Expression of Sox genes in tooth development

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ABSTRACT Members of the Sox gene family play roles in many biological processes including organogenesis. We carried out comparative in situ hybridization analysis of seventeen Sox genes (Sox1-14, 17, 18, 21) during murine odontogenesis from the epithelial thickening to the cytodifferentiation stages. Localized expression of five Sox genes (Sox6, 9, 13, 14 and 21) was observed in tooth bud epithelium. Sox13 showed restricted expression in the primary enamel knots. At the early bell stage, three Sox genes (Sox8, 11, 17 and 21) were expressed in pre-ameloblasts, whereas two others (Sox5 and 18) showed expression in odontoblasts. Sox genes thus showed a dynamic spatio-temporal expression during tooth development.

KEY WORDS: Sox, tooth development, in situ hybridization

Teeth develop from sequential and reciprocal interactions between epithelium and neural crest-derived mesenchyme. The first morphological sign of tooth development is an epithelial thickening on the first branchial arch. The thickened epithelium then progressively takes the form of the bud, cap and bell configurations. Primary enamel knots appear as thickened inner enamel epithelium at the early cap stage, but disappear by the late cap stage. Subsequently, epithelial cells differentiate into enamel-producing ameloblasts and dentin-producing odontoblasts differentiate from mesenchymal cells (dental papilla). It is known that many signaling pathways such as Bmp, Fgf, Wnt, and Shh play critical roles in regulating tooth development (Tucker and Sharpe, 2004).

Sox proteins are characterized by a highly conserved DNA binding motif, HMG (high mobility group) domain, and twenty Sox genes have been identified in mice. Members of the Sox gene family show dynamic and diverse expression patterns during development and mutation analyses in humans and mice provide evidence that they play multiple roles during development (Pevny and Lovell-Badge 1997, Hosking and Koopman 2008, Wegner 1999, Oommen et al., 2012). Sox2 has been shown to be expressed in rodent tooth germs including the incisor cervical loops (Ohazama et al., 2010; Juuri et al., 2012, 2013; Zhang et al., 2012). The expression of other members of Sox family in tooth development however remains unstudied.

We carried out comparative in situ hybridization analysis of sixteen Sox genes (Sox1-14, 17, 18, 21) during murine odontogenesis, and reveal dynamic spatio-temporal expression of Sox 2, 4, 5, 6, 8, 9, 11, 12, 13, 14, 17, 18 and 21 in molar tooth development.

Results

Sox genes are classified into nine subgroups according to homology within the HMG domain and other structural motifs, as well as functional assays (Pevny and Lovell-Badge 1997, Wegner 1999).

Group B

Sox1, Sox2 and Sox3 belong to the B1 group of Sox family. Sox2 expression has been shown in tooth development (Ohazama et al., 2010; Juuri et al., 2012, 2013; Zhang et al., 2012). Sox2 is expressed in tooth epithelium at the initiation stage (E10.5 and E11.5; Fig. 1 F,G). At the bud stage (E13.5) and the cap stage

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(E14.5), Sox2 showed restricted expression in lingual bud epithelium (Fig. 1 H,I). Significant expression of Sox2 is not found in tooth germs at E18.5 (Fig. 1J). Although Sox1 and Sox3 belong to same group (B1) as Sox2, neither Sox1 nor Sox3 expression could be detected in tooth germs from E10.5 to E18.5 (Fig. 1 A-E, 1 K-O). Sox14 and Sox21 belong to the B2 group of Sox family. At the initiation stage, weak expression of Sox14 was observed in presumptive tooth epithelium, whereas Sox21 showed no expression (Fig. 1 P,Q,U,V). At the bud stage (E13.5), Sox21 was weakly expressed in the collar of tooth bud epithelium, although no expression of Sox14 was observed in tooth germs (Fig. 1R,W). At the cap stage (E14.5), neither Sox14 nor Sox21 expression could be detected in tooth germs (Fig. 1S,X). At the cytodifferentiation stages (E18.5), weak Sox21 expression was observed in pre-ameloblasts localized at the presumptive cusp region, and Sox14 showed no expression in tooth germs (Fig. 1T,Y).

