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Expression patterns of WNT/β-CATENIN signaling molecules during human tooth development

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Abstract The WNT/β-CATENIN signaling has been demonstrated to play critical roles in mouse tooth development, but little is known about the status of these molecules in human embryonic tooth. In this study, expression patterns of WNT/β-CATENIN signaling components, including WNT ligands (*WNT3*, *WNT5A*), receptors (*FZD4*, *FZD6*, *LRP5*), transducers (*β-CATENIN*), transcription factors (*TCF4*, *LEF1*) and antagonists (*DKK1*, *SOSTDC1*) were investigated in human tooth germ at the bud, cap and bell stages by in situ hybridization. All these genes exhibited similar but slightly distinct expression patterns in human tooth germ in comparison with mouse. Furthermore the mRNA expression of these genes in incisors and molars at the bell stage was also examined by real-time PCR. Our results reveal the status of active WNT/β-CATENIN signaling in the human tooth germ and suggest these components may also play an essential role in the regulation of human tooth development.

Keywords WNT/β-CATENIN signaling · Human tooth germ · Gene expression · Transcriptional factors · Growth factors

Introduction

Mammalian tooth development depends on the reciprocal and sequential interactions between the ectoderm-derived dental epithelium and cranial neural crest-derived mesenchymal cells. While molecular and cellular mechanisms are being investigated in mice (Du et al. 2012; Feng et al. 2012; Tang et al. 2013; Yang et al. 2013; Lei et al. 2014), there is much less understood in humans. In developing human embryo, the dental placode develops into dental epithelium which thickens at 6 W (weeks of gestation). At 7–8 W, the dental epithelium proliferates and invaginates into the subjacent mesenchyme, forming the epithelial bud. At the cap stage (10–13 W), the epithelial bud undergoes specific folding and forms a transient epithelial cluster, called the enamel knot, in the central region of the bud, which is the signaling center involved in regulating tooth shape. At bell stage (14–18 W), ameloblasts and odontoblasts begin to differentiate (Gary et al. 2008).

Multiple signaling networks composed of numerous amounts of molecules participate in the regulation of tooth development. The Wingless-type MMTV integration site (WNT) signaling is one of the critical regulatory pathways. It has at least three branches, of which the WNT/β-CATENIN branch is the best studied and considered as the canonical WNT pathway. In the absence of WNT ligand, cytoplasmic β-CATENIN is associated with the scaffold proteins AXIN and adenomatous polyposis coli (APC), constitutively phosphorylated by glycogen synthase kinase 3β (GSK3β) and targeted for proteasome-mediated degradation. Binding of WNT ligand to frizzled (FZD) receptor and low-density lipoprotein receptor related proteins (LRP) 5/6 co-receptor inactivates the AXIN/APC/GSK3β/CKI complex and stabilizes cytoplasmic β-CATENIN. Subsequently, β-CATENIN translocates into nucleus and induces transcription of downstream genes through interactions with T cell-specific transcription factor (TCF) and lymphoid enhancer binding factor (LEF) (Huelsken and Behrens 2002).

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The WNT/β-CATENIN signaling regulates tooth development at many levels (Liu and Millar 2010; Liu et al. 2008; Jarvinen et al. 2006). For instance, ligand *Wnt3* shows specific expression in the enamel knot, and the overexpression of *Wnt3* causes progressive loss of amelo-blasts and reduction of enamel in postnatal mouse incisor teeth (Millar et al. 2003). In *Lef1* knockout mice, tooth development arrests at the bud stage (van Genderen et al. 1994).Targeted epithelial overexpression of the secreted WNT antagonist *Dickkopf 1(Dkk1)* arrests tooth development at the lamina stage in mice (Liu et al. 2008), which is slightly earlier than the block to tooth development seen in *Lef1* knockout mice. In embryonic mouse oral epithelium, constitutive activation of *β-catenin* results in multiple tooth buds (Pispa et al. 2004; Mustonen et al. 2003). In addition, mutations in *Sostdc1* (*Wise*, *Ectodin*) encoding an inhibitor of Lrp5- and Lrp6-dependent WNT signaling leads to formation of supernumerary tooth in the diastemal region (Kassai et al. 2005).

Until now, most studies on the molecular mechanisms underlying tooth development were conducted in mice. The expression pattern and function of WNT signaling in human tooth development remain unknown. Different from mouse, human has two sets of teeth (the deciduous teeth and permanent teeth). The function of WNT signaling pathway in mice may not fully mimic that of human. Revealing the molecular mechanism during embryonic tooth development in normal human is essential for studying tooth regeneration or reconstitution of a bioengi-neered tooth organ. In this study, we examined the expression patterns of WNT/β-CATENIN signaling mole-cules during human incisor and molar development. Our data of the specific spatio-temporal patterns of WNT sig-naling in the developing human tooth germ demonstrate the WNT/β-CATENIN signaling cascade is crucial for early human odontogenesis.

Materials and methods

Sample preparation

We obtained 8–15 W (weeks of gestation) human embry-onic tissue samples from aborted fetuses, which were provided by the Hospital for Woman and Children Health of Fujian Province. Written informed consents were obtained from participants for the use of their medically aborted embryos for scientific research. And the obtain-ment and application of human embryos in this study was permitted by the Ethical Committee of Fujian Normal University. The embryonic tissues were fixed in 4 %

paraformaldehyde in phosphate-bufferedsaline (PBS, pH 7.4) at 4 °C overnight and then demineralized with 10 % ethylenediamine tetra-acetic acid (pH 7.4) at 4 °C from 2 days to 1 month depending on stages. Then the samples were processed for paraffin embedding and sectioned at 10 μm.

