up in fibrosis (Grefte et al. 2007). Injury disrupts the connection between the muscle fibers and the adjacent connective tissue, thereby activating the SCs (Thomas et al. 2015). SCs are the stem cells of muscle tissue and mainly responsible for its regenerative capacity. Proliferating SCs express *PAX7* and differentiate into myoblasts that start to express *MYOD* and then fuse to form MYOG-positive myotubes maturing into myofibers. The activity of SCs largely depends on their microenvironment or niche (Grefte et al. 2007; Ten Broek et al. 2010; Hernandez-Hernandez et al. 2017) (Fig. 4). Their activation takes place by growth factors such as FGF6, vascular endothelial growth factor (VEGF), and hepatocyte growth factor (HGF) (Ten Broek et al. 2010). FGF1 and FGF2 secreted by SCs after injury stimulate their own proliferation and the expression of MRFs (Pawlikowski et al. 2017). In addition, both SCs and invading macrophages secrete matrix metalloproteinases (MMP2 and MMP9) to degrade extracellular matrix (ECM) components. Fibroblasts differentiate into myofibroblasts stimulated by TGFβ1 to deposit ECM components, mainly collagen I. TGFβ1 is the main factor inducing fibrosis and reducing SC proliferation and myofiber formation in all adult muscles (Yin et al. 2013; Wynn and Vannella 2016). As discussed earlier, during tongue development, TGFβ1 promotes myofiber formation (Han et al. 2012). Clearly, the role of TGFβ1 is different in muscle development and in the adult muscle.

In mice, freeze injuries of the masseter muscle regenerate less effectively than similar injuries of the tibialis anterior muscle (Pavlath et al. 1998). This was shown by a lower number of regenerated myofibers of smaller diameter. Moreover, much more fibrous tissue was formed. In a model for muscle regeneration in the rat, a circular full-thickness wound was made in the soft palate (Carvajal Monroy et al. 2013; Carvajal Monroy et al. 2015). Large numbers of myofibroblasts were detected in the wound area around 7 d after wounding. Histology also showed a 3-fold increase in collagen content at 56 d, while only few myofibers had regenerated. In vitro, SCs from the masseter muscle of mice show a higher proliferation rate and a delayed differentiation compared with SCs from the extensor digitorum longus muscle (Ono et al. 2010). This was also shown for SCs from other orofacial muscles, including the soft palate muscles in rats (Carvajal Monroy et al. 2015; Carvajal Monroy et al. 2017). Interestingly, when transplanted, mouse masseter-derived SCs regenerated limb muscles as efficiently as those from the EDL itself (Ono et al. 2010). These data indicate that orofacial muscles regenerate less than limb and trunk muscles and develop more fibrosis. Other adult connective tissues derived from the ectoderm such as that in skin and heart also show extensive fibrosis after injury (Parfejevs et al. 2018). It appears that ectoderm-derived fibroblasts respond more strongly to TGFβ1, readily differentiate into myofibroblasts, and produce ECM components at a higher rate. Therefore, the ectodermal origin of the connective tissue in orofacial muscles might explain why these muscles develop more fibrosis and regenerate less than other skeletal muscles.

Promoting Orofacial Muscle Regeneration

Strategies to promote orofacial muscle regeneration should target both the low differentiation ability of the SCs and the strong tendency for fibrosis. Previous work on trunk and limb muscles indicates that SC differentiation is enhanced by growth factors such as FGF1, FGF2, and FGF6 (Thomas et al. 2015; Pawlikowski et al. 2017). Other factors that stimulate muscle regeneration in trunk and limbs are HGF, insulin-like growth

