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ABSTRACT

The tongue exerts crucial functions in our daily life. However, we know very little about the regulatory mechanisms of mammalian tongue development. In this review, we summarize recent findings of the molecular and cellular mechanisms that control tissue-tissue interactions during tongue morphogenesis. Specifically, cranial neural crest cells (CNCC) lead the initiation of tongue bud formation and contribute to the interstitial connective tissue, which ultimately compartmentalizes tongue muscles and serves as their attachments. Occipital somite-derived cells migrate into the tongue primordium and give rise to muscle cells in the tongue. The intimate relationship between CNCC- and mesoderm-derived cells, as well as growth and transcription factors that have been shown to be crucial for tongue myogenesis, clearly indicate that tissue-tissue interactions play an important role in regulating tongue morphogenesis.

KEY WORDS: craniofacial biology/genetics, developmental biology, muscle biology myoblasts, neural crest, tongue.

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Molecular and Cellular Regulatory Mechanisms of Tongue Myogenesis

INTRODUCTION

The tongue is a muscular organ located on the floor of the mouth of most vertebrates. It is composed of a complex array of muscles with a coating of sensors on the dorsal surface for taste, temperature, pain, and tactile information (Gilroy *et al.*, 2008; Moore and Persaud, 2008). The tongue is also necessary to mix, control, and propel the food bolus toward the oropharynx and to sweep around the mouth to clear food debris. In humans, a crucial function of the tongue is phonetic articulation (Löfqvist and Lindblom, 1994). The complex anatomy and physiology of the tongue are the result of complex processes occurring during embryonic development. Here we review the current concepts on tongue morphogenesis, emphasizing the molecular basis of the tissue-tissue interactions leading to the formation of a functional tongue.

TONGUE ANATOMY

The mammalian tongue is constituted by striated muscle, cranial neural crest cell (CNCC)-derived mesenchyme, and a stratified, squamous, non-keratinized epithelium. The anterior two-thirds of the tongue are located in the oral cavity (Fig. 1A) and are mobile, whereas the posterior third, known as the pharyngeal part, is relatively immobile (Fig. 1A). The eight muscles of the tongue are classified as either intrinsic or extrinsic according to their location (Fig. 1B). All the muscles are bilateral, being partially separated by a median septum (Fig. 1B). The tongue receives its blood supply largely from the lingual artery, whereas blood from the tongue drains into the lingual veins (Gilroy *et al.*, 2008). All muscles of the tongue are innervated by the hypoglossal nerve, except for the palatoglossus, whose innervation comes from the vagus nerve (Moore and Persaud, 2008). The sensory innervation for the mucosa of nearly the entire anterior two-thirds of the tongue comes from the trigeminal nerve, which is the nerve of the first branchial arch (BA). Although the facial nerve is the nerve for the second BA, it innervates the taste buds in the anterior two-thirds of the tongue, except for the vallate papillae. The vallate papillae in the anterior part of the tongue are supplied by the glossopharyngeal nerve of the third BA. The posterior third of the tongue is innervated largely by the glossopharyngeal nerve of the third BA. A small area of the tongue anterior to the epiglottis is supplied by the superior laryngeal branch of the vagus nerve of the fourth BA (Moore and Persaud, 2008).

TONGUE DEVELOPMENT

The tongue is derived from all BAs. Tongue development begins with the formation of a medial elevation on the floor of the pharynx anlagen at the end

of the 4th wk in humans and at E10.5 in mice (Fig. 2B). This elevation is called the median lingual swelling of the first BA. Next, lateral lingual swellings form on each side of the median tongue bud (Fig. 2B). These lateral swellings fuse with each other and overgrow the medial lingual swelling, which does not form any identifiable part of the adult tongue. The merged lateral lingual swellings develop into the anterior two-thirds of the tongue (Fig. 1A). The fibrous CNCC-derived lingual septum is the fusion site of those lateral swellings (Fig. 1B). Two other outgrowths, the copula and the hypopharyngeal eminence, develop caudal to the foramen cecum from the third BA and will constitute the posterior third of the tongue (Fig. 1A). As development proceeds, the copula is progressively overgrown by the hypopharyngeal eminence and disappears. Consequently, the posterior third of the tongue forms from the rostral part of the hypopharyngeal eminence. The terminal sulcus is the fusion site of the anterior and posterior parts of the tongue (Moore and Persaud, 2008). The early tongue undergoes rapid enlargement and differentiates into a muscular organ (Fig. 2D) (Huang *et al.*, 1999).