Probes and in situ mRNA hybridization

All the probes (*WNT3*, *WNT5A*, *FZD4*, *FZD6*, *LRP5*, *CTNNB1*, *TCF4*, *LEF1*, *DKK1* and *SOSTDC1*) used for examining human gene expression in this study were pur-chased from Open Biosystems (Thermo Scientific Inc). cDNA fragments were subcloned into pBluesscript for ri-boprobe transcription.

Sections for in situ hybridization with DIG-labeled ri-boprobe were performed as previously described (Lin et al. 2007; Hu et al. 2013; Zhang et al. 2012). Sections were de-waxed in xylene, subsequently rehydrated through a graded series of alcohol and post-fixed in 4 % PFA. Digoxigenin (DIG) labeled RNA probes were prewarmed at 75 °C and hybridized to sections overnight at the temperature according to the TM value of the gene. Sense probes were used as negative control.

RNA isolation and real-time RT-PCR

Human embryonic tooth germs at 15 W (the bell stage) were isolated and two types of tooth germs (incisor and premolar) from one quadrant were respectively subjected to total RNA extraction for 3 times using RNeasy Mini Kit (QIAGEN) according to the manufacturer's instructions. Template cDNAs were synthesized by reverse transcription of 1ug total RNAs using oligo (dT) primer and Revert-Aid^{TM} M-MuLV Reverse Transcriptase (Fermentas, EU). Real-time PCR was carried out using 2×DyNAmo Color-Flash SYBR Green qPCR Kit (Thermo Scientific), and performed with ABI StepOnePlus Real-time PCR System (Applied Biosystems). Primers used in this assay were chosen from previous reports (Zhang et al. 2012). GAPDH was used as an internal control for correcting the expres-sion levels of target genes. Relative mRNA expression levels of target genes in human molar germs were achieved using the ΔΔCT method in comparison with incisor germs. For each RNA sample, three replicates of real-time PCR were performed. Experimental data was shown as Mean and SD from at least three independent experiments. *T* test was analysis with ANOVA using the SigmaPlot 12.5 software. Differences with $P < 0.05$ or $P < 0.01$ were considered as statistically significant change.

Results

Human tooth morphogenesis and expression patterns of *WNT3* and *WNT5A* ligands in the developing human tooth germ

Firstly, H&E staining was used to detect the human incisor and molar germ morphogenesis. At around 8 W (Weeks of gestation, bud stage), the dental epithelium invaginated into the subjacent mesenchyme, and dental mesenchymal cells condensed around the bud cells forming the epithelial bud of the primary teeth (Fig. 1a, d). At 12 W (cap stage), the epithelial bud folded and formed a transient epithelial cluster called the enamel knot in the central region of the bud (Fig. 1b, e). During the subsequent W bell stage, the epithelium and mesenchyme started to differentiate into ameloblasts and odontoblasts respectively, and the tooth germs assume bell-like structure (Fig. 1c, f). These data suggest the normal tooth morphogenesis in human tooth germ resembles that of mice.

Then in situ hybridization was performed to investigate the expression patterns of WNT signaling ligands, *WNT3* and *WNT5A* in human embryonic tooth development (8–15 W). Ligand *Wnt5a* used to be considered as noncanonical WNT family members. But recent studies showed that *Wnt5a* could also regulate canonical WNT signaling via Ror2 or Frizzled4/Lrp5 receptors, suggesting a dual role for Wnt5a signaling in a receptor dependent manner (Mikels and Nusse 2006a; Lin et al. 2011). In the developing human incisor and molar germs, *WNT3* was rarely expressed throughout the tooth germ at 8 and 12 W (Fig. 1g–h, j–k), and its expression was restricted in the internal enamel epithelium at the 15 W bell stage (Fig. 1i, l). However, *WNT5A* transcripts showed strong expression in oral epithelium and dental epithelium, and weaker expression throughout the remaining dental mesenchyme at the bud stage. At the 12 W cap stage, *WNT5A* highly expressed all around the tooth germ, including oral epithelium, dental epithelium, dental papilla mesenchyme and enamel knot. At the 15 W bell stage, expression of *WNT5A* transcripts was continually dense in outer enamel epithelium, inner enamel epithelium and dental mesenchyme (Fig. 1m–r).

Expression patterns of *FZD4*, *FZD6* and *LRP5* receptors in the developing human tooth germ

In situ hybridization was also used to determine the expression patterns of WNT signaling receptors *FZD4*, *FZD6* and *LRP5*. Receptor *FZD4* was not detected in dental epithelium and mesenchyme during 8 and 12 W in human incisor and molar germ (Fig. 2a–b, d–e). But at the bell stage (15 W), *FZD4* was not detected in the incisors, while the expression was well localized in the internal enamel epithelium of molars (Fig. 2c, f). Another receptor *FZD6* showed exclusively weak localization at dental epithelium and enamel knot of incisor and molar at 8 and 12 W, but was clearly observed in the internal and outer enamel epithelium and the surrounding mesenchymal cells during 15 W (Fig. 2g–l). In addition, the *FZD6* signaling in incisor was stronger than that of molar. Low density lipoprotein receptor *LRP5* was weakly detected in the dental epithelium and dental mesenchyme during the 8 W bud stage and the 12 W cap stage, but was obviously expressed in the internal enamel epithelium at the 15 W bell stage in both the human incisor and molar (Fig. 2m–r).