factor (IGF), VEGF, platelet-derived growth factor (PDGF), and stromal cell–derived factor (SDF) (Grefte et al. 2007; Ten Broek et al. 2010; Von den Hoff et al. 2019). These factors are present in the ECM of skeletal muscles and regulate the proliferation and differentiation of SCs (Grefte et al. 2007, Ten Broek et al. 2010). These growth factors might be used to improve the regenerative ability of orofacial SCs. Strategies to promote regeneration in trunk and limb muscles include the inhibition of fibrosis with microRNAs (miRNAs), decorin, and small molecules such as losartan that target the TGFβ1/myofibroblast axis (Garg et al. 2015; Vancheri et al. 2018; Von den Hoff et al. 2019). As discussed earlier, the proliferation and differentiation of ectoderm-derived fibroblasts into myofibroblasts are strongly stimulated by TGFβ1. The implantation of SCs or nonmyogenic stem cells in the site of the injury might not be the best approach since they generally do not survive for long in vivo (Von den Hoff et al. 2019). A more efficient strategy might be the delivery of growth factors and antifibrotic components to the impaired tissue in scaffolds or hydrogels. This might also be more cost-effective for future clinical applications.

Future strategies to promote orofacial muscle regeneration should target the specific aspects of orofacial muscle development and regeneration. Local SCs and other stem cells with myogenic potential may be stimulated by applying growth factors such as FGFs and HGF. Simultaneously, the TGFβ1/myofibroblasts axis should be targeted by using small-molecule inhibitors or miRNAs to prevent fibrosis. This may enhance orofacial muscle regeneration and the treatment of orofacial muscle defects.

Conclusion

Orofacial muscle development is regulated by CNCCs that will eventually form the intramuscular connective tissue and the tendons. Thus, the connective tissue of orofacial muscles is of ectodermal origin. In contrast, the development of trunk and limb muscles is induced by the notochord in the thoracic and lumbar somites. In contrast to the CNCCs, the NCCs in trunk and limb muscle development do not differentiate into the intramuscular connective tissue. Instead, the connective tissue is of mesodermal origin. In general, connective tissue of ectodermal origin has a strong tendency for fibrosis after injury. Similarly, the ectodermal origin of the connective tissue in orofacial muscles might increase fibrosis. In addition, SCs from orofacial muscles have a lower differentiation capacity compared to those from trunk and limb muscles. Thus, the improvement of orofacial muscle regeneration should therefore focus on both the stimulation of SC differentiation and the reduction of fibrosis. Therapeutic strategies to improve the regeneration of orofacial muscles (e.g., after surgery in orofacial clefting) should include antifibrotic agents as well as growth factors that stimulate satellite cell differentiation such as FGFs and HGF. These approaches may greatly improve the outcome of orofacial cleft surgery and reconstructive surgery after tumor resection or trauma in the orofacial region.

Author Contributions

D.H. Rosero Salazar, contributed to conception, drafted the manuscript; P.L. Carvajal Monroy, J.W. Von den Hoff, contributed to conception and design, critically revised the manuscript; F.A.D.T.G. Wagener, contributed to design, critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

Acknowledgments

This study was supported by a grant from the researcher program of Colciencias, Colombia (contract 2017-756). The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

References

Adeyemo WL, Butali A. 2017. Genetics and genomics etiology of nonsyndromic orofacial clefts. Mol Genet Genomic Med. 5(1):3–7.

Andersson EM, Sandvik L, Abyholm F, Semb G. 2010. Clefts of the secondary palate referred to the Oslo cleft team: epidemiology and cleft severity in 994 individuals. Cleft Palate Cran J. 47(4):335–342.

Aziz A, Sebastian S, Dilworth FJ. 2012. The origin and fate of muscle satellite cells. Stem Cell Rev. 8(2):609–622.

Beaty TH, Marazita ML, Leslie EJ. 2016. Genetic factors influencing risk to orofacial clefts: today's challenges and tomorrow's opportunities. F1000Res. 5:2800.

Bismuth K, Relaix F. 2010. Genetic regulation of skeletal muscle development. Exp Cell Res. 316(18):3081–3086.

Buckingham M. 2017. Gene regulatory networks and cell lineages that underlie the formation of skeletal muscle. Proc Natl Acad Sci USA. 114(23):5830–5837.

Carvajal Monroy PL, Grefte S, Kuijpers-Jagtman AM, Helmich MP, Wagener FA, Von den Hoff JW. 2015. Fibrosis impairs the formation of new myofibers in the soft palate after injury. Wound Repair Regen. 23(6):866–873.

Carvajal Monroy PL, Grefte S, Kuijpers-Jagtman AM, Helmich MPAC, Ulrich DJO, Von den Hoff JW, Wagener FADTG. 2013. A rat model for muscle regeneration in the soft palate. PLoS One. 8(3):e59193.

