The Crucial Role of Wntless in Osteogenesis and Odontogenesis

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Wnt signalling pathways have been the focus of intense research activity for decades due to their fundamental role in skeletal and dental development. Wntless, an exclusive chaperone protein for the exocytotis of Wnt ligands, was identified in 2006. In the last decade, the molecular biological studies of Wntless and its genetic studies in human and mice have highlighted the importance of this protein in mineralised tissues, including bone, cartilage and teeth. This article reviews recent developments and discrepancies in the role of Wntless in skeletal and dental development based on mutant phenotypes, as well as the underlying mechanism involved in its molecular and physiological regulation. We conclude that, though some controversial phenotypes exist due to different Cre line resources, Cre recombinase activity and detection time points, Wntless undeniably exerts a context- and stage-dependent regulatory function during the development and homeostasis of both skeletal and dental tissue.

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It is widely accepted that the Wnt signalling pathway plays an important role in the entire development process of mineralised tissue. Linkage analysis in several monogenic bone disorders, such as osteoporosis pseudoglioma (OPPG), high bone mass (HBM), sclerosteosis and Paget's disease, have yielded important advances in recent years and highlighted the importance of the Wnt signalling pathway in the regulation of bone mass and bone turnover[1-9]. With regard to tooth development, it has been found that excessive activated and inactivated Wnt signalling pathways can cause supernumerary teeth with odontomas (tumour-like malformations consisting of multiple small teeth)[10,11] or a decreased number of teeth, respectively[12-14]. Wnt proteins (Wnts), a family of secreted cysteine-rich glycoproteins, are the major ligands that activate the membrane receptor complex of the Wnt signalling pathway[15,16]. Thus far, a total of 19 Wnts have been found in the mammalian genome during cell–cell communication. Most of them are reported to be involved in skeletal and dental development in vivo and in vitro[4,5,7-9,17-25].

As an exclusive chaperone protein for transporting Wnts, Wntless (WLS), also called G protein-coupled receptor 177 (GPR177), was first identified in Drosophila by three independent groups in 2006[26-28]. It belongs to a family of highly conserved proteins present in both vertebrate and invertebrate genomes and is evolutionarily conserved among all mammals[26,27]. More recently, it has been consistently confirmed that WLS is indispensable to the activation of Wnt signalling pathways

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throughout embryonic development[29,30]. Studies on the function of WLS during mineralised tissue development did not raise any concern until the past ten years. Due to the advancements made in molecular biology, transgenic approaches and others, an increasing number of studies have highlighted the important role of WLS in mineralised tissue development and the pathogenesis of bone metabolic diseases. This review places emphasis on the role of WLS during skeletal and dental development, and both the characteristics of *WLS* mutations and the underlying mechanisms will be summarised systemically.

WLS mutation/deletion interrupts osteogenesis and odontogenesis

In mammals, *WLS* is mapped to the short arm (p) of chromosome 1 at position 31.3 (1p31.3) in humans and 3;3H4 in mice. In humans, the gene contains 30 distinct gt-ag introns and 55 alternative exons, whereas in mice, it contains 13 distinct gt-ag introns and 22 alternative exons. Its genetic transcription can be detected in developing mineralised tissues, including skeletons, incisors and molars, via in situ hybridisation[30]. Clinical studies using techniques like genome-wide association studies (GWAS) and gene mutation analysis found that *WLS* mutation may be closely related to the pathological development of several bone diseases in humans. Additionally, as laboratory studies of WLS have progressed, it has been found that inactivation of WLS could severely impair the development of the craniofacial, trunk and appendicular skeletons, as well as the teeth. These findings indicated that WLS is required during intramembranous and endochondral ossification and tooth development.