Expression patterns of *β-CATENIN*, *TCF4* and *LEF1* in the developing human tooth germ

Transducer *β-CATENIN*, also known as *CTNNB1*, is a key molecule in canonical WNT pathway. The mRNA expression pattern of *CTNNB1* during human tooth germ development was also examined by in situ hybridization. *CTNNB1* was distinctively present in dental epithelial cells from the bud to bell stage. At 8 W, strong *CTNNB1* expression was detected at the tip of the budding epithelium (Fig. 3a, d). At 12 W, the expression was observed in the dental epithelium, enamel knots and the mesenchyme surrounding epithelium (Fig. 3b, e). But at 15 W bell stage, *CTNNB1* expression was limited in the inner enamel epithelium (Fig. 3c, f). The transcription factors *TCF4* showed no expression during the bud and cap stages (8 and 12 W) (Fig. 3g, h, j, k), but positive *TCF4* staining predominantly localized in internal enamel epithelium during the 15 W bell stage (Fig. 3i, l). Another transcription factor *LEF1* was not detected in dental epithelium and mesentchyme at 8 W in both incisor and molar (Fig. 3m, p). However, *LEF1* was expressed throughout the dental epithelium and mesenchyme of the incisor and molar at the cap stage (Fig. 3n, q), and had high expression in the internal and outer enamel epithelium at the bell stage in the developing human tooth germ (Fig. 3o, r).

Expression patterns of *DKK1* and *SOSTDC1* inhibitor in the developing human primary dentition

Dkk1 inhibits the WNT signaling pathway by binding to the WNT receptor complex. As in situ hybridization results showed, *DKK1* was expressed throughout the epithelium at 8 W in the human incisor and molar (Fig. 4a, d). And the expression expanded to all of the tooth germ, including

Fig. 1 Human tooth morphogenesis and expression patterns of WNT signaling ligands during the developing human tooth germs. Morphogenesis of the early tooth germ development at 8-week bud stage (**a**), 12-week cap stage (**b**) and 15-week bell stage (**c**) in human primary incisor, and molar germ (**d–f**). *WNT3* expression was rarely detected at 8.5-week bud stage and 12-week cap stage in the human primary incisor germ (**g**, **h**), and molar germ (**j**, **k**), but at the bell stage from a 15-week-old embryo exhibited *WNT3* expression in the inner enamel epithelium in the human primary incisor (**i**) and molar germ (**l**). *WNT5A* strongly expression was detected at the 8.5-week bud stage, 12-week cap stage and 15-week bell stage in the human primary incisor and molar germ (**m–r**). *DE* dental epithelium, *DP* dental papilla, *EK* enamel knot, *IEE* inner enamel epithelium. *Scale bar* 50 μm (**a**, **b**, **d**, **g**, **h**, **j**, **m**, **n**, **p**), 100 μm (**c**, **e**, **f**, **i**, **k**, **l**, **o**, **q**, **r**)
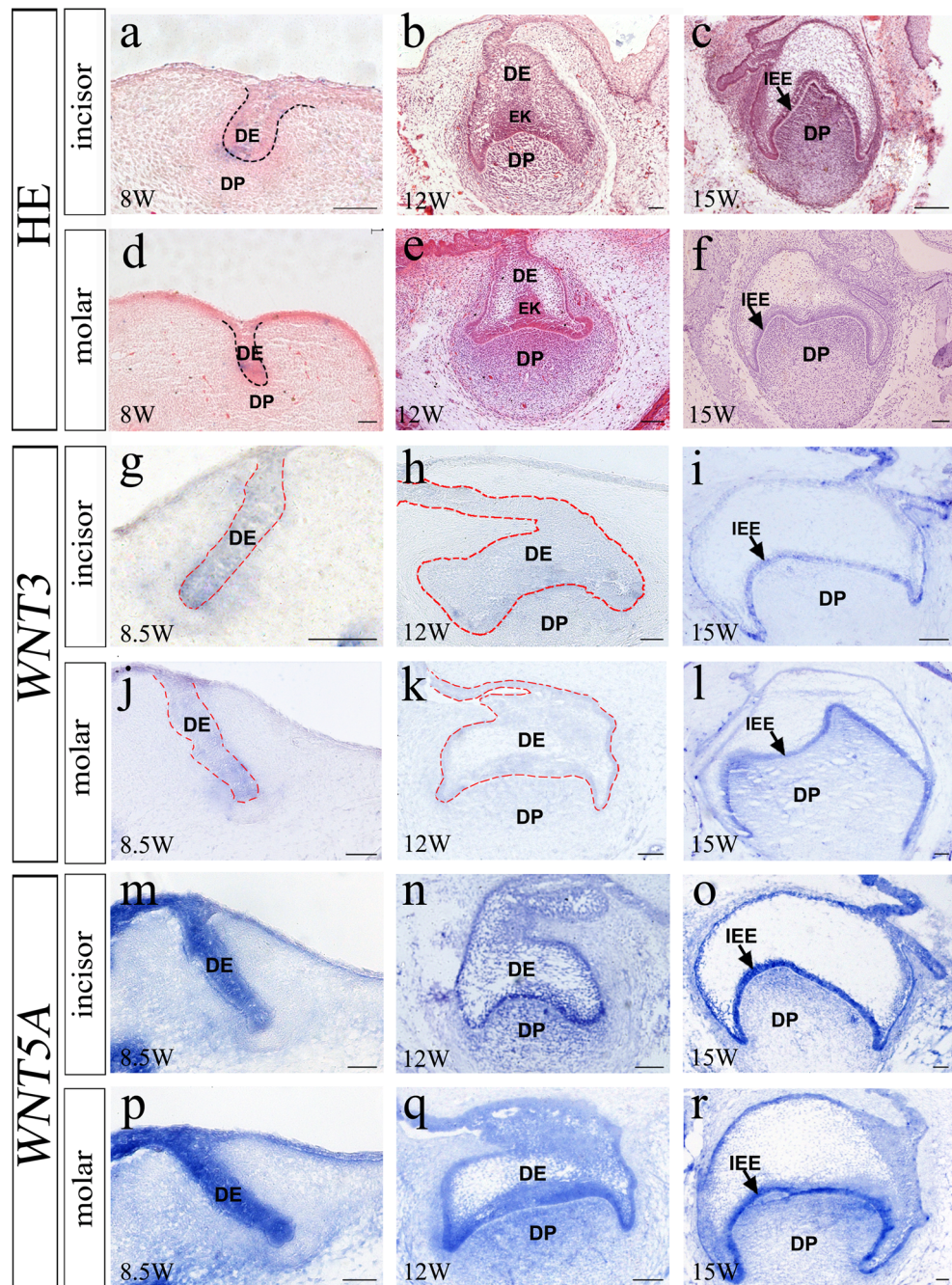