Carvajal Monroy PL, Grefte S, Kuijpers-Jagtman AM, Von den Hoff JW, Wagener FADTG. 2017. Neonatal satellite cells form small myotubes in vitro. J Dent Res. 96(3):331–338.

Carvajal Monroy PL, Grefte S, Kuijpers-Jagtman AM, Wagener FA, Von den Hoff JW. 2012. Strategies to improve regeneration of the soft palate muscles after cleft palate repair. Tissue Eng Part B Rev. 18(6):468–477.

Chang CN, Kioussi C. 2018. Location, location, location: signals in muscle specification. J Dev Biol. 6(2). pii: E11.

Cheng X, Song L, Lan M, Shi B, Li J. 2018. Morphological and molecular comparisons between tibialis anterior muscle and levator veli palatini muscle: a preliminary study on their augmentation potential. Exp Ther Med. 15(1):247–253.

Czajkowski MT, Rassek C, Lenhard DC, Brohl D, Birchmeier C. 2014. Divergent and conserved roles of Dll1 signaling in development of craniofacial and trunk muscle. Dev Biol. 395(2):307–316.

Du W, Prochazka J, Prochazkova M, Klein OD. 2016. Expression of FGFs during early mouse tongue development. Gene Expr Patterns. 20(2):81–87.

El Haddad M, Notarnicola C, Evano B, El Khatib N, Blaquiere M, Bonnieu A, Tajbakhsh S, Hugon G, Vernus B, Mercier J, et al. 2017. Retinoic acid maintains human skeletal muscle progenitor cells in an immature state. Cell Mol Life Sci. 74(10):1923–1936.

Frisdal A, Trainor PA. 2014. Development and evolution of the pharyngeal apparatus. Wiley Interdiscip Rev Dev Biol. 3(6):403–418.

Garg K, Corona BT, Walters TJ. 2015. Therapeutic strategies for preventing skeletal muscle fibrosis after injury. Front Pharmacol. 6:87.

Grefte S, Kuijpers-Jagtman A, Torensma R, Von den Hoff J. 2007. Skeletal muscle development and regeneration. Stem Cells Dev. 16(5):857–868.

Grenier J, Teillet M-A, Grifone R, Kelly RG, Duprez D. 2009. Relationship between neural crest cells and cranial mesoderm during head muscle development. PLoS One. 4(2):e4381.

Grifone R, Jarry T, Dandonneau M, Grenier J, Duprez D, Kelly RG. 2008. Properties of branchiomeric and somite-derived muscle development in tbx1 mutant embryos. Dev Dyn. 237(10):3071–3078.

Han A, Zhao H, Li J, Pelikan R, Chai Y. 2014. Alk5-mediated transforming growth factor beta signaling in neural crest cells controls craniofacial muscle development via tissue-tissue interactions. Mol Cell Biol. 34(16):3120–3131.

