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Expression of forkhead box transcription factor genes *Foxp1* and *Foxp2* during jaw development

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ABSTRACT

Development of the face is regulated by a large number of genes that are expressed in temporally and spatially specific patterns. While significant progress has been made on characterizing the genes that operate in the oral region of the face, those regulating development of the aboral (lateral) region remain largely unknown. Recently, we discovered that transcription factors LIM homeobox (LHX) 6 and LHX8, which are key regulators of oral development, repressed the expression of the genes encoding forkhead box transcription factors, *Foxp1* and *Foxp2*, in the oral region. To gain insights into the potential role of the *Foxp* genes in region-specific development of the face, we examined their expression patterns in the first pharyngeal arch (primordium for the jaw) of mouse embryos at a high spatial and temporal resolution.

Foxp1 and *Foxp2* were preferentially expressed in the aboral and posterior parts of the first pharyngeal arch, including the developing temporomandibular joint. Through double immunofluorescence and double fluorescent RNA in situ hybridization, we found that *Foxp1* was expressed in the progenitor cells for the muscle, bone, and connective tissue. *Foxp2* was expressed in subsets of bone and connective tissue progenitors but not in the myoblasts. Neither gene was expressed in the dental mesenchyme nor in the oral half of the palatal shelf undergoing extensive growth and morphogenesis. Together, we demonstrated for the first time that *Foxp1* and *Foxp2* are expressed during craniofacial development. Our data suggest that the *Foxp* genes may regulate development of the aboral and posterior regions of the jaw.

1. Introduction

The forkhead box (FOX) proteins are an ancient family of transcription factors, characterized by the conserved DNA binding domain called a forkhead domain (also known as a winged helix domain). They play important roles in a wide variety of biological processes, such as development, carcinogenesis, metabolism, and immunity (Hannenhalli and Kaestner, 2009; Jackson et al., 2010; Lam et al., 2013). There are 50 members of FOX family in humans and 44 members in mice. The FOX proteins are further divided into subgroups, FOXA through FOXS, with 1–6 members in each subgroup (Jackson et al., 2010).

The FOXP subgroup is encoded by four genes (*Foxp1-Foxp4*), and they contain a leucine zipper motif and a zinc finger domain in addition to the forkhead domain (Takahashi et al., 2009). Among the four FOXP proteins, FOXP1 and FOXP2 show a particularly high homology to each other throughout their amino acid sequences (Takahashi et al., 2009). *Foxp1* and *Foxp2* are expressed in diverse organs during embryonic development, often in partially overlapping but distinct patterns (Shu et al., 2001, 2007; Wang et al., 2004; Shu et al., 2007; Takahashi et al., 2009; Leishman et al., 2013; Zhao et al., 2015). Analyses of mouse mutants have shown that *Foxp1* plays critical roles in development of the spinal motor neurons, lymphocytes, and the cardiomyocytes (Wang et al., 2004; Hu et al., 2006; Dasen et al., 2008; Zhang et al., 2010). In addition, *Foxp1* and *Foxp2* co-regulate the development of the foregut and the skeleton (Shu et al., 2007; Zhao et al., 2015).

In humans, mutations affecting *FOXP1* and *FOXP2* have been found in patients with language and speech defects, often accompanied by additional intellectual disability and/or autism spectrum disorder (Lai et al., 2001; Feuk et al., 2006; Zeesman et al., 2006; Takahashi et al., 2009; Horn et al., 2010; Fevre et al., 2013, Lozano

