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Dkk1, -2, and -3 expression in mouse craniofacial development

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Summary

The Dickkopf family is important for embryogenesis and postnatal development and growth. Dkk1 is a strong head inducer and knockout of this gene leads to absence of anterior head structures, which are predominantly formed through neural crest migration. During early craniofacial development, *Dkk1* to *Dkk3* show developmentally regulated expression in a number of elements. However, their expression and roles in late times of craniofacial development are largely unknown. This study focuses on the expression profile of *Dkk1-3* on late embryonic and early postnatal stages. It was found that *Dkks* were involved in a variety of craniofacial developmental processes, including facial outgrowth, myogenesis, osteogenesis, palatogenesis, olfactory epithelium and tooth development; and the expression persisted to postnatal stage in the muscles and bones. Their expression patterns suggest important roles in these processes; further study is warranted to elucidate these roles.

Introduction

Dkk genes were identified through the initial characterization of Xenopus Dkk1, a strong head and axis inducer and a Wnt signalling antagonist (Glinka *et al.* 1998). Expressed in anterior endomesoderm of the Spermann organizer, Dkk1 was shown to be sufficient to induce head formation. Injection of Dkk1 mRNA into Xenopus embryos induces axes and head formation, while microinjection of anti-Dkk1 antibody leads to microcephaly (Glinka *et al.* 1998). Later, several other members were also identified. The Dkk family comprises four members (Dkk1 to Dkk4) and a Dkk3-related protein named Soggy (Kawano and Kypta 2003). Most members show a role in modulating the Wnt/β-catenin pathway. Dkk1 is the most extensively studied member. Dkk1 acts as a strong antagonist of the Wnt/β-catenin pathway by binding to LRP5/LRP6 components of the Wnt receptor complex (Mao *et al.* 2001, Semenov *et al.* 2001, Kawano and Kypta 2003). Dkk2, however, can both inhibit and activate Wnt signalling depending on cellular context (Mao *et al.* 2002, Mao and Niehrs 2003). In addition to receptor LRP5/6, Dkk1 and Dkk2 interact with another class of receptor, Krm1 and Krm2, which have a synergistic role with Dkks in regulating Wnt receptor Lrp6 (Davidson *et al.* 2002, Mao *et al.* 2002). Dkk4 behaves similar to Dkk1 in Wnt inhibition and Krm co-operation. Dkk3, on the other hand, binds neither LRP nor Krm and shows no effect on Wnt/β-catenin pathway (Mao *et al.* 2002). Recently, Dkk1 was shown to be able to act independent of Wnt/β-catenin pathway (Lee *et al.* 2004).

Dkk genes are important for embryogenesis. Their expression is present throughout gastrulation and organogenesis (Glinka *et al.* 1998, Monaghan *et al.* 1999). As a head inducer, Dkk1 is critical for anterior neural plate patterning and forebrain specification (Hashimoto *et al.* 2000, Kazanskaya *et al.* 2000, Shinya *et al.* 2000). Dkks are now found to serve broad roles in multiple developmental processes, involved in the formation of heart, limb, lung, and a number of other organs (Monaghan *et al.* 1999, Kazanskaya *et al.* 2000, Mukhopadhyay *et al.* 2001, Grotewold and Ruther 2002a, De Langhe *et al.* 2005). They also appear important in many physiological processes in adulthood (Monaghan *et al.* 1999, Heller *et al.* 2003). Dkk1 has recently been found to be important in stem cell regulation (Horwitz, 2004, Byun *et al.* 2005). In line with the oncological role of the Wnt/β-catenin pathway, expression of *DKKs* is altered in many kind of human cancers (Wirths *et al.* 2003, Kurose *et al.* 2004, Gonzalez-Sancho *et al.* 2005).

Besides the critical role of early head induction, Dkks are also important for subsequent craniofacial development. In vertebrate, the craniofacial structures are the most anatomically sophisticated parts and evolutionary novelty. The most striking feature is the emergence of neural crest and its contribution to the