HYBRID CELL ORIGINS OF THE TONGUE

The cell origins of the tongue are a hybrid. Tongue connective tissue and vasculature are derived from CNCC, whereas most of the tongue muscles originate from myoblasts that have migrated from the occipital somites (Noden, 1983; Noden and Francis-West, 2006) (Fig. 2A). The intimate relationship between these cell lineages suggests that reciprocal interactions between CNCC and myogenic cells may occur during tongue development. Chick/quail recombination experiments have previously suggested that CNCC surround the myogenic cell lineage at a very early stage, but do not penetrate the myogenic core (Bogusch, 1986; Noden, 1986; Noden and Francis-West, 2006). Our recent results demonstrate that CNCC populate the tongue primordium before the invasion of myogenic progenitors in mouse embryos (our unpublished observations, Figs. 2A, 2B), suggesting that CNCC are the cell type initiating and directing tongue

Figure 1. Tongue anatomy. (A) Drawing of the adult tongue showing the branchial arch (BA) derivation of the nerve supply of its mucosa. The anterior two-thirds are known as the oral part of the tongue, whereas the posterior third is called the pharyngeal part. (B) Drawing of a coronal section of the adult tongue with intrinsic and extrinsic muscles indicated. The CNCC-derived lingual septum is depicted. Abbreviations: 1st, first BA; 3rd, third BA; 4th, fourth BA.

Figure 2. Early tongue development. (A) Schematic diagram showing the contributions of migrating myogenic progenitors from occipital somites (pink) and CNCC-derived mesenchyme (blue) to the tongue primordium at E10.5. Dark-red line shows the hypoglossal cord. (B-D) Schematic diagrams of coronal sections of mouse heads at E10.5, E11.5, and E12.5, respectively. In (B), the lateral lingual swellings (arrows) are shown. Note that at this stage the tongue primordium is comprised only of CNCC-derived mesenchyme (blue), which is covered by the lingual epithelium (red). In (C), the lateral lingual swellings have fused, and the invasion of the tongue mesenchyme by myogenic progenitors (pink) has begun. (D) At E12.5, myogenic progenitors have organized into the intrinsic and extrinsic muscles seen in the future adult tongue. Abbreviations: H, heart; HC, hypoglossal cord; NT, neural tube; OS, occipital somites; PM, cranial paraxial mesoderm; PS, palatal shelves.

development. From previous studies and our own work, we hypothesize that CNCC-derived mesenchyme has two main functions during tongue development: (1) It acts as a scaffolding structure for the organization of migrating myoblasts into the

Figure 3. Scheme showing the phases of limb, tongue, and craniofacial muscle development and the regulatory factors involved. Limb and tongue muscles share initial mechanisms such as myogenic specification and migration. Factors implicated in these processes appear to be the same, although the final morphology of limb and tongue muscles is dramatically different. Tongue and head muscle development is influenced by CNCC molecules (Blue) and myogenic factors (Pink). Function of factors with question marks is assumed from expression patterns or our unpublished observations.

myogenic core, and (2) it operates as a source of molecular instruction to direct survival, proliferation, and differentiation of myogenic progenitors.

Migratory Myogenic Progenitor Cell Contribution

Because tongue myogenic progenitors originate at the boundary of the trunk and head mesoderm, it is not clear whether these migrating progenitors share more characteristics with trunk or head mesoderm. Tongue muscles originate from the segmented paraxial mesoderm, known as somites (Noden, 1983; Huang *et al.*, 1999). Within the occipital somites 2 to 5, cells of the ventral dermomyotomal lip lose their epithelial morphology and delaminate as single cells, which are called myogenic progenitors. Myogenic progenitors from different somites migrate as a single stream to the tongue primordium, taking a specific pathway, the hypoglossal cord (Fig. 2A) (Huang *et al.*, 1999). CNCC are not required for myogenic progenitor migration toward their presumptive destinations in the BA and tongue primordium (Huang *et al.*, 1999; von Scheven *et al.*, 2006). However, as these

myogenic progenitors first enter the craniofacial region, they immediately establish intimate contact with the CNCC (Figs. 2C, 2D). This close association between the two cell types continues throughout the entire course of tongue morphogenesis (Fig. 2), suggesting that tissue-tissue interactions may play an important role in regulating cell fate determination.