dental epithelium, dental mesenchyme and enamel knot at 12 W (Fig. 4b, e). At 15 W, *DKK1* was observed in the inner enamel epithelium, outer enamel epithelium and dental mesenchyme (Fig. 4c, f). Another antagonist *SOSTDC1* was also detected by in situ hybridization at the bud, cap and bell stages. From 8 to 15 W, the expression of *SOSTDC1* was strongly detected in the dental epithelium and mesenchyme of incisor and molar germ (Fig. 4g–l). Negative controls for in situ hybridization in the developing human incisor and molar were shown in Fig. 4m–r.

Comparison of the mRNA expression of WNT/β-CATENIN signaling component in the developing human incisors and molars

To further confirm the mRNA expression levels of WNT/β-CATENIN signaling components in the developing human incisors and molars, we pooled these two types of tooth germs at the 15 W bell stage from all the available embryonic mandibular quadrant and isolated total RNAs from each pool. We then determined the expression levels

Fig. 2 Expression patterns of WNT/β-CATENIN signaling receptors during the developing human tooth germs. *FZD4* expression was not detected at 8.5-week bud stage and 12-week cap stage in the human primary incisor (**a**, **b**), and molar germ (**d**, **e**). *FZD4* expression was weekly detected at 15-week bell stage in the incisor germ (**c**), but obviously detected in the inner enamel epithelium at 15-week-old embryo in the human primary molar germ (**f**). *FZD6* expression was weekly detected in epithelium at 8-week (**g**), 12-week (**h**) in human primary incisor germ, and molar germ (**j**, **k**). *FZD6* expression was well located at 15-week human primary incisor (**i**) and molar germ (**l**). *LRP5* expression was weekly detected at the 8-week bud stage, and 12-week cap stage in human primary incisor (**m**, **n**) and molar germ (**p**, **q**), but at the bell stage from a 15-week-old embryo exhibited *LRP5* expression in the inner enamel epithelium in the human primary incisor (**o**) and molar germ (**r**). *DE* dental epithelium, *DP* dental papilla, *IEE* inner enamel epithelium. *Scale bar* 100 μm (**a–l**), 50 μm (**m–r**)
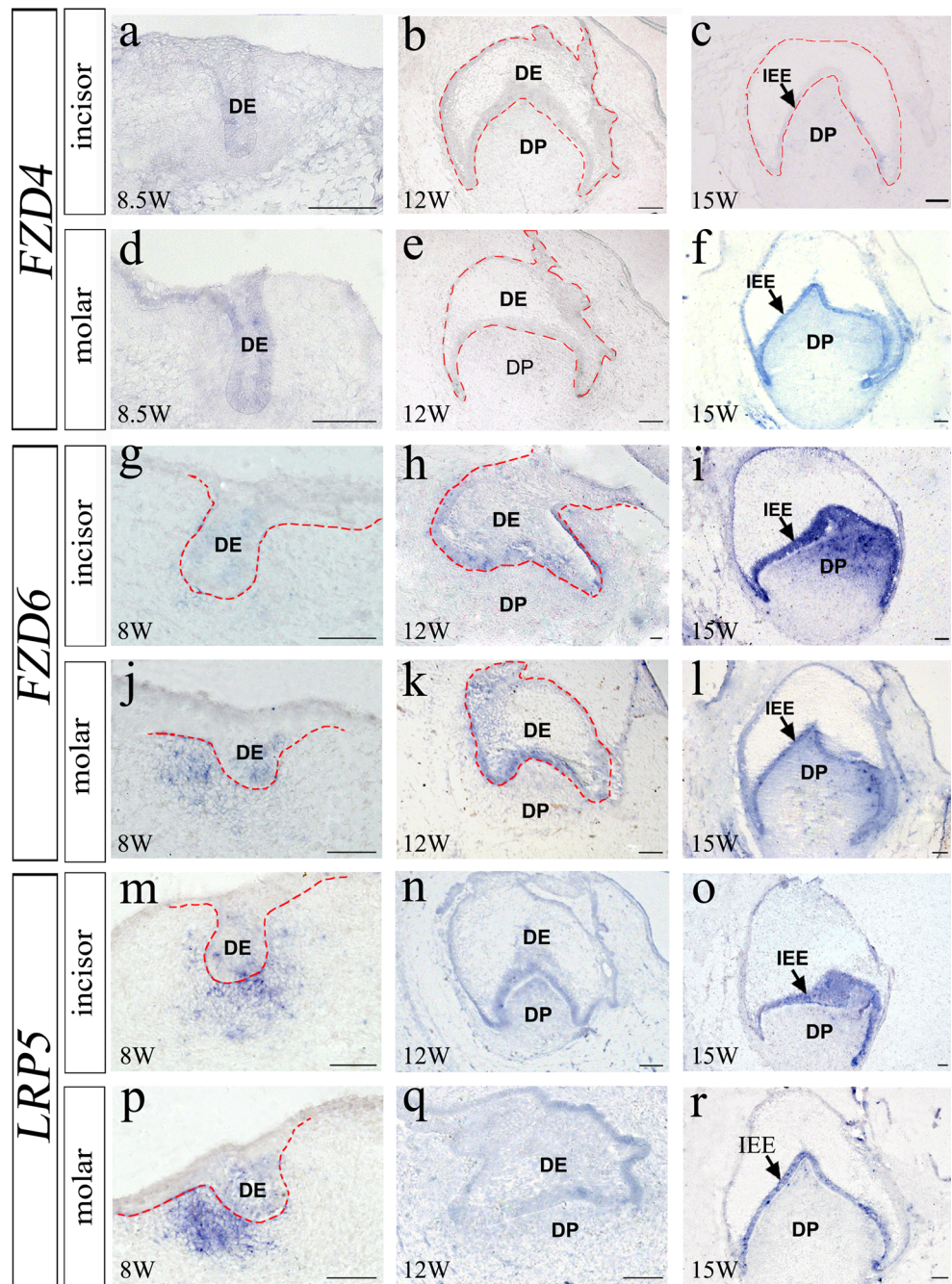