craniofacial development. How this conserved Dkk signalling is involved in the formation of the evolutionary new elements is a very interesting issue. Evidence indicates that Dkks are also used in the formation of the newly emerged layer, the neural crest, and are important for the formation of craniofacial structures through participating epithelial-mesenchymal interaction (Monaghan *et al.* 1999). Knockout of Dkk1 in mice results in absent of anterior head structures, including eyes, olfactory placodes, frontonasal and mandibular processes, and skull derivatives anterior of the parietal bone (Mukhopadhyay *et al.* 2001). In line with this, *Dkk1* is expressed in the cranial neural crest, which gives rise to most of the craniofacial structures (Monaghan *et al.* 1999). The molecular mechanism of Dkks in craniofacial development remains to be defined. Expression of Dkk1–3 is highly regulated in early facial primordia, palate, tooth, eye, and hair follicles (Monaghan *et al.* 1999, Ang *et al.* 2004, Fjeld *et al.* 2005). However, the roles and expression of *Dkks* in late times of development are unknown in many of the craniofacial structures. In order to address the roles of Dkks in the development of craniofacial elements, a preliminary expression study of *Dkk1* to *Dkk3* was performed from E12, the time that most of the craniofacial structures are being formed in mice, to early postnatal stages.

Materials and methods

Preparation of tissues

All the procedures involving mouse use were approved by The Animal Welfare Committee of the University of Bergen. The stage of the embryos was determined by the day of appearance of vaginal plug and confirmed by morphological criteria. NMRI Mice were used in this study. The appearance of a vaginal plug was taken as day 0 of embryogenesis (E0). Delivery of NMRI mice takes place at E19, which corresponds to newborn stage (P0). The mice were killed by cervical dislocation and decapitation. Embryonic (E) and postnatal (P) mice (E10 to E18, P0, P3, P5) were dissected in PBS and fixed in 4% paraformaldehyde (PFA) overnight at 4 °C. Mice embryos harvested at E14 or older were decalcified with 12.5% EDTA–2.5%PFA in PBS. They were then dehydrated in a serial of ethanol and embedded in paraffin. Sagital sections of 7 μm from the midline of mouse heads were cut and mounted, dried overnight at 37 °C and stored at 4 °C.

In situ *hybridization*

Fragment cDNAs was generated by RT-PCR and subcloned in pGEM-T easy vector (Fjeld *et al.* 2005). ^{35}S-labeled sense and anti-sense riboprobes are made through *in vitro* transcription from the linealized cDNA-containing vectors. Following transcription, cDNA templates were digested with RNase-free DNase I. Riboprobes were quantified in a liquid scintillation analyzer.

Sagital sections of mouse head were deparaffinized in xylene, rehydrated through serial ethanol, washed in PBS, treated for 30 min with proteinase K (Promega Corp., Madison, WI, USA), post-fixed in 4% PFA for 30 min, followed by 1 min in PBS containing glycine at 2 mg/ml. After 25 min washes in PBS, the sections were acetylated with freshly prepared 0.25% acetic anhydride in 0.1 M triethanolamine–HCl (pH 8) for 10 min at room temperature, followed by 2 water washes of 5 min each. The sections were dehydrated by dipping in a series of ethanol solutions (30, 50, 70 and 95%) for 30 s each, air-dried, and used for hybridization. The probes was pipetted onto the sections and covered with parafilm. The sections were hybridized for about 15 h at 55 °C. Following hybridization, the sections were washed under high stringent conditions with 20 mM DTT in 50% formamide and 2× SSC for 1 h at 65 °C. Unhybridized probe was digested with RNase A at 37 °C for 20 min. The sections were then washed for 1 h at 55 °C in 0.1× SSC, dehydrated in 70% ethanol, air-dried, and exposed to X-ray film overnight. They were then dipped in NTB-2 emulsion (Eastman Kodak, New Haven, CT, USA) for autoradiography, exposed for 2–4 week at 4 °C depending on the signal intensity on the X-ray film, and developed in D-19 (Kodak). Finally, the sections were counterstained with haematoxylin and mounted with Depex. No specific signal was detected in sections hybridized with the control sense probes.

Image processing

Images were taken with a SPOT Insight digital camera (Diagnostic Instruments, Sterling Heights, MI, USA) mounted on a Zeiss Axioskop2 microscope (Carl Zeiss Jena GmbH, Jena, Germany). The bright-field and dark-field images of each section were digitized separately and processed with Adobe Photoshop 6.0 software (Adobe Systems, San Jose, CA, USA). Signals from dark field were pseudocoloured to red and imposed into the bright field.