Genes That Regulate the Migration of Myogenic Progenitors

The molecular regulation of myogenic progenitor migration has been well-studied for skeletal muscle development. This involves the activity of at least three different molecules that act as guidance cues: c-met (proto-oncogene that encodes a protein known as Hepatocyte Growth Factor Receptor), Gab1 (encodes the GRB2-associated-binding protein 1), and Lbx1 (encodes the transcription factor Ladybird homeobox 1) (Fig. 3). c-Met is essential for the migration of myogenic progenitors from the somites not only into the limbs, but also into the tongue-forming region (Bladt *et al.*, 1995; Amano *et al.*, 2002). In *c-met* homozygous mutants, both skeletal limb muscles and intrinsic tongue muscles fail to develop. A detailed examination of *Gab1* mutant mice indicates that c-met signals mediated by Gab1 are fundamental not only for delamination, but also for migration and survival of myogenic progenitors (Sachs *et al.*, 2000). *Lbx1* is expressed

exclusively in migrating myogenic progenitors that will form hypaxial muscle. *Lbx1* deficiency severely alters migration of those progenitors that move to the limbs, whereas other populations of migrating muscle progenitors find their targets (Schafer and Braun, 1999; Brohmann *et al.*, 2000; Gross *et al.*, 2000). This indicates that different subpopulations of migrating progenitors respond to distinct guidance cues during migration.

Myogenic Regulatory Factors (MRFs)

A network of four myogenic regulatory factors (MRFs), consisting of myogenic factor 5 (Myf5), muscle-specific regulatory factor 4 (MRF4 or Myf6), myoblast determination protein (MyoD), and myogenin, governs the determination and terminal differentiation of muscle cells, including tongue myoblasts (Berkes and Tapscott, 2005). *Myf5* and *MyoD* are thought to act as determination genes. Expression of *Myf5* or *MyoD* is required for the commitment of multipotential somite cells to the myogenic lineage, because disruption of both genes results in the absence of skeletal myoblasts (Rudnicki *et al.*, 1993). Myogenin is essential for the terminal differentiation of committed myoblasts but is dispensable for

establishing the myogenic lineage. Mice lacking myogenin have very poorly developed skeletal muscle tissue, even though myoblasts are present (Hasty *et al.*, 1993; Nabeshima *et al.*, 1993). *MRF4* seems to have a dual role: It is a differentiation gene, acting in post-mitotic maturing cells; but it is also expressed by undifferentiated proliferating cells, possibly acting as a determination gene (Kassar-Duchossoy *et al.*, 2004). Myogenesis can be partially rescued in *myogenin*^{-/-} embryos by a *myogenin* promoter-driven *MRF4* transgene (Zhu and Miller, 1997), supporting a role for MRF4 in terminal differentiation. In *Myf5*^{-/-}:*MyoD*^{-/-} double-null mice, skeletal muscle is present only when *Mrf4* expression is not compromised. This finding contradicts the widely held view that myogenic identity is conferred exclusively by *Myf5* and *MyoD*, and identifies *Mrf4* as a determination gene acting in the early stages of myogenesis (Kassar-Duchossoy *et al.*, 2004), demonstrating a role for MRF4 in the early stages of myogenesis [for expression patterns of *myogenin*, *MyoD*, and the myosin heavy chain (MHC) in the developing tongue, see Fig. 4].