WLS mutation related to severe skeletal and dental disease in humans

Genetic studies analysing the pathogenic mechanisms of bone diseases have demonstrated that WLS may be a bone mineral density (BMD)–related gene that regulates bone metabolism or an oncogene for osteosarcoma. BMD is a highly heritable trait and a major predictor for the risk of osteoporotic fracture[31]. Mutants within *WLS* have been identified and associated with BMD in several previous GWAS, some of which have even observed more than one independent association signal originating from the locus[32-38]. A large meta-analysis identified the rs2566752 single nucleotide polymorphism (SNP) as the maximally associated variant in WLS for BMD in the lumbar spine and femoral neck[39]. Moreover, investigators have demonstrated that *WLS* is a BMD-related gene common to both Europeans and East Asians irrespective of race[40,41]. Osteosarcoma is an aggressive bone tumour that preferentially develops in adolescents. Disordered Wnt signalling pathways and frequent overexpression of *WLS* were reported to be found in various osteosarcoma sets, suggesting that *WLS* could be a new candidate oncogene involved in osteosarcoma tumorigenesis[42-45]. Foetuses with mutants on chromosome 1p32-p31, where *WLS* and others localised, presented intrauterine growth restriction, macrocephaly, facial dysmorphism and other malformations[46,47]. In addition, nonsyndromic cleft lip with or without cleft palate and early onset osteoporosis in osteogenesis imperfecta (OI) have been proven to be associated with genetic variations in several Wnts in humans[22,48]. These reported cases that were involved with mutations of *WLS* or *Wnts* suggested WLS may play a role during skeletal development.

Intricate control of Wnt signalling activity is also necessary for normal tooth development, since both inhibition and stimulation of Wnt signalling caused by *AXIN2* or *WNT10B* mutations lead to tooth agenesis in humans[12,49]. In addition, *WNT10A* nonsense mutation was shown to be related with odonto-onycho-dermal dysplasia, a rare autosomal recessive inherited form of ectodermal dysplasia[21]. Until now, however, there has been no direct evidence of odontogenesis defects caused by *WLS* mutations in humans.

Wls impairs the development of mineralised tissue in mice

Homozygous inactivation of *Wls* in mice resulted in embryonic lethality due to impairment of the patterning of the anterior–posterior axis[29]. To overcome the early embryonic lethality associated with the inactivation of *Wls* globally, mice carrying a *Wls-flox* ($Wls^{flox/flox}$) allele, permitting the ablation of *Wls* by Cre-mediated recombination, have been created. So far, there are two lines of $Wls^{flox/flox}$ mice. In one of the alleles, exon 1 is chosen as a target[50], while in the other, an loxP site is inserted flanking exon 3, since exon 3 encodes the first transmembrane domain of WLS and its removal would cause an out-of-frame deletion[51]. In this review, mouse models with Wls deficiency in specific tissue and cell types were generated by crossing $Wls^{flox/flox}$ mice with diverse Cre lines, as summarised in Table 1.

Using conditional knockout (cKO) methods, numerous groups reported that WLS is required for the development of each germinal layer during early embryo development, and its inactivation in any germinal layer would interrupt skeleton formation. Mesenchymal *Wls*

Table 1 Summary of skeletal and dental phenotypes in mouse models with *Wls* alterations.