Results

Consistent with a previous study (Monaghan *et al.* 1999), *Dkk1* was highly expressed in the lower part of the nasal septum below vomeronasal organ at E12, where it would fuse with the primary palate and paired palatal shelves (Figure 1A). The earlier study also showed that the expression was only located on the left–right sides (Monaghan *et al.* 1999). *Dkk1* was also found in the ventral plate of the brain and the notochord in the

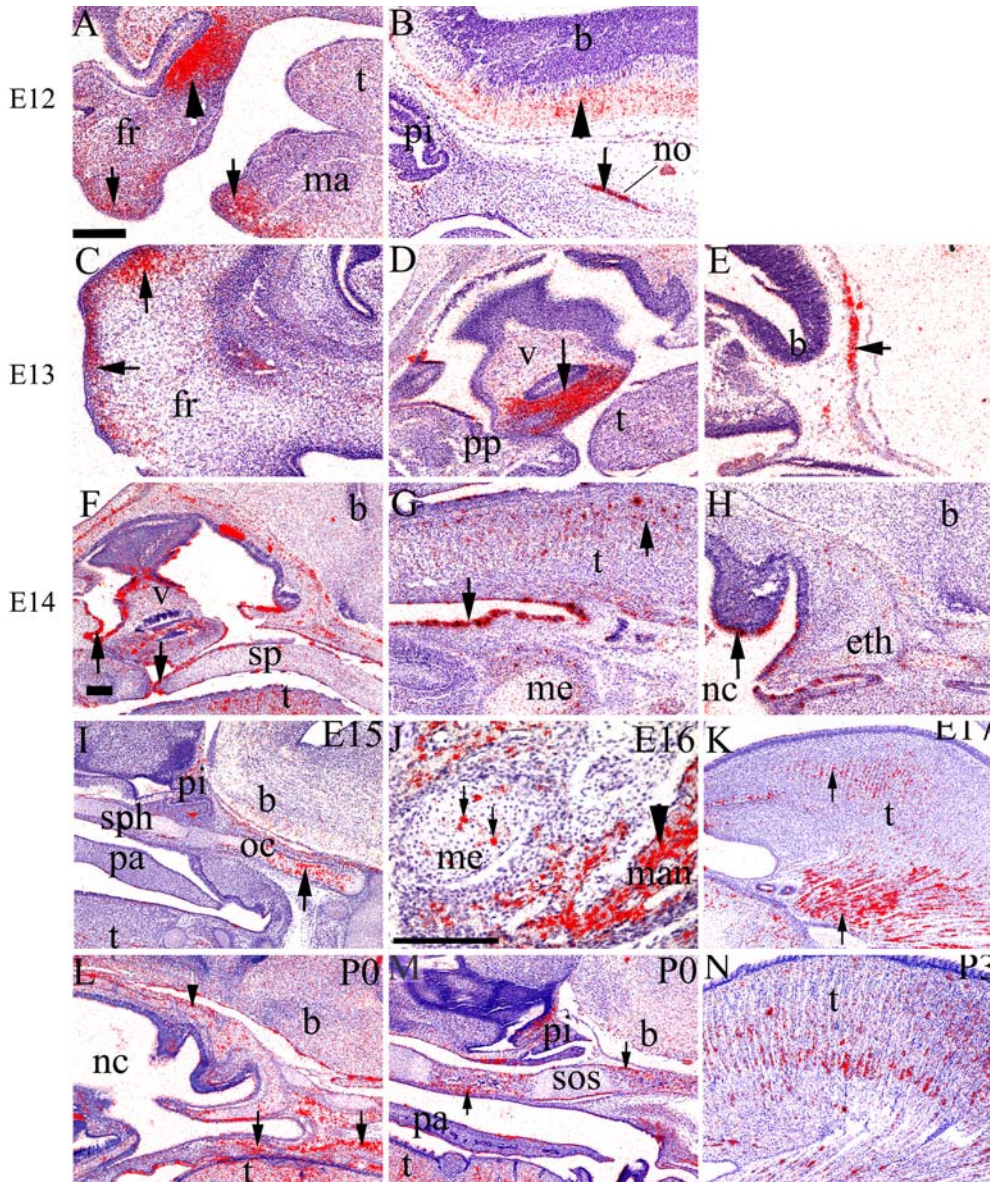

Figure 1. *Dkk1* expression in developing face. At E12, *Dkk1* was seen in the mesenchyme of the first branchial arch (A, arrows), lower region of vomeronasal organ (A, arrow head), notochord (B, arrow), and ventral plate of the brain (B, arrow head). At E13, its expression was restricted to the sub-epithelial mesenchyme in the frontonasal mass (C, arrows) and intensified in the lower part of the vomer (D, arrow). It was also highly present in the squamous occipital precursors at E13 (E, arrow). *Dkk1* appeared in the oral epithelia, developing tongue, and perichondral mesenchyme of Meckel cartilage at E14 (F, G, arrows). It was also expressed in the olfactory epithelium from E14 (H, arrow). At E15, it was seen in the migrated cells in the basioccipital cartilage (I, arrow). At E16, it was highly expressed in the osteogenic cells in the mandible (J, arrow head); it was also detected in individual chondrocytes within the Meckel cartilage (J, arrows). From late embryogenesis *Dkk1* was highly expressed in the muscles of the tongue and the craniofacial bones (K, L, M, N). Expression was detected in both intramembranous bones, i.e. palatine bone (L, arrows), and endochondral basicranium (M, arrows). Scale bar represents 200 μm; scale bar in (A) applies to (A, B, C, D, E, G, and H); scale bar in (F) applies to (I, K, L, M, and N). b: brain; eth: ethmoid; fr: frontonasal mass; ma: mandibular arch; man: mandible; me: Meckel cartilage; n: notochord; na: nasal cartilage; nc: nasal cavity; no: notochord; pa: palate; pi: pituitary gland; pp: primary palate; oc: occipital; sos: sphenoid–occipital synchondrosis; sp: secondary palate; sph: sphenoid; t: tongue; v: vomer.