Pax3 and Pax7

Paired-box genes *Pax3* and *Pax7* are critical for myogenic potential, survival, and expansion of mammalian muscle progenitors (Fig. 3). *Pax3* is required for the formation of hypaxial muscles of the trunk and for the delamination and migration of myogenic progenitor cells to other sites of myogenesis (Tajbakhsh and Buckingham, 2000). *Pax3*-mutant *Splootch* embryos have somite defects, including abnormalities in segmentation (Schubert *et al.*, 2001) as well as loss of the epaxial and hypaxial dermomyotome (Tajbakhsh and Buckingham, 2000). Limb and tongue muscles are defective in *Pax3*-mutant embryos (Bober *et al.*, 1994; Goulding *et al.*, 1994). As in the limb, tongue migrating myogenic progenitors express *Pax3* and are mitotically active (Amthor *et al.*, 1996; Huang *et al.*, 1999). *Pax3* has an essential function in regulating the gene hierarchy that leads to the activation of *MyoD* and the formation of skeletal muscle (Tajbakhsh *et al.*, 1997). Ectopic expression of *Pax3* is sufficient to induce the expression of *MyoD*, *Myf5*, and *myogenin* in the absence of inducing tissues (neural tube and paraxial surface epithelium) in both the paraxial and lateral plate mesoderm of chicken embryos and in the neural tube (Maroto *et al.*, 1997; Mennerich and Braun, 2001). Moreover, transient activation of *Pax3* expression in cultures of primary myoblasts results in enhanced proliferation of these cells (Conboy and Rando, 2002).

Pax7, a paralogue of *Pax3*, is crucial for the specification and survival of satellite cells, which are quiescent mononucleated myogenic cells, located between the sarcolemma and basement

Figure 4. TGF β and FGF signaling pathways in tongue development. **(A)** Schematic diagram of the mouse tongue at E13.5, showing the expression of CNCC-derived mesenchyme markers Scleraxis (green) and FGF10 (blue), and the muscle differentiation marker MHC (pink). Arrows indicate influences from CNCC-derived mesenchyme to myoblasts. **(B)** Model for the mechanism of TGF β signaling pathway regulation of tongue development. TGF β signaling controls the expression of Scleraxis and Fgf10 in the CNCC-derived mesenchyme. In turn, FGF10 is necessary for the induction of proliferation of myogenic progenitors and muscle organization (see also arrows in A), which highlights the relevance of tissue-tissue interactions in tongue morphogenesis. **(C)** Schematic diagram of the mouse tongue at E13.5, showing the expression of two MRFs involved in myogenic differentiation (myogenin and MyoD), which is coincident with the expression pattern of FGFR (pink). **(D)** Model for the activity of FGF in myoblasts of the tongue. Previous work and our unpublished observations suggest that FGF signaling controls differentiation and fusion of myoblasts in a cell-autonomous manner via the regulation of myogenin expression during tongue development. Abbreviations: PS, palatal shelves.

membrane of terminally differentiated muscle fibers (Seale *et al.*, 2000). These are normally quiescent in adult muscle, but act as a reserve population of cells, able to proliferate in response to injury and give rise to regenerated muscle and to more satellite cells (Morgan and Partridge, 2003). Interestingly, *Pax7* germline mutant mice do not show overt muscle defects during embryonic mouse development. Adult *Pax7*^{-/-} mice have few or no myofiber-associated cells resembling satellite cells, and display severely compromised muscle regeneration capability (Oustanina *et al.*, 2004). Furthermore, ectopic expression of *Pax7* leads to enhanced proliferative and survival potential of myoblasts (Seale *et al.*, 2004). Interestingly, overexpression of *Pax7* also seems to down-regulate *MyoD* and promote cell-cycle withdrawal from the proliferating state, therefore playing a critical role in the maintenance of the satellite cell pool (Olguin and Olwin, 2004). Together, these findings support the notion that *Pax3/Pax7* directly or indirectly activate the transcription of MRFs. This function is performed in collaboration with two other transcription factors, Pitx2 and Tbx1 (for a detailed description, see the Appendix).