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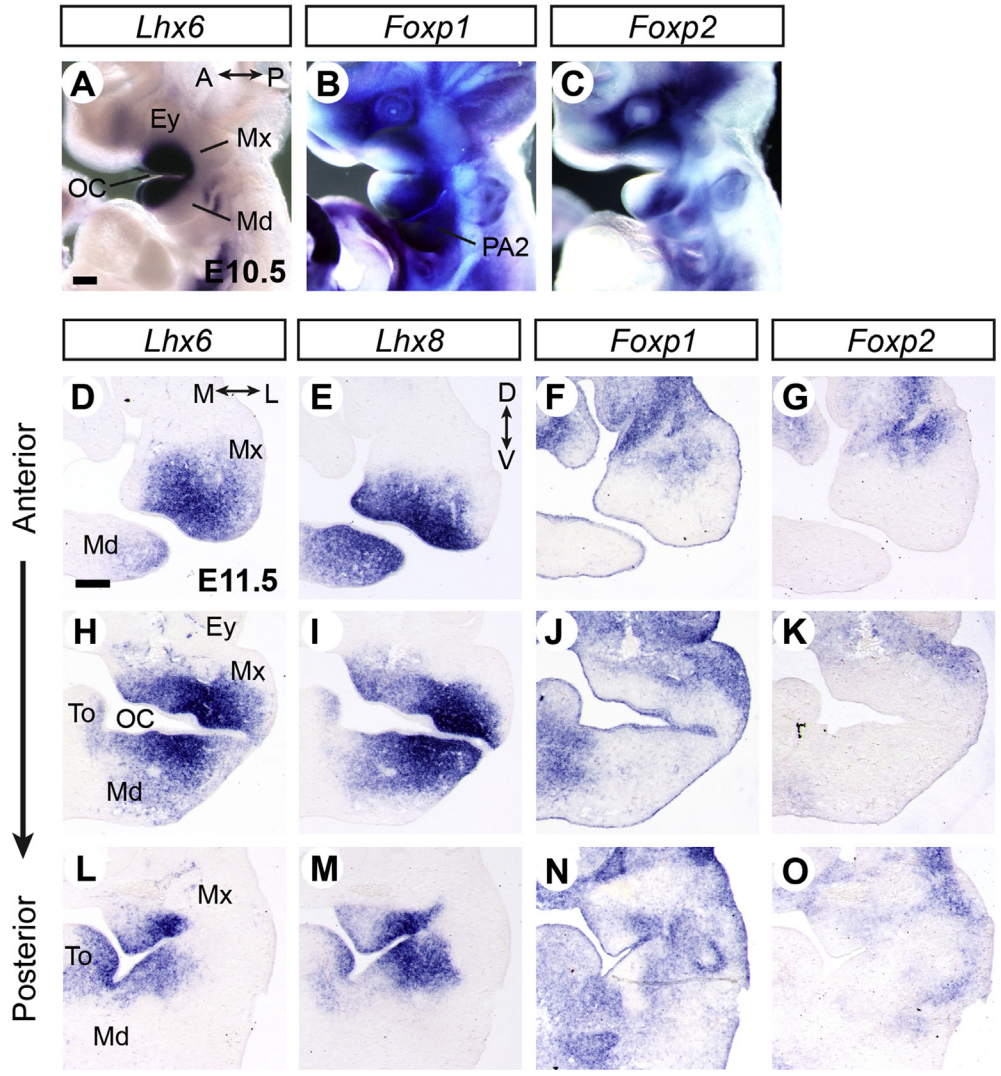

Fig. 1. Complementary patterns of expression between *Lhx* and *Foxp* genes in PA1. (A–C) Lateral views of the head of E10.5 embryos processed by whole mount RNA in situ hybridization. (D–O) Coronal sections of the head of an E11.5 mouse embryo processed by RNA in situ hybridization. Only the right half of the face is shown. The anterior–posterior axis, medial–lateral axis, and the dorsal–ventral axis are indicated in panels (A), (D), and (E), respectively. Ey, eye; Md, mandibular arch; Mx, maxillary arch; OC, oral cavity; PA2, second pharyngeal arch; To, tongue. Bar, 0.2 mm.

et al., 2015). Many of the patients also exhibited generalized functional deficiencies of the face and neck muscles (difficulties in chewing, swallowing, coughing, laughing), and characteristic facial features (triangular face, prominent forehead, short and broad nose, low set ears, downward slanting eyes, high-arched palate, wide-spaced teeth) (Feuk et al., 2006; Zeesman et al., 2006; Horn et al., 2010; Fevre et al., 2013, Lozano et al., 2015). The language and mental defects clearly implicate problems in brain development, and the musculoskeletal phenotypes of the face may be secondary to the disruption in the motor control from the brain for the muscles. Alternatively, the latter phenotypes can also be explained by abnormal development of the face itself. To date, potential roles of *Foxp1* and *Foxp2* in orofacial development have not been examined.

In vertebrates, the face develops from the embryonic primordia called frontonasal prominence and the first pharyngeal arches (PA1) (Minoux and Rijli, 2010; Frisdal and Trainor, 2014; Cesario et al., 2015a). PA1 gives rise to the jaw including the palate and the teeth, and it is further divided into the mandibular arch, which is the prospective lower jaw, and the maxillary arch, which is the prospective upper jaw. The mesenchyme of the developing face is of dual origin: the mesoderm-derived cells occupy the core of PA1, and they become skeletal muscles (Noden, 1983; Noden and Trainor, 2005). On the other hand, the cells that have migrated from the neural crest (called ectomesenchyme cells) give rise to all the bone and cartilage of the face, as well as connective tissues such as tendons and dermis (Noden, 1978; Le Douarin and Kalcheim, 1999).

Recently, we discovered that *Lhx6* and *Lhx8* repressed *Foxp1* and *Foxp2* in PA1, where the *Lhx* (LIM homeobox) genes are crucial to development of the palate and the molars on the oral side (Cesario et al., 2015b). To gain insights into the biological significance of the repression of *Foxp* by LHX, we examined the expression patterns of *Foxp1* and *Foxp2* during normal development of PA1 in mouse embryos. Our results suggest that the *Foxp* genes may be involved in development of the muscle, bone, and connective tissue in the face.

2. Results

At embryonic day (E) 10.5, *Lhx6* and *Lhx8* are expressed in the areas of PA1 facing the oral cavity, with the broad expression