area of basioccipital precursor (Figure 1B). *Dkk1* expression in the mesenchyme of the first branchial arch continued during E12 and E13, but was restricted to the rostra area (Figure 1A, C). In the maxillary arch, it was located in the mesenchyme that was directly underneath the overlying ectoderm (Figure 1C). The expression of *Dkk1* in the lower part of nasal septum was intensified during E12 and E13 (Figure 1A, D). At E13, it was also observed in the precursor of squamous occipital bone (Figure 1E). At E14, it appeared in the epithelia of the primary and secondary palate, ventral tongue, oral floor, and nasal cavity at a high level (Figure 1F, G, H). It was also seen in the perichondria of the chondrocranium and the perichondral mesenchyme of the Meckel cartilage (Figure 1G). From this time, *Dkk1* transcripts start to be clustered within the tongue (Figure 1G); this

expression was upregulated and maintained in subsequent development (Figure 1K, N). High expression was also observed in osteogenic cells of both endochondral and intramembranous bones from late embryogenesis (Figure 1I, J). In the intramandubular part of Meckel cartilage, *Dkk1* was also localized in the differentiated chondrocytes (Figure 1J). At postnatal stage, *Dkk1* was mainly seen in the tongue and bones (Figure 1L, M, N). In sagital sections, it was particularly clear in the palatine bone, calvaria, and basicranium (Figure 1L, M).

Dkk2 was highly expressed in the epithelia and underlining mesenchyme in the cranial vault at E12 (Figure 2A). It was also seen in the first branchial mesenchyme adjacent to the facial epithelia. At E13, it was observed in the perichondrium in the anterior chondrocranium (Figure 2B). From E14 transcripts also clustered within the tongue. At E16, *Dkk2* appeared in the olfactory epithelia in nasal cavities (Figure 2C). Transcripts were predominantly located in the cells of the basal layer (Figure 2C). Thereafter, *Dkk2* expression was mainly seen in the muscles of the tongue (Figure 2D).

Dkk3 showed weak and restricted expression in comparison to *Dkk1* and *Dkk2*. It overlapped with *Dkk1* and *Dkk2* in the frontonasal mesenchyme, whereas absent from the mandibular arch at E12 and E13 (Figure 2E). It was also expressed in the occipital–vertebral joint during E13 to E14 (Figure 2F). Its transcripts were barely detectable in the perichondral area in the anterior chondrocranium as well (data not shown). Thereafter, it was mainly expressed in the developing tongue and oral epithelia (Figure 2G, H).