Cranial Neural Crest Cell Contribution

One of the key characteristics of craniofacial development is the formation of CNCC. Neural crest cells (NCC) are a migratory

cell population that is unique to vertebrate embryos and gives rise to a wide variety of differentiated cell types. The NCC are induced at the dorso-lateral border of the neural folds. Simultaneously with their induction, NCC undergo epithelial to mesenchymal transformation, which results in their delamination and subsequent migration (Sauka-Spengler and Bronner-Fraser, 2006, 2008). Upon the arrival of CNCC to their destination in the ventral region of the embryo, their proliferative activity produces the swellings that demarcate the first and second BAs as well as the frontonasal prominence. Using a two-component genetic model for indelibly marking the progeny of CNCC, *Wnt1-Cre;R26R*, previous studies have shown that tendons and connective tissues in the tongue are derived from CNCC, which are critical for shaping the tongue muscular architecture and, consequently, its function (Hosokawa *et al.*, 2010; for details regarding the *Wnt1-Cre;R26R* model, see Chai *et al.*, 2000).

Dlx Genes

CNCC and mesodermal cells that colonize the first BA, which constitute a great part of the tongue primordium, do not express Hox genes (Couly *et al.*, 1998). Instead, a distal-less homeobox (*Dlx*) code provides CNCC with patterning information and intra-arch polarity along the dorsoventral/proximodistal axis. In the first BA, *Dlx1* and *Dlx2* are expressed in both the maxillary and mandibular processes, whereas *Dlx5* and *Dlx6* are expressed only in the mandibular process, where the anterior two-thirds of the tongue develop (Depew *et al.*, 2005).

Recently, Heude and co-workers demonstrated that craniofacial myogenesis depends on *Dlx5/6* expression by CNCC, because inactivation of *Dlx5* and *Dlx6* results in loss of jaw muscles and compromised tongue development (Heude *et al.*, 2010). Since *Dlx5/6* are not expressed by the myogenic component, this result indicates an instructive role for *Dlx5/6*-positive CNCC in muscle formation. However, the alteration in muscularization is not necessarily a consequence of the loss of mandibular identity, both of which occur in *Dlx5/6*^{-/-} mice, because masticatory muscles are still present in Endothelin Receptor type A-null mice (*EdnRA*^{-/-}) that display a similar jaw transformation. In *Dlx5/6*^{-/-}, the intrinsic muscles of the tongue and sublingual muscles are severely affected: The genioglossus and the geniohyoid are absent, and other intrinsic muscles of the tongue are reduced and disorganized. However, the remaining tongue muscles express determination and differentiation markers. Interestingly, limb and trunk muscles in *Dlx5/6* mutant mice are not affected, indicating a specific function of *Dlx* genes in tissue-tissue interactions involving neural crest derivatives. *Dlx5/6* expression by CNCCs is necessary for interactions between CNCC-derived mesenchyme and mesoderm to occur, which result in myogenic determination, differentiation, and patterning.

The transcription factor *Hand2* also plays an important function in the establishment of proximal-distal patterning of the lower jaw. This occurs through a negative-feedback loop in which *Hand2* represses *Dlx5/6* expression in the distal arch ectomesenchyme following *Dlx5*- and *Dlx6*-mediated induction of *Hand2* expression in the same region. Interestingly, failure to

inhibit distal *Dlx5/6* expression leads to the absence of lateral lingual swelling expansion, from which the tongue arises, resulting in aglossia. Thus, *Hand2* seems to determine a distal mandibular arch domain that is favorable for lower jaw development, including the induction of tongue morphogenesis (Barron *et al.*, 2011). Thus, the importance of *Dlx* genes in tongue development is two-fold: (1) They establish the dorso-ventral pattern of the first BA and, indirectly, that of the tongue; and (2) they regulate myogenic determination and differentiation processes, including those affecting the tongue myogenic core (Fig. 3).

Transforming Growth Factor-beta (TGFβ) and Fibroblast Growth Factor (FGF) Signaling Pathways

TGFβ family members have a critical function in regulating skeletal muscle development through tissue-tissue interactions. In the head, bone morphogenetic proteins (BMPs) act to repress skeletal muscle differentiation. Myogenic differentiation of the cranial paraxial mesoderm is initiated following CNCC production of BMP inhibitors such as Noggin and Gremlin (Tzahor *et al.*, 2003). Specific deletions of TGFβ pathway members in NCC with the *Wnt1-Cre* system lead to severe tongue defects, consistent with the conclusion that CNCC influence tongue morphogenesis and tongue muscle formation and organization. For example, loss of TGFβ receptor 2 (*Tgfr2*) in CNCC results in microglossia, with defects in CNCC-derived connective tissue and tongue muscle development (Hosokawa *et al.*, 2010). Other muscles in the craniofacial region, such as the soft palate muscles and masseter, are also affected in *Tgfr2* mutant mice (our unpublished observations).