Knockout site	Flox exon/Cre	Stage	Skeletal and dental phenotype(s)
Conventional germline	-/-	E10.5	Embryonic lethality (E10.5) due to impairment of the patterning of the anterior-posterior axis[29]
	Exon1/*Ella-Cre*	E6, E8.5	Embryo arrested at E6, and 9 of 10 were reabsorbed at E8.5[50]
Mesenchyme	Exon1/*Wnt1-Cre*	E10.5	Shortened anteroposterior axis at E10.5[50]
	Exon3/*Wnt1-Cre*	E18.5	Severe abnormalities in the craniofacial skeleton at E18.5[51]
	Exon3/*Prx1-Cre*	E12.5, E16.5	Hypoplastic and shortened skeletons with truncated autopods at E16.5 with delayed skeletal ossification[52]
	Exon3/*Dermo1-Cre*	E15.5	Severely impaired development of the craniofacial skeleton and appendicular long bones at E15.5 with defective intramembranous ossification and endochondral ossification[54]
	Exon1/*Dermo1-Cre*	E15.5	Embryo lethality after E15.5; reduction in mineralised bone without ectopic cartilage formation[55]
	Exon1/*En1-Cre*	E13.5–E18.5	Survive until birth; reduced cranial bone differentiation and mineralisation[55]
	Exon3/*Wnt1-Cre*	E11.5–E18.5	The anterior palatal shelves (PS) failed to grow vertically at E13.5 and wide open cleft secondary palate at E16.5[56]
	Exon1/*Dermo1-Cre*	E14.5	Outgrowth defect in limbs and digit specification; little mineralisation and decreased cartilage content at E14.5; did not survive beyond E15.5[53]
	Exon3/*Wnt1-Cre*	E10.5	Minor but no noticeable defects with formation of the nasal pit at E10.5[57]
Ectoderm	Exon3/*Msx2-Cre*	E12.5, P0	Truncated limbs at the level of autopod and zeugopod; impaired intramembranous ossification and suture fusion in the skull at P0[52]
	Exon3/*K14-Cre*	E16.5, E18.5	Arrested tooth development at the early cap stage; abrogated tooth-forming capability of the dental epithelium, without impairing odontogenic capability in the mesenchyme[69]
	Exon1/*Crect*	E18.5	Perinatal lethality, hypoplastic face without upper or lower jaw, no mineralisation in the skull vault; ectopic cartilage[55]
	Exon1/*K14-Cre*	E18.5	Normal skull bone ossification[55]
	Exon3/*K14-Cre*^{ER}	2 m after TAM	Digital bone resorption[58]
	Exon3/*Foxg1-Cre*	E16.5	Severe facial deformities; failed to form several key features within the upper face, including upper nasal, upper jaw and ocular structures at E16.5[57]
	Exon3/*Shh-Cre*	E18.5, P0	Smaller molars at E17.5 with thinner dentine matrix and reduced enamel matrix proteins at P0[70]
Endoderm	Exon1/*Shh-Cre*	E11.5–E18.5	Inhibited formation of tracheal–bronchial cartilaginous rings; loss of tracheal mesenchymal dorsal–ventral patterning[59]
Osteoblast progenitors	Exon3/*Osx-Cre*	E15.5, P0	No obvious defect in development and mineralisation of the craniofacial bones at E15.5; delayed calvaria mineralisation (most likely caused by the Cre transgene but not the Gpr177 deletion) at birth[54]
	Exon1/*Osx-Cre*	E18.5, P0	Shorter bones; obvious constrictions with some broken at femurs, tibias, humeri, radii and ulnae and ribs[62]
Immature osteoblast	Exon3/*Col1a1-Cre*	P14	Dose-dependent dwarfism; decrease in bone mass accrual[64]
	Exon3/*Col1a1-Cre*	E17.5, P10–P14	Slight delay in chondrocyte hypertrophy at E17.5; significant defects in skull ossification and vertebral organisation coupled with ectopic cartilage formation after P10; severe osteopenia at P14[63]
	Exon3/*Col1a1-Cre*	E15.5, P0	Mutants' calvaria and limb bone development did not show any deformities as embryos or at birth[54]