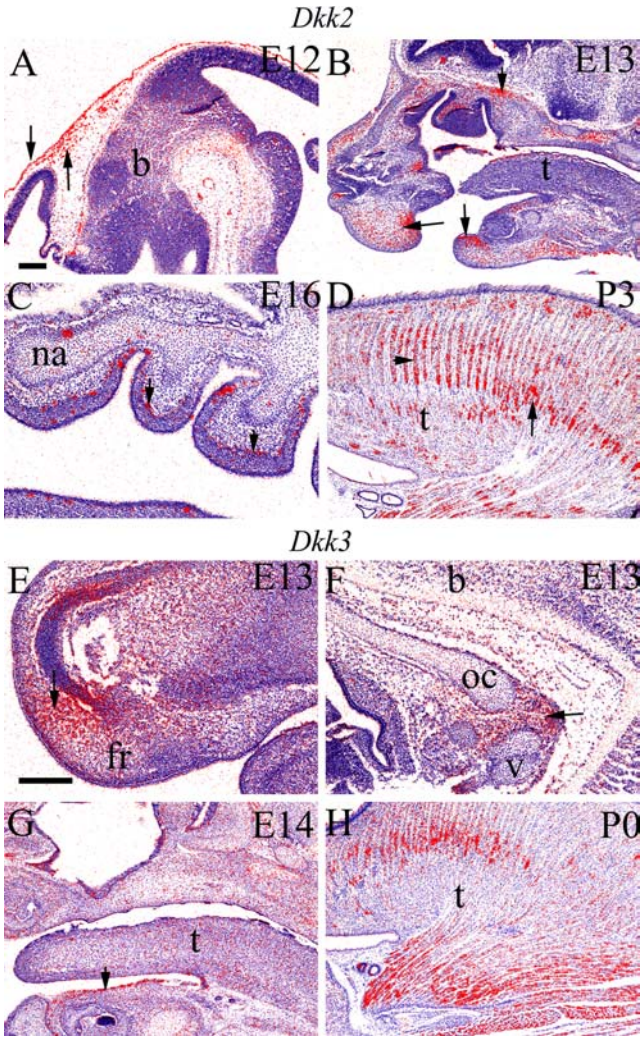

Figure 2. *Dkk2* and *Dkk3* expression in the developing face. At E12, *Dkk2* was seen in the sutural mesenchyme and the epithelia of the calvaria (A, arrows). At E13, it was seen in the first branchial mesenchyme and anterior chondrocranium (B). *Dkk2* transcripts were clustered in the olfactory epithelia at E16 (C, arrows). Postnatally, it was seen in the tongue (D). *Dkk3* was only seen in the frontonasal mass in the first branchial arch (E). At E13, it was moderately expressed in the occipital–vertebral joint (F, arrow). At E14, it appeared in the epithelium of the oral floor (G, arrow). Later it was expressed in the tongue (H). Scales bar represents 200 μm. Scale bar in (A) applies to (A, B, C, D, H). Scale bar in (E) applies to (F, G). Arrows indicate the expression. b: brain; fr: frontonasal mass; na: nasal cartilage; oc: occipital bone; t: tongue.

Discussion

Recent studies provide evidence that Dkks are important participants in the development of a number of craniofacial structures like eye and tooth (Ang *et al.* 2004, Fjeld *et al.* 2005). Study on early stage showed that they are also present in other craniofacial elements, where its roles remain to be defined (Monaghan *et al.* 1999). This study focused on *Dkk* expression at late times of craniofacial development to test whether these genes are still acting after initial pattern and induction process. The results show that *Dkk1–3* show both overlapping and differing expression during craniofacial development. Generally, *Dkk1* shows a widespread expression, while *Dkk2* overlaps to *Dkk1* in many sites; expression of *Dkk3* is restricted. The spatiotemporal expression of *Dkks* implicates important roles in multiple processes.

A major finding of this study is the high and continuous expression of *Dkks* in craniofacial muscles from the time of differentiation, as clearly seen in the tongue. The tongue is formed through outgrowth and midline fusion of paired lateral lingual swellings and a median eminence. The early tongue bud undergoes vigorous cell proliferation and expansion at early stage (Nie 2005). Myogenic differentiation starts from around E15 in tongue, earlier than trunk muscles (Shuler and Dalrymple 2001). All the studied *Dkks* show high expression within the tongue from E14 and the expression is upregulated in subsequent development, overlapping to the decreased proliferation and increased myogenic differentiation in the tongue muscles. Dkk1 was shown a negative role in myogenesis by a functional study, in which mRNA microinjection of Dkk1 inhibits head muscle differentiation and leads to small musculature in

Xonoplus (Glinka *et al*. 1998). This role is most likely through its anti- Wnt effect, for Wnt signalling is commonly a positive regulator of cell proliferation. It should be noted that members of the Wnt family were found to be functional divergent in regulating myogenic differentiation in avian (Anakwe *et al*. 2003), and the Wnt signalling shows distinct roles in regulating myogenesis in the trunk and head (Tzahor *et al*. 2003). In the craniofacial region, evidence suggests that inhibition of Wnt signalling by Frzb is required for cranial skeletal myogenesis (Tzahor *et al*. 2003). In subsequent development, Wnts undoubted play a role in regulating myogenic differentiation, and the expression of Dkks also suggested an important role in this process. Therefore, it appears that Dkk modulated Wnt signalling is an important mechanism in regulating the pace of myogenic cell proliferation and differentiation. Overlapping expression pattern and knockout approach suggests functional redundancies among Dkks in myogenic differentiation in muscles (Li *et al*. 2005).