Our recent study addressed the molecular and cellular mechanisms underlying the microglossia in *Wnt1-Cre;Tgfr2*^{fl/fl} mice (Hosokawa *et al.*, 2010). Muscle cells are disorganized and present in low density in *Tgfr2* mutant mice, although they are able to express MHC. The reduction in tongue size in *Tgfr2* mutant mice is due to a decrease in proliferation activity of myogenic cells, which is associated with down-regulation of *Fgf10* expression. In contrast, proliferation is unaffected in CNCC cells in *Tgfr2* mutant mice. Interestingly, exogenous FGF10 reverses the reduction of tongue muscle cell numbers in *Tgfr2* mice *in vitro*, which suggests a non-cell autonomous activity, because FGF10 is expressed only by CNCC (Figs. 3, 4A, 4B). A function for FGF10 during tongue development is consistent with reports from previous studies showing that FGF signaling is required for skeletal muscle formation (Flanagan-Steet *et al.*, 2000; de Alvaro *et al.*, 2005). For instance, the viral expression of truncated FGFR1 causes muscle defects in the chick limb (Flanagan-Steet *et al.*, 2000). *Fgf4* and *Fgf8* are expressed in muscle and tendon boundary regions during limb development, suggesting a potential role for the FGF signaling pathway in muscle and tendon interactions (Eloy-Trinquet *et al.*, 2009). In addition, FGFs also function in a cell-autonomous manner in the myogenic cells of limb muscles (Floss *et al.*, 1997) and the tongue (our unpublished observations, Figs. 3, 4C, 4D). In wild-type mice, FGF6 is up-regulated after skeletal muscle injury, implying that FGF6 functions in muscle regeneration. Moreover, *Fgf6*^{-/-} mice have a severe regeneration defect with fibrosis and myotube degeneration. The number of

MyoD- and Myogenin-expressing activated satellite cells after injury is significantly reduced in these mice. This reduction is caused not by a reduced pool of quiescent satellite cells, but presumably by a lack of activation or proliferation. Thus, FGF6 appears to be a critical component of the muscle regeneration machinery in mammals, possibly by stimulating or activating satellite cells (Floss *et al.*, 1997).

Tendon-Muscle Interaction and the Influence of the TGF β Pathway

The initial relationship between the CNCC and myogenic mesoderm is maintained throughout myogenesis and helps determine the attachment of head muscles during craniofacial development. Both muscle and tendon cells appear to be involved in the formation of the attachment between tendons and skeletal muscle. For example, the loss of *Stripe*, which is involved in early steps of tendon differentiation, results in the disruption of the entire somatic muscle pattern (Frommer *et al.*, 1996). A defect in tendon formation also results in compromised muscle formation in *Drosophila* and zebrafish (Frommer *et al.*, 1996; Kudo *et al.*, 2004). The loss of Periostin, an adhesion molecule, in the myoseptum causes a differentiation defect in myoblasts (Kudo *et al.*, 2004). These results show again that the reciprocal interaction between myogenic and surrounding cells, including tendon cells, is crucial for skeletal muscle development.

Despite distinct embryological origins, interactions between muscles and tendons during craniofacial development are similar to those observed in the limb and trunk. In the branchiomeric and extra-ocular regions, muscles are not necessary for the initiation of tendon formation but are required for further tendon development (Grenier *et al.*, 2009). A molecular framework for tendon formation is also beginning to emerge. The condensation and differentiation of tendon progenitors are dependent on the activity of *Scleraxis* (*Scx*) (Murchison *et al.*, 2007). All the forming tendons associated with the tongue are of neural crest origin and express the *Scx* marker in chick and mouse embryos (Grenier *et al.*, 2009; Hosokawa *et al.*, 2010). Tendon formation is diminished in *Scx* knockout mice (Murchison *et al.*, 2007). *Scx* is a transcription factor involved in controlling collagen expression. Pro- α 1(I) collagen possesses TSE1 (tendon-specific elements) and TSE2 sites in its promoter region, where SCX is able to bind (Léjard *et al.*, 2007). Studies of TSE1 and TSE2 LacZ transgenic mice indicate that these regions are active in the fibroblast cells giving rise to tendon cells, including those of craniofacial muscles such as the masseter (Terraz *et al.*, 2002).