Han D, Zhao H, Parada C, Hacia JG, Bringas P, Jr., Chai Y. 2012. A TGFβ-Smad4-Fgf6 signaling cascade controls myogenic differentiation and myoblast fusion during tongue development. Development. 139(9):1640–1650.
Harandi N, Woo J, Stone M, Abugharbieh R, Fels S. 2017. Variability in muscle activation of simple speech motions: a biomechanical modeling approach. J Acoust Soc Am. 141(4):2579.
Harel I, Maezawa Y, Avraham R, Rinon A, Ma HY, Cross JW, Leviatan N, Hegesh J, Roy A, Jacob-Hirsch J, et al. 2012. Pharyngeal mesoderm regulatory network controls cardiac and head muscle morphogenesis. Proc Natl Acad Sci USA. 109(46):18839–18844.
Harel I, Nathan E, Tirosh-Finkel L, Zigdon H, Guimaraes-Camboa N, Evans S, Tzahor E. 2009. Distinct origins and genetic programs of head muscle satellite cells. Dev Cell. 16(6):822–832.
Hernandez-Hernandez JM, Garcia-Gonzalez EG, Brun CE, Rudnicki MA. 2017. The myogenic regulatory factors, determinants of muscle development, cell identity and regeneration. Semin Cell Dev Biol. 72:10–18.
Hernandez-Torres F, Rodriguez-Outeirino L, Franco D, Aranega AE. 2017. Pitx2 in embryonic and adult myogenesis. Front Cell Dev Biol. 5:46.
Hosokawa R, Oka K, Yamaza T, Iwata J, Urata M, Xu X, Bringas P, Jr., Nonaka K, Chai Y. 2010. TGF-beta mediated FGF10 signaling in cranial neural crest cells controls development of myogenic progenitor cells through tissue-tissue interactions during tongue morphogenesis. Dev Biol. 341(1):186–195.
Huang R, Zhi Q, Izpisua-Belmonte JC, Christ B, Patel K. 1999. Origin and development of the avian tongue muscles. Anat Embryol. 200(2):137–152.
Ishii K, Mukherjee K, Okada T, Liao EC. 2018. Genetic requirement of talin1 for proliferation of cranial neural crest cells during palate development. Plast Reconstr Surg Glob Open. 6(3):e1633.
Kablar B, Krastel K, Tajbakhsh S, Rudnicki MA. 2003. Myf5 and MyoD activation define independent myogenic compartments during embryonic development. Dev Biol. 258(2):307–318.
Kong P, Racedo SE, Macchiarulo S, Hu ZJ, Carpenter C, Guo TW, Wang T, Zheng DY, Morrow BE. 2014. Tbx1 is required autonomously for cell survival and fate in the pharyngeal core mesoderm to form the muscles of mastication. Hum Mol Genet. 23(16):4215–4231.
Le Reverend B, Edelson L, Loret C. 2014. Anatomical, functional, physiological and behavioural aspects of the development. Br J Nutr. 111(3):403–414.
Leslie EJ, Taub MA, Liu H, Steinberg KM, Koboldt DC, Zhang Q, Carlson JC, Hetmanski JB, Wang H, Larson DE, et al. 2015. Identification of functional variants for cleft lip with or without cleft palate in or near PAX7, FGFR2, and NOG by targeted sequencing of GWAS loci. Am J Hum Genet. 96(3):397–411.
Li D, Zhang H, Ma L, Han Y, Xu M, Wang Z, Jiang H, Zhang W, Wang L, Pan Y. 2016. Associations between microRNA binding site SNPs in FGFs and FGFRs and the risk of non-syndromic orofacial cleft. Sci Rep. 6:31054.
McCarthy N, Liu JS, Richarte AM, Eskiocak B, Lovely CB, Tallquist MD, Eberhart JK. 2016. Pdgfra and Pdgfrb genetically interact during craniofacial development. Dev Dyn. 245(6):641–652.
McGurk PD, Swartz ME, Chen JW, Galloway JL, Eberhart JK. 2017. In vivo zebrafish morphogenesis shows cyp26b1 promotes tendon condensation and musculoskeletal patterning in the embryonic jaw. PLoS Genet. 13(12):e1007112.
Michailovici I, Eigler T, Tzahor E. 2015. Craniofacial muscle development. Curr Top Dev Biol. 115:3–30.
Moncaut N, Cross J, Siligan C, Keith A, Taylor K, Rigby P, Carvajal J. 2012. Musculin and TCF21 coordinate the maintenance of myogenic regulatory factor. Development. 139(5):958–967.
Mourikis P, Sambasivan R, Castel D, Rocheteau P, Bizzarro V, Tajbakhsh S. 2012. A critical requirement for notch signaling in maintenance of the quiescent skeletal muscle stem cell state. Stem Cells. 30(2):243–252.