of WNT signaling genes by Real-time RT-PCR. As shown in Fig. 5, the expression levels of *FZD4* and *LEF1* in molars were markedly higher than that of incisors, but *FZD6* in molars was obviously lower than that of incisors. And there were no significant changes in *WNT5A*, *LRP5*, *β-CATENIN*, *TCF4*, *DKK1* and *SOSTDC1* of molars when compared with incisors. The changes in expression levels of these genes at the bell stage were basically matched with that detected by in situ hybridization.

Discussion

In mice, WNT signaling pathway has previously been shown to play important roles at various periods during tooth development, from the initiation to the bud, cap, and differentiation stages (Liu and Millar 2010). In the current study, the spatial and temporal expression patterns of WNT signaling components including the ligands (WNT3 and WNT5A), receptors (FZD4, FZD6 and LRP5), transducers

Fig. 3 Expression patterns of WNT/β-CATENIN transducers and transcription factors during the developing human tooth germs. *CTNNB1* (*β-CATENIN*) expression was detected in the epithelium at 8-week human primary incisor (**a**) and molar germ (**d**). *CTNNB1* expression was seen in both the epithelium and the surrounding mesenchyme at 12-week human primary incisor (**b**) and molar germ (**e**). *CTNNB1* expression was seen in the inner epithelium at 15-week human primary incisor (**c**) and molar germ (**f**). *TCF4* showed no expression during the 8-week bud stage and 12-week cap stage in human primary incisor (**g**, **h**) and molar germ (**j**, **k**), but positive *TCF4* staining was predominantly localized in internal enamel epithelium during the 15-week bell stage (**i**, **l**). *LEF1* expression was not detected in the human tooth germ at the 8-week human primary incisor (**m**) and molar germ (**p**). *LEF1* was expressed throughout the dental epithelium and mesenchyme of the incisor and molar at the 12-week cap stage (**n**, **q**), and had high localized expression in the internal and outer enamel epithelium at the 15-week bell stage in the developing human tooth germ (**o**–**r**). *DE* dental epithelium, *DP* dental papilla, *EK* enamel knot, *IEE* inner enamel epithelium. *Scale bar* 100 μm
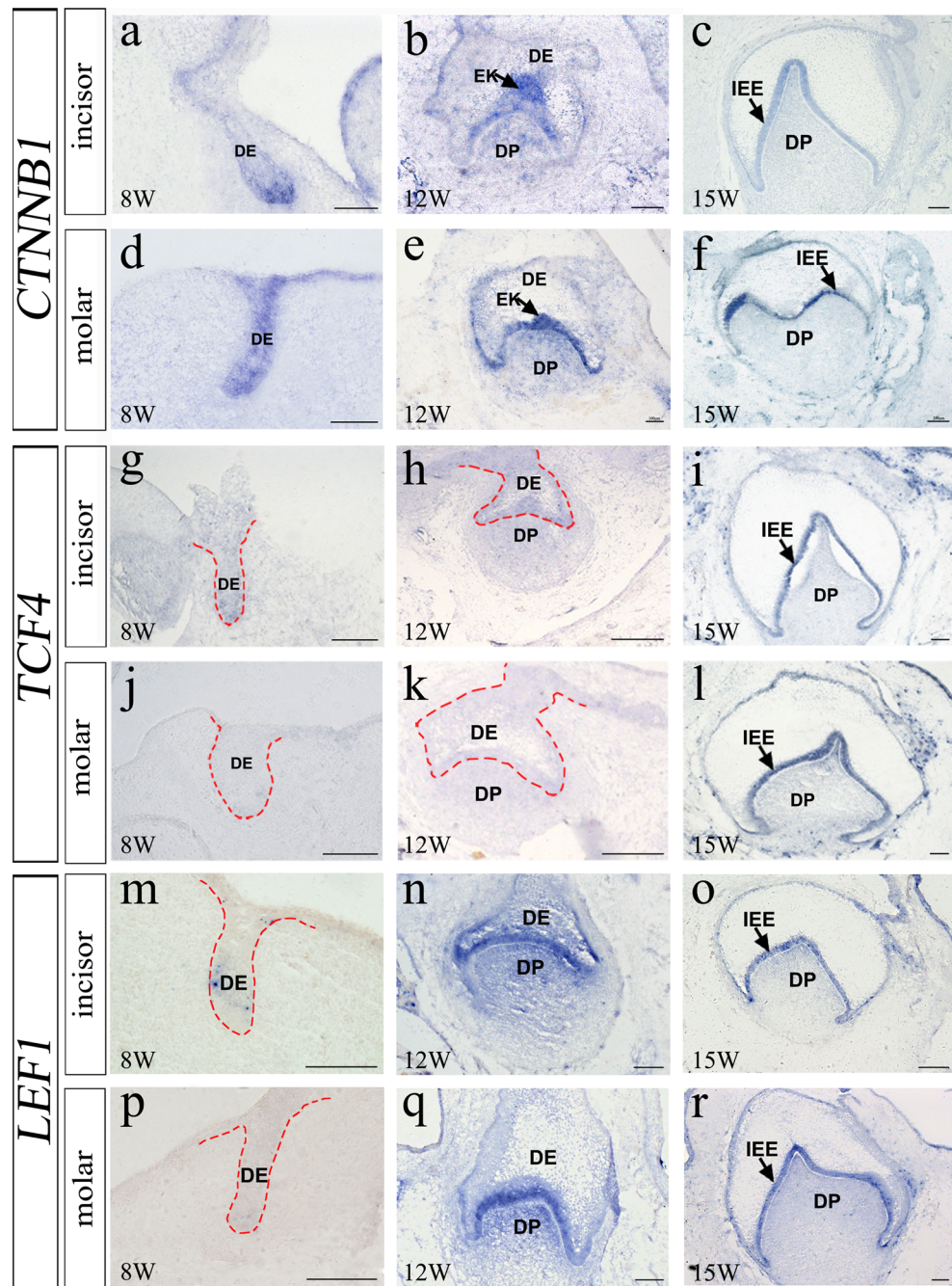
and transcription factors (CTNNB1, TCF4 and LEF1), and antagonists (DKK1 and SOSTDC1) were examined in the developing human tooth germ during the bud, cap and bell stages. The expression of *WNT3*, *FZD4*, *FZD6* and *LRP5* in the developing human tooth germ was distinctly restricted in the dental epithelium at the bell stage. These observations indicate similar expression patterns of these genes in the developing human and mouse tooth germs. However, *WNT5A* showed strong localization not only around dental mesenchyme, but also in oral epithelium, dental epithelium during all of the stages in our study (Fig. 2m–r). And our immunohistochemistry data further confirmed the expression pattern of the WNT5A in human primary incisor and molar germ (data not shown), which is identical with our data of in situ hybridization and consistent with previous publciation that WNT5A protein was expressed in enamel epithelium cells, enamel knot and and stellate reticulum in developing human tooth germ (Peng et al. 2010a). In mice, *Wnt5a* mRNA is only expressed in mesenchymal cells with no overlying expression in the dental or oral epithelium (Kettunen et al. 2005; Sarkar and Sharpe 1999). These data suggests that *WNT5A* gene may be one of the key genes