FGF and TGF β are also involved in the induction of tendon formation (Tozer and Duprez, 2005; Pryce *et al.*, 2009). TGF β signaling is essential for the maintenance of the early tendon progenitors. Disruption of TGF β signaling in *Tgfb2*^{-/-};*Tgfb3*^{-/-} double-mutant embryos or through inactivation of the type II TGF β receptor results in the loss of most tendons and ligaments in the limbs, trunk, tail, and head (Pryce *et al.*, 2009). In *Wnt1-Cre*;*Tgfr2*^{fl/fl} mice, *Scx* expression is diminished in the tendon of the tongue muscle (Hosokawa *et al.*, 2010). Bead implantation experiments indicate that TGF β signaling induces *Scx* expression in CNCC during tongue development. Type I collagen expression is also compromised following the loss of TGF β signal-

ing. Thus, TGF β signaling is required for the cell-autonomous induction of *Scx* and *type I collagen* expression and regulation of the fate of CNCC during the development of tongue muscles as well as other craniofacial skeletal muscles.

Sonic Hedgehog (Shh) and Wnt

Hh signaling in NCC is also crucial for tongue development. For instance, *Wnt1-Cre*;*Smon/c* embryos lack a tongue. Jeong and co-workers (2004) have shown that a tongue defect in these mutants is first detectable at E10.75. At this stage, occipital somite-derived myogenic progenitors accumulate around the midline of the mandibular arch, where the tongue will form. These myogenic progenitors are not detectable in *Wnt1-Cre*;*Smon/c* embryos. Interestingly, the position of the tongue appears to have been specified in the epithelium, as judged by the expression of *Shh*, a marker for tongue epithelium. Thus, Hh signaling in the CNCC-derived mesenchyme might be involved in transmitting information from the epithelium to the myogenic progenitors to coordinate tongue formation (Jeong *et al.*, 2004).

Similarly, conditional inactivation of β -catenin in the lingual epithelium causes severe craniofacial malformations, including retardation of tongue growth. The two lateral swellings of the primitive tongue are smaller and remain separated. In addition, the merging between the tuberculum impar and two lingual swellings is defective. At E14.5, the tongue is much smaller, deformed, and completely lacks taste papillae. The number of CNCC-derived mesenchymal cells is severely reduced, which appears to be the cause of microglossia. Interestingly, the inactivation of β -catenin is associated with down-regulation of *Shh* expression in the tongue epithelium and reduction of *Ptch1* and *Gli1* expression in the underlying mesenchyme. *Shh* conditional inactivation right at the stage of tongue initiation results in underdevelopment of the tongue, confirming that lack of *Shh* signaling is sufficient to cause defective tongue formation *in vivo* (Lin *et al.*, 2011).

DISEASE AND THERAPEUTIC PERSPECTIVES

The prevalence of tongue cancer is fairly high. Tongue squamous cell carcinoma is one of the most prevalent malignant cancers of the head and neck region. Surgical management of tongue cancer remains the mainstay of treatment. After excision of the lesions, reconstruction is required to maintain function. However, almost half of the patients have difficulties with eating and drinking (Prince and Bailey, 1999). The ideal regeneration would restore the normal tongue volume and shape, have a supple, non-hairy surface, be highly innervated, and move precisely (Bokhari and Wang, 2007). A comprehensive understanding of the regulatory mechanisms that control tongue development and the functional significance of tissue-tissue interaction during tongue morphogenesis will provide the opportunity to strive toward an ideal regeneration approach. This will likely be based on stem cell and tissue-engineering technologies, using isolated CNCC-derived stem cells and skeletal muscle satellite cells, under the precise control of the proper molecular regulatory mechanisms, and will ultimately generate a functional tongue.

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