Knockout site	Flox exon/Cre	Stage	Skeletal and dental phenotype(s)
Mature osteoblast, odontoblasts, cementoblasts, ameloblasts	Exon1/*OC-Cre*	P20	Gradual severe low bone mass phenotype at both trabecular and cortical bone as early as P20 (20 d of age); frequent spontaneous fractures early in life with decreased bone formation and increased matrix resorption[65]
	Exon3/*OC-Cre*	120 d 7 m	Reduction in cortical bone thickness while no changes in other bone properties within trabecular and cortical bone at 7 months old; significant decrease in body weight after P120 [63]
	Exon1/*OC-Cre*	2 m 3 m	Dramatic reduction in bone volume and BMD of craniofacial skeleton, including both cranial neural crest–derived and mesoderm-derived skeletal elements, without affecting the size of skeleton[66]
	Exon1/*OC-Cre*	2 m 3 m	Significant increase in dentine volume and density[66]
	Exon1/*OC-Cre*	3 m	Pathologically increased periodontal width, thinner alveolar bone[73]
	Exon1/*OC-Cre*	1 m 3 m	Pathological root resorption with an increase in osteoclast activity and decrease in osteoblast activity[71]
	Exon3/*OC-Cre*	8 m	Strikingly rescued the wavy mineralised structures in incisors of Tgfbr2 mutant mice[68]
	Exon1/*OC-Cre*	P8, P14, P28, P56	Severe defects in dentine formation and root elongation with remarkably decreased dentine thickness, enlarged pulp chambers and root canals[74]
	Exon1/*OC-Cre*	E18.5, P5, P50	No gross defects in the skeleton up to E18.5, but showed less bone mass in the skull (but not long bones) from P5[53]
	Exon1/*OC-Cre*	2 m	Malformation of cementum-type transition with less apical cellular cementum[72]
Mature osteoblast, osteocyte	Exon3/*Dmp1-Cre*	3 w–6 m	No apparent defects in early bone formation; disrupted bone remodelling with severely low BMD at a later stage[67]
Chondrocyte	Exon3/*Col2a1-Cre*	E16.5, E18.5, P0	Dwarfism; defective perichondrial mineralisation and endochondral bone formation; agenesis of craniumbase bone at embryos; delayed suture fusion in frontal bones at P0[64]
	Exon3/*Col2a1-Cre*	E15.5, E17.5	Impaired endochondral ossification with defective axial and appendicular bone formation[54]
	Exon1/*Col2a1-Cre*	E16.5, E18.5	Delayed mineralisation in humerus and other bones (except calvarial bone) at E16.5 and E18.5; impaired cartilage development at E16.5; died shortly after birth due to poor mineralised ribs[53]

knockout mice exhibited a shortened anteroposterior axis[50], hypoplastic skeletons with truncated autopods[52,53] and severe abnormalities in the craniofacial skeleton, including clefts involving the secondary palate[51,54-56]. In addition, the deletion of ectodermal *Wls* could result in distal limb agenesis, abnormal digital bone regression, impaired upper facial structures, intramembranous ossification and suture fusion in the skull[52,55,57,58]. Moreover, deletion of *Wls* in endoderm inhibited the formation of tracheal–bronchial cartilaginous rings with the abnormal dorsal–ventral patterning of tracheal mesenchyme, tracheal cartilage and smooth muscle[59]. This demonstrated that, although skeletal tissue is derived from mesoderm and neural crest, *Wls* in ectoderm and endoderm can also regulate their development via the secretion of Wnt ligands.

The osteoblastic lineage is a heterogenous population with signature gene expression at diverse differentiation stages. In perinatal mice, Osterix (OSX) appears to be expressed in osteolineage-restricted progenitors and continues to be expressed as the cells divide and differentiate into osteoblasts[60,61]. Osteoblasts begin expressing Col1a1 at an immature stage, followed by osteocalcin (OC) expression as they fully mature, before some of them are eventually embedded in the bone matrix and become osteocytes[62]. Therefore, several related Cre lines mediated *Wls* knockout mice models constructed to study the role of WLS at diverse osteoblastic differentiation stages. Maruyama et al[54] found that WLS may be dispensable for the osteoblast precursors, as the mutants' calvaria and limb bone did not show any deformities at embryos E15.5 or P0 in the cKO mice using *Osx-Cre*. However, other investigators have demonstrated that Wls mutation in OSX-expressing osteoblast precursors could result in shorter bones with obvious constrictions and breaks at E18.5 and P0[62]. Wls mutation in the *Col1a1-Cre* line could cause severe osteopaenia with significant defects in the