A major function of Dkks in craniofacial development is to regulate osteogenesis and bone homeostasis, as suggested by this study and other functional analysis. Both *Dkk1* and *Dkk2* show high and widespread expression in craniofacial bones during development. Even though Dkk1 and Dkk2 are closely related to each other, evidence indicates that they have divergent roles in osteogenesis. *Dkk1* appears to be a negative regulator of osteogenesis by inhibiting Wnt/β-catenin pathway. In human multiple myeloma, expression of DKK1 in tumour cells was shown to be responsible for osteolytic lesions (Tian *et al*. 2003). Deficiency of DKK1 activity due to a mutation in Wnt receptor LPR5 is the cause of a high bone mass disorder in humans, characterized by a thickened mandible and palatine bone (Boyden *et al*. 2002, Little *et al*. 2002). Dkk2, on the other hand, is a positive regulator of osteogenesis. Dkk2 null mice are osteopenic and do not have increased bone mass, contrary to the conventional prediction (Li *et,al*. 2005). In further analysis, these authors demonstrated that Dkk2 is a stimulator of osteogenic differentiation (Li *et al*. 2005). Dkk2 is also seen in the suture mesenchyme of the cranial vault, indicating a role in the formation and regulation of the calvarial sutures. Clearly, Dkk1 and Dkk2 are critical players in maintaining the pace of osteogenic cell differentiation by differently modulating the Wnt/β-catenin pathway and other unknown mechanisms during bone development and growth. The mechanism of different functions of Dkk1 and Dkk2 in regulating bone development remains to be elucidated. These functions of Dkks raise the possibility of treating and preventing osteoporosis through regulating Dkk expression level. In addition, Dkk1 is transitory observed in hypertrophic chondrocytes during endochondral ossification of the basicranium and intramandibular part of the Meckel cartilage. These chondrocytes undergo apoptosis in subsequent development. Dkks have a role in apoptosis in limb development (Grotewold and Ruther 2002b); whether it is a apoptotic signal to chondrocytes needs further investigation.

Dkks also showed a widespread expression in the oral epithelia. This is particular clear in the oral floor and nasal olfactory epithelia. In the olfactory epithelia, *Dkk1* and *Dkk2* transcripts were widely clustered at late embryonic stage, while *Dkk3* was not observed. Olfactory epithelia is enriched in neural stem cells and characterized of being capable of regeneration after injury (Murrell *et al*. 2005). Thus, this site provides an important source of human stem cells for potential clinical therapy. So far, the roles of Dkks in olfactory epithelium development have not been studied. Whether Dkks have a specific role in regulating sensory neural progenitor differentiation within the olfactory epithelia or just show a role in epithelia development in general is unknown. Widespread expression throughout the oral epithelia also suggests a role in epithelial specification. Recent evidence suggests that Dkk1 is important for stem cell regulation (Horwitz 2004, Byun *et al*. 2005). The epithelia is enriched with stem cells, thus Dkks might also regulate the stem cell differentiation in the epithelia.

Dkk1 was observed in the mesenchyme of early palate before fusion (Monaghan *et al*. 1999). Here the author observed a high expression in the epithelia of both primary and secondary palate around the period of palatal fusion, and its expression below vomeronasal organ continued until the time of palatal fusion. The expression of *Dkk1* in restricted regions of nasal septum is an interesting observation. These parts of nasal septum fuse to the palatal shelves of the secondary palate. Thus, it is tempting to speculate that Dkk1, specifically expressed in the lower part of nasal septum, is a guiding signal for the fusion of the palatal shelves and vomer.

In this study, *Dkk1* was found in the notochord and ventral plate of the brain. In line with this expression, functional studies showed that *Dkk1* is not essential for notochord development but enhances its formation (Glinka *et al*. 1998, Kazanskaya *et al*. 2000). Notochord is an important structure that defines the formation of axial structures. Dkk1, as a strong antagonist of the Wnt/β-catenin pathway, might also play a role in the induction of its related structures. In the notochord, other critical signal such as *Shh* is also intensely expressed. It is still not clear how these signals interact to coordinate the induction process.

To sum up, Dkks are involved in craniofacial development in multiple stages. Their expression in craniofacial elements is developmentally regulated. At embryonic stage, *Dkks* are present in many kinds of tissues and organs such as the olfactory epithelia, palate, muscles, bones, and tooth. Postnatally, they are mainly present in bones and muscles suggesting long-term roles in regulating these tissues. The function and molecular mechanism need to be further elucidated in future studies.