Fig. 4 Expression patterns of WNT/β-CATENIN inhibitors during the developing human tooth germs. *DKK1* expression was mainly detected in the epithelium at the bud stage in human primary incisor (**a**) and molar germ (**d**). *DKK1* expression was detected in the epithelium, mesenchyme and enamel knot at 12-week human primary incisor (**b**) and molar germ (**e**). *DKK1* was observed in the inner enamel epithelium, outer enamel epithelium and dental mesenchyme at 15-week human primary incisor (**c**), and molar germ (**f**). *SOSTDC1* expression was detected in dental epithelium and mesenchyme at the bud (**g**, **j**), cap (**h**, **k**) and bell (**i**, **l**) stages of the primary incisor and molar germ. Negative control for in situ hybridization in the developing human tooth germ (**m**–**r**). *DE* dental epithelium, *DP* dental papilla, *IEE* inner enamel epithelium. *Scale bar* 100 μm
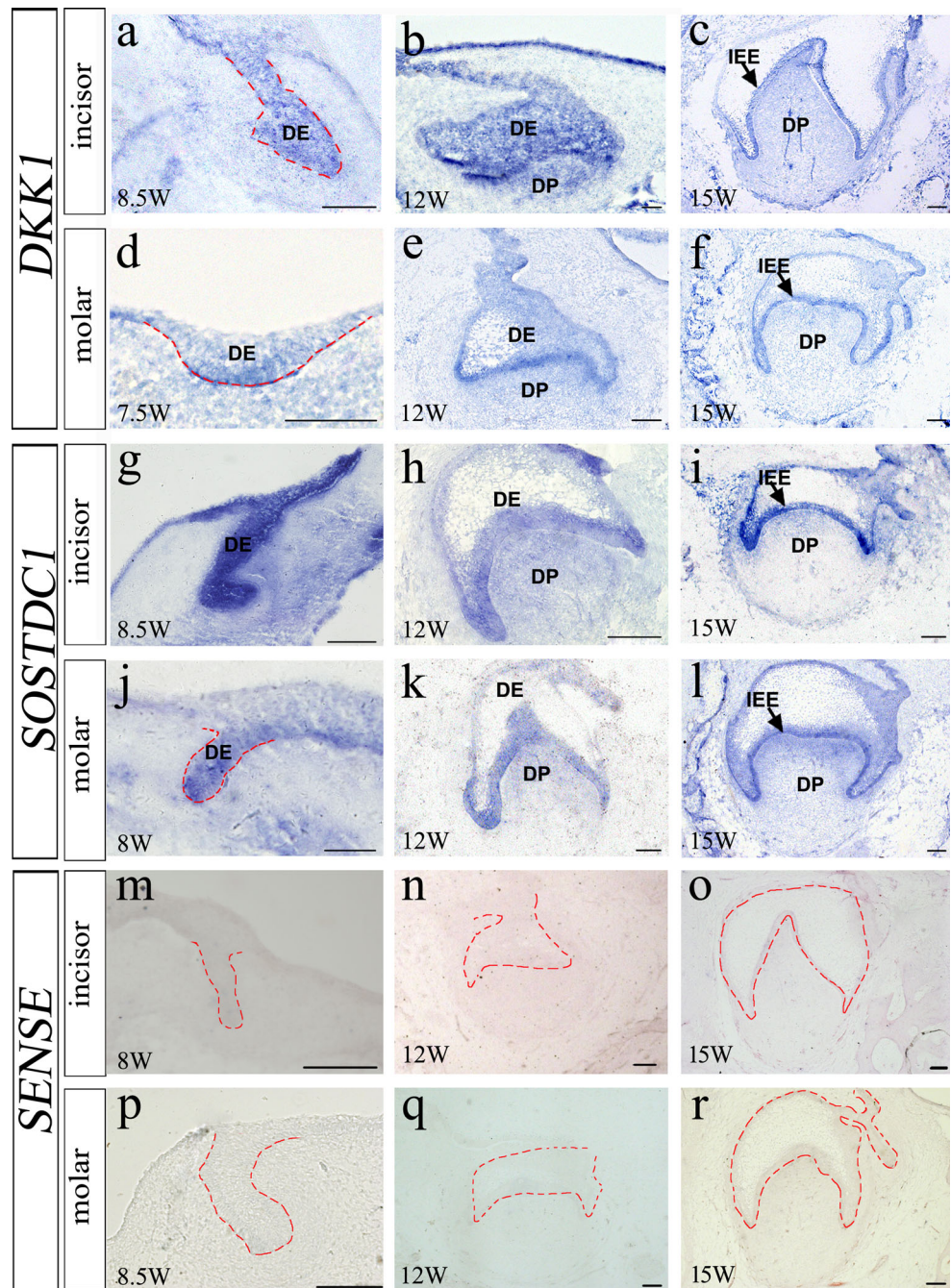
differ between human and mice in teeth. It was reported that *Wnt5a* plays dual role in noncanonical and canonical WNT pathway depending on the receptor (Mikels and Nusse 2006a; Lin et al. 2011). It was recently reported that WNT5A promoted differentiation and inhibited migration and proliferation in human dental papilla cells.