Munsterberg AE, Kitajewski J, Bumcrot DA, McMahon AP, Lassar AB. 1995. Combinatorial signaling by sonic hedgehog and Wnt family members induces myogenic bHLH gene expression in the somite. Genes Dev. 9(23):2911–2922.
Nassari S, Duprez D, Fournier-Thibault C. 2017. Non-myogenic contribution to muscle development and homeostasis: the role of connective tissues. Front Cell Dev Biol. 5:22.
Noden D, Marcucio R, Borycki A, Emerson CP J. 1999. Differentiation of avian craniofacial muscles: I. Patterns of early regulatory. Dev Dyn. 216(2):96–112.
Ono Y, Boldrin L, Knopp P, Morgan JE, Zammit PS. 2010. Muscle satellite cells are a functionally heterogeneous population in both somite-derived and branchiomeric muscles. Dev Biol. 337(1):29–41.
Parfejevs V, Antunes AT, Sommer L. 2018. Injury and stress responses of adult neural crest–derived cells. Dev Biol. 444 Suppl 1:S356–S365.
Pavlath GK, Thaloor D, Rando TA, Cheong M, English AW, Zheng B. 1998. Heterogeneity among muscle precursor cells in adult skeletal muscles with differing regenerative capacities. Dev Dyn. 212(4):495–508.
Pawlikowski B, Vogler TO, Gadek K, Olwin BB. 2017. Regulation of skeletal muscle stem cells by fibroblast growth factors. Dev Dyn. 246(5):359–367.
Saket M, Saliminejad K, Kamali K, Moghadam FA, Anvar NE, Khorram Khorshid HR. 2016. Bmp2 and bmp4 variations and risk of non-syndromic cleft lip and palate. Arch Oral Biol. 72:134–137.
Scarpa E, Mayor R. 2016. Collective cell migration in development. J Cell Biol. 212(2):143–155.
Schiaffino S, Rossi AC, Smerdu V, Leinwand LA, Reggiani C. 2015. Developmental myosins: expression patterns and functional significance. Skelet Muscle. 5:22.
Schubert FR, Singh AJ, Afoyalan O, Kioussi C, Dietrich S. 2018. To roll the eyes and snap a bite—function, development and evolution of craniofacial muscles. Semin Cell Dev Biol. 91:31–44.
Shih H, Gross M, Kioussi C. 2007. Cranial muscle defects of pitx2 mutants result from specification defects in the first branchial arch. Proc Natl Acad Sci U S A. 104(14):5907–5912.
Sugii H, Grimaldi A, Li J, Parada C, Vu-Ho T, Feng J, Jing J, Yuan Y, Guo Y, Maeda H, et al. 2017. The dlx5-fgf10 signaling cascade controls cranial neural crest and myoblast interaction during oropharyngeal patterning and development. Development. 144(21):4037–4045.
Szabo A, Mayor R. 2018. Mechanisms of neural crest migration. Annu Rev Genet. 52:43–63.
Ten Broek R, Grefte S, Von den Hoff J. 2010. Regulatory factors and cell populations involved in skeletal muscle regeneration. J Cell Physiol. 224(1):7–16.
Thomas K, Engler AJ, Meyer GA. 2015. Extracellular matrix regulation in the muscle satellite cell niche. Connect Tissue Res. 56(1):1–8.
Tzahor E, Kempf H, Mootoosamy RC, Poon AC, Abzhanov A, Tabin CJ, Dietrich S, Lassar AB. 2003. Antagonists of wnt and bmp signaling promote the formation of vertebrate head muscle. Genes Dev. 17(24):3087–3099.
Vancheri C, Kreuter M, Richeldi L, Ryerson CJ, Valeyre D, Grutters JC, Wiebe S, Stansen W, Quaresma M, Stowasser S, et al. 2018. Nintedanib with add-on pirfenidone in idiopathic pulmonary fibrosis: results of the INJOURNEY trial. Am J Respir Crit Care Med. 197(3):356–363.
Von den Hoff JW, Carvajal Monroy PL, Ongkosuwito EM, van Kuppevelt TH, Daamen WF. 2019. Muscle fibrosis in the soft palate: delivery of cells, growth factors and anti-fibrotics. Adv Drug Deliv Rev. 146:60–76.
Wehby GL, Cassell CH. 2010. The impact of orofacial clefts on quality of life and healthcare use and costs. Oral Dis. 16(1):3–10.
Wynn TA, Vannella KM. 2016. Macrophages in tissue repair, regeneration, and fibrosis. Immunity. 44(3):450–462.
Yin H, Price F, Rudnicki MA. 2013. Satellite cells and the muscle stem cell niche. Physiol Rev. 93(1):23–67.