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References

Anakwe K, Robson L, Hadley J, Buxton P, Church V, Allen S, Hartmann C, Harfe B, Nohno T, Brown AM, Evans DJ, Francis-West P (2003) Wnt signalling regulates myogenic differentiation in the developing avian wing. *Development* **130**(15): 3503–3514.

Ang SJ, Stump RJ, Lovicu FJ, McAvoy JW (2004) Spatial and temporal expression of Wnt and Dickkopf genes during murine lens development. *Gene Expr Patterns* **4**(3): 289–295.

Boyden LM, Mao J, Belsky J, Mitzner L, Farhi A, Mitnick MA, Wu D, Insogna K, Lifton RP (2002) High bone density due to a mutation in LDL-receptor-related protein 5. *N Engl J Med* **346**(20): 1513–1521.

Byun T, Karimi M, Marsh JL, Milovanovic T, Lin F, Holcombe RF (2005) Expression of secreted Wnt antagonists in gastrointestinal tissues: potential role in stem cell homeostasis. *J Clin Pathol* **58**(5): 515–519.

Davidson G, Mao B, del Barco Barrantes I, Niehrs C (2002) Kremen proteins interact with Dickkopf1 to regulate anteroposterior CNS patterning. *Development* **129**(24): 5587–5596.

De Langhe SP, Sala FG, Del Moral PM, Fairbanks TJ, Yamada KM, Warburton D, Burns RC, Bellusci S (2005) Dickkopf-1 (DKK1) reveals that fibronectin is a major target of Wnt signaling in branching morphogenesis of the mouse embryonic lung. *Dev Biol* **277**(2): 316–331.

Fjeld K, Kettunen P, Furmanek T, Kvinnsland IH, Luukko K (2005) Dynamic expression of Wnt signaling-related Dickkopf1, -2, and -3 mRNAs in the developing mouse tooth. *Dev Dyn* **233**(1): 161–166.

Glinka A, Wu W, Delius H, Monaghan AP, Blumenstock C, Niehrs C (1998) Dickkopf-1 is a member of a new family of secreted proteins and functions in head induction. *Nature* **391**(6665): 357–362.

Gonzalez-Sancho JM, Aguilera O, Garcia JM, Pendas-Franco N, Pena C, Cal S, de Garcia Herreros A, Bonilla F, Munoz A (2005) The Wnt antagonist DICKKOPF-1 gene is a downstream target of beta-catenin/TCF and is downregulated in human colon cancer. *Oncogene* **24**(6): 1098–1103.

Grotewold L, Ruther U (2002a) Bmp Fgf and Wnt signalling in programmed cell death and chondrogenesis during vertebrate limb development: the role of Dickkopf-1. *Int J Dev Biol* **46**(7): 943–947.

Grotewold L, Ruther U (2002b) The Wnt antagonist Dickkopf-1 is regulated by Bmp signaling and c-Jun and modulates programmed cell death. *Embo J* **21**(5): 966–975.

Hashimoto H, Itoh M, Yamanaka Y, Yamashita S, Shimizu T, Solnica-Krezel L, Hibi M, Hirano T (2000) Zebrafish Dkk1 functions in forebrain specification and axial mesendoderm formation. *Dev Biol* **217**(1): 138–152.

Heller RS, Klein T, Ling Z, Heimberg H, Katoh M, Madsen OD, Serup P (2003) Expression of Wnt, Frizzled, sFRP, and DKK genes in adult human pancreas. *Gene Expr* **11**(3–4): 141–147.

Horwitz EM (2004) Dkk-1-mediated expansion of adult stem cells. *Trends Biotechnol* **22**(8): 386–388.

Kawano Y, Kypta R (2003) Secreted antagonists of the Wnt signalling pathway. *J Cell Sci* **116**(Pt 13): 2627–2634.

Kazanskaya O, Glinka A, Niehrs C (2000) The role of Xenopus dickkopf1 in prechordal plate specification and neural patterning. *Development* **127**(22): 4981–4992.

Kurose K, Sakaguchi M, Nasu Y, Ebara S, Kaku H, Kariyama R, Arao Y, Miyazaki M, Tsushima T, Namba M, Kumon H, Huh NH (2004) Decreased expression of REIC/Dkk-3 in human renal clear cell carcinoma. *J Urol* **171**(3): 1314–1318.