(Peng et al. 2010b; Wang et al. 2013). The essential role of Wnt5A in the developing human tooth germ and human dental papilla might suggest its potential in the research of human regenerative dentistry (Peng et al. 2010b). Hence, more attention should be paid to noncanonical WNT pathway in

the future to find the differences between human and mice in tooth germ.

In the WNT/β-CATENIN pathway, binding of the WNT ligands to the FZD receptors complex with LRP5/6 stabilizes β-CATENIN, leading to the transcription of its target genes through interaction with the transcription factors, TCF and LEF (Liu and Millar 2010). In mouse, *β-catenin* and *Lef1* expression in the dental buds epithelium overlaps with expression of *Pitx2*, an essential transcription factor in the developing tooth (Sarkar and Sharpe 1999; Hjalt et al. 2000). Strong *Lef1* expression is also detected in the dental
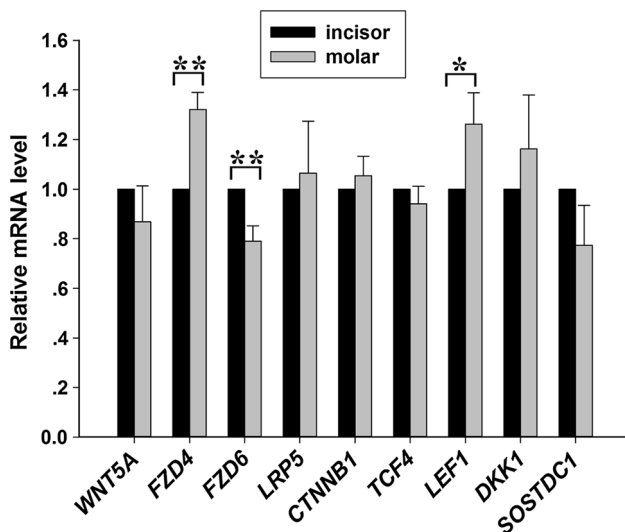
Fig. 5 Real-time RT-PCR was used to detect the mRNA expression levels (mean ± SEM) of WNT/β-CATENIN signaling components, including WNT ligands (*WNT5A*), WNT receptors (*FZD4*, *FZD6*, *LRP5*), WNT transducers (*β-CATENIN*), WNT transcription factors (*TCF4*, *LEF1*) and WNT antagonists (*DKK1*, *SOSTDC1*) in the 15-week primary incisor and molar. Data were normalized to the average mRNA level of *GAPDH*. Statistical difference is indicated by asterisks (*$P < 0.05$, **$P < 0.01$)