**Group C**

Sox4, Sox11 and Sox12 belong to the C group of Sox family. Sox4 and Sox11 were expressed in presumptive tooth epithelium and mesenchyme at both E10.5 and E11.5, whereas Sox12 showed no expression (Fig. 2 A,B,F,G,K,L). At E13.5, Sox4 was strongly expressed in tooth mesenchyme and the centre of bud epithelium, and Sox11 expression was observed in basal epithelium of tooth bud epithelium (Fig. 2C,H). Punctate expression of Sox12 was observed in both tooth epithelium and mesenchyme (Fig. 2M). At the cap stage, Sox4 was expressed in inner enamel epithelium, stellate reticulum, dental papillae and mesenchyme facing buccal outer enamel epithelium, whereas outer tooth enamel epithelium showed weak expression (Fig. 2D). Sox11 was expressed in the cervical loop of molar tooth epithelium and outer enamel epithelium, whereas Sox12 expression could not be detected in tooth germs at this stage (Fig. 2I,N). At cytodifferentiation stages, Sox11

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**Fig. 1.** The expression of Sox genes (Group B) in rodent tooth development. In situ hybridisation of Sox1, Sox2, Sox3, Sox14 and Sox21 on frontal head sections at E10.5, E11.5, E13.5, E14.5 and E18.5. Tooth epithelium is outlined in red. Arrowheads indicate the presumptive tooth region.
expression was observed in pre-ameloblasts, whereas neither Sox4 nor Sox12 show expression in tooth germs (Fig. 2 E,J,O).

**Group D**

Sox5, Sox6 and Sox13 belong to the group D Sox genes. At E10.5, Sox5 showed restricted expression in tooth mesenchyme, whereas Sox6 and Sox13 expression were observed in both presumptive tooth epithelium and mesenchyme (Fig. 3 A,F,K). At E11.5, expression of Sox6 was observed in tooth epithelium, whereas Sox5 showed weak expression in mesenchyme (Fig. 3 B,G). Faint expression of Sox13 was observed in both tooth epithelium and mesenchyme at this stage (Fig. 3L). At the bud stage, Sox6 and Sox13 showed restricted expression in lingual bud epithelium and at the tip of bud epithelium, respectively (Fig. 3 H,M). No expression of Sox5 could be detected in tooth germs (Fig. 3C). At the cap stage, Sox5 was weakly expressed in dental papillae, whereas Sox13 expression was observed in the primary enamel knot (Fig. 3 D,N). Sox6 showed restricted expression in lingual outer enamel epithelium (Fig. 3I). At cytodifferentiation stages, Sox5 showed weak expression in dental papillae and odontoblasts, whereas neither Sox6 nor Sox13 were expressed in tooth germs (Fig. 3 E,J,O).

![Fig. 2. The expression of Sox genes (Group C) in rodent tooth development.](image)
**Group E**

Sox8, Sox9 and Sox10 belong to the group E Sox genes. Sox10 showed no expression in tooth germs from E10.5 to E18.5 (Fig. 4 K-O). Sox9 showed expression in both tooth epithelium and mesenchyme at E10.5, which became weak at E11.5 (Fig. 4 F,G, Mitsiadis et al., 1998). No expression of Sox8 could be detected in tooth germs at E10.5 or E11.5 (Fig. 4 A,B). At bud stage, Sox9 was weakly expressed in tooth epithelium, whereas no Sox8 expression was observed in tooth germs (Fig. 4 C,H). At the cap stage, weak expression of Sox8 was observed in inner enamel epithelium and dental papillae, whereas Sox9 showed expression in outer enamel epithelium and collar of tooth epithelium (Fig. 4 D,I). At E18.5, weak expression of Sox8 was observed in pre-ameloblasts, whereas Sox9 was expressed in rostral developing pulp and caudal stellate reticulum (Fig. 4 E,J).

**Group F**

Sox7, Sox17 and Sox18 belong to the group F Sox genes. A punctate expression pattern of Sox7 and Sox18 were seen throughout the mesenchyme at E10.5-E14.5 (Fig. 5 A-D, 5 K-N).
Sox17 showed similar expression, but weaker than those of Sox7 and Sox18 at these stages (Fig. 5 F-I). At E18.5, Sox17 was expressed in pre-ameloblasts, whereas Sox18 showed restricted expression in mesenchyme underneath presumptive cusp and facing cervical loops (Fig. 5 J,O). Sox7 showed no expression in tooth germs at E18.5 (Fig. 5E).

**Transgenic mice**

It has been shown that epithelial conditional Sox2 mutation using ShhCre led to no significant changes of molars (Juuri et al., 2013). In common with previous reports, significant anomalies could not be detected in molars in Sox2 mutants using K14Cre mice (Fig. 6B). To further analyze the role of Sox2 in tooth development, we examine mice overexpressing under the keratin 5 promoter (Krt5-Cre;Rosa26Sox2/+) (Fig. 6C). Our data from in situ hybridization analysis shows Sox6 showed similar an expression pattern to Sox2 in tooth development (Fig. 1F-J). In order to investigate the role of Sox6 in tooth development, we examined Sox6 mutant mice (p100H)

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**Fig. 4. The expression of Sox genes (Group E) in rodent tooth development.** In situ hybridisation of Sox8, Sox9 and Sox10 on frontal head sections at E10.5, E11.5, E13.5, E14.5 and E18.5. Tooth epithelium is outlined in red. Arrowheads indicate the presumptive tooth region.
homozygotes). Significant differences however could not be detected in mutant molars (Fig. 6D).