Lee AY, He B, You L, Xu Z, Mazieres J, Reguart N, Mikami I, Batra S, Jablons DM (2004) Dickkopf-1 antagonizes Wnt signaling independent of beta-catenin in human mesothelioma. *Biochem Biophys Res Commun* **323**(4): 1246–1250.

Li X, Liu P, Liu W, Maye P, Zhang J, Zhang Y, Hurley M, Guo C, Boskey A, Sun L, Harris SE, Rowe DW, Ke HZ, Wu D (2005) Dkk2 has a role in terminal osteoblast differentiation and mineralized matrix formation. *Nat Genet* **37**(9): 945–952.

Little RD, Carulli JP, Del Mastro RG, Dupuis J, Osborne M, Folz C, Manning SP, Swain PM, Zhao SC, Eustace B, Lappe MM, Spitzer L, Zweier S, Braunschweiger K, Benchekroun Y, Hu X, Adair R, Chee L, FitzGerald MG, Tulig C, Caruso A, Tzellas N, Bawa A, Franklin B, McGuire S, Nogues X, Gong G, Allen KM, Anisowicz A, Morales AJ, Lomedico PT, Recker SM, Van Eerdewegh P, Recker RR, Johnson ML (2002) A mutation in the LDL receptor-related protein 5 gene results in the autosomal dominant high-bone-mass trait. *Am J Hum Genet* **70**(1): 11–19.

Mao B, Niehrs C (2003) Kremen2 modulates Dickkopf2 activity during Wnt/LRP6 signaling. *Gene* **302**(1–2): 179–183.

Mao B, Wu W, Davidson G, Marhold J, Li M, Mechler BM, Delius H, Hoppe D, Stannek P, Walter C, Glinka A, Niehrs C (2002) Kremen proteins are Dickkopf receptors that regulate Wnt/beta-catenin signalling. *Nature* **417**(6889): 664–667.

Mao B, Wu W, Li Y, Hoppe D, Stannek P, Glinka A, Niehrs C (2001) LDL-receptor-related protein 6 is a receptor for Dickkopf proteins. *Nature* **411**(6835): 321–325.

Monaghan AP, Kioschis P, Wu W, Zuniga A, Bock D, Poustka A, Delius H, Niehrs C (1999) Dickkopf genes are co-ordinately expressed in mesodermal lineages. *Mech Dev* **87**(1–2): 45–56.

Mukhopadhyay M, Shtrom S, Rodriguez-Esteban C, Chen L, Tsukui T, Gomer L, Dorward DW, Glinka A, Grinberg A, Huang SP, Niehrs C, Belmonte JC, Westphal H (2001) Dickkopf1 is required for embryonic head induction and limb morphogenesis in the mouse. *Dev Cell* **1**(3): 423–434.

Murrell W, Feron F, Wetzig A, Cameron N, Splatt K, Bellette B, Bianco J, Perry C, Lee G, Mackay-Sim A (2005) Multipotent stem cells from adult olfactory mucosa. *Dev Dyn* **233**(2): 496–515.

Nie X (2005) Apoptosis proliferation and gene expression patterns in mouse developing tongue. *Anat Embryol* (DOI (10).1007/s00429-005-0009-5) Sep 6: 1–8.

Semenov MV, Tamai K, Brott BK, Kuhl M, Sokol S, He X (2001) Head inducer Dickkopf-1 is a ligand for Wnt coreceptor LRP6. *Curr Biol* **11**(12): 951–961.

Shinya M, Eschbach C, Clark M, Lehrach H, Furutani-Seiki M (2000) Zebrafish Dkk1, induced by the pre-MBT Wnt signaling, is secreted from the prechordal plate and patterns the anterior neural plate. *Mech Dev* **98**(1–2): 3–17.

Shuler CF, Dalrymple KR (2001) Molecular regulation of tongue and craniofacial muscle differentiation. *Crit Rev Oral Biol Med* **12**(1): 3–17.

Tian E, Zhan F, Walker R, Rasmussen E, Ma Y, Barlogie B, Shaughnessy JD Jr (2003) The role of the Wnt-signaling antagonist DKK1 in the development of osteolytic lesions in multiple myeloma. *N Engl J Med* **349**(26): 2483–2494.

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.

Wirths O, Waha A, Weggen S, Schirmacher P, Kuhne T, Goodyer CG, Albrecht S, Von Schweinitz D, Pietsch T (2003) Overexpression of human Dickkopf-1, an antagonist of wingless/WNT signaling, in human hepatoblastomas and Wilms' tumors. *Lab Invest* **83**(3): 429–434.