mesenchyme in developing mouse tooth bud (Sarkar and Sharpe 1999). Similar expression patterns of *β-CATENIN* were found in dental epithelium of the embryonic human. However, *LEF1* did not appear at the bud stage, instead was highly expressed in the dental epithelium and mesenchyme including stellate reticulum in human tooth germ at 12 weeks of gestation (cap stage), which had the same expression patterns with that of the bud stage in mice. This implies that *LEF1* plays important role from the cap stages in the regulation of human tooth development, which is later than that in mice.

In mice, antagonist *Dkk1* expression persists in the dental epithelium during the tooth initiation and bud stages, and then transfer to the dental mesenchyme from the cap stage to the differentiate stage (Liu et al. 2008; Fjeld et al. 2005). Another antagonist *Sostdc1*, also known as *Ectodin*, *Wise* or *Usag-1*, was identified as a gene encoding a conserved secreted protein capable of regulating canonical WNT signaling (Itasaki et al. 2003). But a recent study showed that it also binds to a subset of BMPs in vitro and influences Bmp signaling (Laurikkala et al. 2003). In mice, *Sostdc1* is expressed in the dental epithelium and dental mesenchyme, except for the forming enamel knot at the tip of the upper tooth bud. The expression is fully negative from the enamel knot as well as the epithelium and mesenchyme surrounding at the cap stage (Laurikkala et al. 2003). In human, the expression patterns of *DKK1* were strongly detected in the dental epithelium as that of mice at

the bud stage, but the signaling expanded to the dental epithelium and mesenchyme at the cap and bell stages, which was slightly different from that of mice. *SOSTDC1* transcripts were strongly detected in the dental epithelium and mesenchyme, and enamel knot from the 12th to 15th weeks of human tooth germs (Fig. 5h, i, k, l), which also was slightly different from that in mice. Hence, the WNT signals, and inhibitors probably act in concert to maintain a homeostasis for the regulation of tooth development.

Taken together, these results suggest that, the canonical WNT signaling pathway components exhibited similar but slightly distinct expression patterns in human tooth germ in comparison with mouse, suggesting that these components may also play an essential role in the regulation of human tooth development.

It is well known that the incisors and molars possess several differences in shape and size in the human tooth germ. For instance, molar is larger and has several cusps, while incisors only has one cusp. Therefore, the molecular mechanisms underlying the development of molar and incisors may be different. In order to investigate whether the WNT signaling molecules are responsible for the differential histomorphogenesis of the incisors and molars, we performed Real-time RT-PCR to determine the gene expression levels of WNT signaling in the human incisors and molars at 15 W (bell stage). We found that the gene expression levels of receptors *FZD4* and *FZD6*, transcription factors *LEF1* in molar were markedly different that of incisors, suggesting *FZD4*, *FZD6* and *LEF1* of WNT/β-CATENIN signaling components may be the key molecules make difference between incisors and molars. More studies will be needed to study the functional roles of FZD4 and FZD6 during the development of molars and incisor, which may provide in-depth understanding of the developmental processes of the tooth germs.

In addition to its role in tooth development and morphogenesis, the WNT/β-CATENIN signaling pathway is also associated with the pathogenesis of human oral diseases. For example, the role of WNT pathway in various types of oral tumor is well established. Differential expression of several WNT components including WNT3, WNT5A, WNT10A, LRP5, β-CATENIN, AXIN2, APC and DKK1, have been reported in normal tooth tissues and oral cancers (Adaimy et al. 2007; Blanton et al. 2004; Bohring et al. 2009; Foulkes 1995; Niemann et al. 2004; Rickels et al. 2005; Roessler et al. 2000; Sekine et al. 2002, 2003). Nuclear localized β-CATENIN is detected in follicular and plexiform-type ameloblastomas, and these tumors are occasionally linked to gain of function mutation of β-CATENIN or loss of function mutation of APC (Kawabata et al. 2005; Mikels and Nusse 2006b; Siriwardena et al. 2009). WNT signaling components are overexpressed in adenoid cystic carcinoma (ACC), another common

salivary gland tumor subtype, and nuclear localized β-CATENIN and mutations in WNT signaling genes including β-CATENIN, APC and AXIN1 are detected in some cases (Daa et al. 2004, 2005; Frierson et al. 2002). This finding revealed that Wnt/β-CATENIN signaling is critical in formation of some oral tissue tumors, as well as in embryonic development of the various structures of the oral cavity (Liu and Millar 2010).

In conclusion, the specific spatio-temporal patterns showed that canonical WNT signaling components including *WNT3*, *WNT5A*, *FZD4*, *FZD6*, *LRP5*, *β-CATENIN*, *LEF1*, *DKK1* and *SOSTDC1* exhibit similar expression patterns in the developing human and mouse tooth germs. Among them, *WNT5A*, *DKK1* and *SOSTDC1* genes showed wider spatial expression in the developing human tooth germ as compared to that in mice. And *FZD4*, *FZD6* and *LEF1* showed difference in the expression levels of incisors and molars at the bell stage. All of our data revealed that WNT/β-CATENIN signaling cascade is crucial for early human tooth patterning during morphogenesis.

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