**Discussion**

Members of the Sox gene family show dynamic and diverse expression patterns during development of many organs, and analysis of mutations in mice suggest that member of Sox gene family play multiple roles during development (Pevny and Lovell-Badge 1997). Our results also show dynamic spatio-temporal expression of Sox genes in developing tooth germs.

It has been shown that Sox2 plays a critical role in regulating molar dental lamina growth (Juuri et al., 2013). Sox2 is also expressed in the lingual bud and cap epithelium, although Sox2 mutant molars show no significant morphological changes (Juuri et al., 2013). We found that Sox6 have a similar expression pattern to Sox2 in tooth development. No significant anomalies however could be detected in Sox2 mutant molars. It has been shown that there is the redundancy between different Sox group members, and it is possible that Sox2 function is compensated by Sox6 in

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**Fig. 5.** The expression of Sox genes (Group F) in rodent tooth development. In situ hybridisation of Sox7, Sox17 and Sox18 on frontal head sections at E10.5, E11.5, E13.5, E14.5 and E18.5. Tooth epithelium is outlined in red. Arrowheads indicate the presumptive tooth region.
molar tooth development (Ito 2010). Oligodontia have been shown in patients with Sox5 haploinsufficiency (Lamb et al., 2012). We found that the expression of Sox5 was observed in tooth mesenchyme at early stages of tooth development. Although the first tooth inductive signals are known to be derived from tooth epithelium at E9.5 and E10.5, mesenchymal cells provide signals back to the tooth epithelium at E11.5 (Ferguson et al., 2000). Sox5 has been shown to be associated with Bmp and Shh signaling (Chimal-Monroy et al., 2003, Hojo et al., 2013). Both signaling pathways are known to be activated in tooth mesenchyme at early stages, and are essential for tooth development (Yang et al., 2014, Hardcastle et al., 1998, Li et al., 2011, Jeong et al., 2004). Sox5 might play a critical role in initiation of tooth development by regulating these signaling pathways. The primary enamel knot is known to play a role in regulating tooth shape. Expression of many molecules including Shh have been identified in the primary enamel knots (Tucker and Sharpe, 2004). Our results showed the expression of Sox13 in the primary enamel knots, and Sox13 has been shown to be involved in Shh signaling (Katoh and Katoh 2008). It is possible that Sox13 regulate tooth shape through involving Shh.

Sox18 mutations have been shown to result in the extensive detachment of developing oral epithelium from the underlying mesenchymal tissue due to abnormal hemidesmosome formation (Oommen et al., 2012). Abnormal teeth including enamel hypoplasia and extensive dental caries have been described in blistering diseases such as epidermolysis bullosa that is caused by disorders of hemidesmosomes (Kirkham et al., 2000, Wright et al., 1993). It is known that the interaction between odontoblasts, ameloblasts, and basement membrane play a critical role in enamel/dentin formation (Tucker and Sharpe 2004, Fukumoto et al., 2005). We found Sox18 expression in odontoblasts. It is possible that Sox18 is involved in enamel/dentin formation.

Materials and Methods

Production and analysis of transgenic mice

The production of mice with mutation of Sox6 (p100H) have previously been described (Hagiwara et al., 2000). Krt5-Cre;Rosa26loxP-STOP-loxP-Sox2-IRES-eGFP (Krt5Cre;Rosa26Sox2+/+), Keratin(K)K14Cre and Sox2fl/fl mice were bred as described by Liu et al., (2013). Andl et al., (2004) and Teranov et al., (2006), respectively. CD1 mice were used for radioactive in situ hybridization. The day on which vaginal plugs were found was considered as embryonic day (E) 0.5. To accurately assess the age of embryos, somite pairs were counted and the stage confirmed using morphological criteria such as relative size of maxillary and mandibular primordia, extent of nasal placode invagination, and the size of limb buds. Mouse heads were fixed in 4% paraformaldehyde, embedded and serially sectioned at 8 µm. Sections were split over 4-10 slides and prepared for histology and radioactive in situ hybridisation. Decalcification using 0.5M EDTA was performed after fixation of E18.5 mice.

In situ hybridization

Radioactive in situ hybridization with 35-S-UTP-radiolabelled riboprobes was performed as described previously by Ohazama et al., (2008).

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