skull ossification and vertebral organisation at P10-14, although they found no obvious bone defects at the embryo or newborn stage[54,63,64]. Using the *OC-Cre* line, Wan et al[63] proved that mice with osteoblast-specific WLS mutation only displayed reductions in cortical bone thickness with no changes in the other properties of trabecular and cortical bone at 7 months old, whereas Zhong et al[53] demonstrated that their mutants showed less bone mass in the skull from 5 days old, and a significantly lower BMD after weaning, with most spontaneous fractures in early life[65]. Additionally, Lim et al[66] demonstrated that osteoblast-specific Wls mutants caused a dramatic reduction in bone volume and BMD of both cranial neural crest–derived skeletal elements and mesoderm-derived skeletal elements at 2 and 3 months old. Our previous study also showed that the *Wls* mutant in mature osteoblasts and osteocytes led to severe osteoporosis in both long bone and craniofacial bone at a late stage[67]. As for chondrogenesis and endochondral ossification, after the depletion of Wls in the chondrocytes and perichondrium, the *Col2a1-Cre*;*Wls*$^{flox/flox}$ mouse exhibited dwarfism with defective endochondral ossification and ectopic chondrogenesis along with agenesis of the cranium base bones and delayed suture fusion in the frontal bones[53,54,64]. We noticed that the controversial phenotypes existed with the same Cre line during osteogenesis, such as *Osx-Cre*[54,62] and *OC-Cre*[53,63,65,66], perhaps related to the two different condition alleles of *Wls* they used. In general, the phenotypes seem more severe when using the conditional null allele with loxP sites flanking exon 1[53,62,65,66] compared with exon3[54,63].

Taken together, during the morphogenesis of skeletal tissues, though some controversial phenotypes existed due to different Cre line resources, conditional null alleles, Cre recombinase activity and detection time points, it is undeniable that WLS have an indispensable function in bone development and homeostasis.

WLS was found to be expressed throughout the dental epithelium and mesenchyme[68], suggesting it may play a role in odontogenesis. Zhu et al[69] reported that inactivation of Wls in the whole oral and dental ectoderm by *K14-Cre* leads to arrest of early tooth development at the early cap stage of E14.5-E16.5 and regresses at E18.5, and *Wls* mutation in the odontogenic *Shh-Cre* line leads to smaller molars at E17.5 with a thinner dentine matrix and reduced enamel matrix proteins at P0[70]. In addition to osteoblasts, odontoblasts, cementoblasts and ameloblasts also express OC[66]. As a result, deletion of *Wls* using the *OC-Cre* line caused pathological root resorption[71], malformation of cementum-type transition with less apical cellular cementum[72] and thinner alveolar bone with a wider and disordered periodontal ligament space at 1, 2 and 3 months old[73]. In particular, Lim et al[66] found that loss of Wls in OC-expression cells leads to a significant increase in dentine volume and density in mouse incisors at 2 and 3 months old. In similar mouse models, however, Bae et al[74] discovered that loss of Wls in OC-expression cells leads to decreased dentine thickness, enlarged pulp chambers and root canals, and shortened roots in mouse molars at P28. In addition, Yang et al[68] confirmed that WLS may be involved in the regulation of dentine structures since *Wls* mutants could dramatically decrease the number of wavy mineralised structures caused by a Tgfbr2 deficiency.

Molecular mechanism of WLS

WLS is predicted to contain a long N-terminal region, seven or eight transmembrane segments and an intracellular C terminus based on amino acid sequence analysis[30]. It belongs to a family of highly conserved glycoproteins, and is predominantly localised in the Golgi apparatus in a variety of tissues and cells[30]. Studies have confirmed that the N-linked glycosylation of WLS is necessary for proper transportation in the secretory pathway[27,29]. In recent years, significant progress has been made in studies on the regulation mechanisms of WLS during mineralised tissue development.

Related signalling pathway regulation mechanism

It is reported that the expression level of WLS may contribute to the promotion or inhibition of Wnt signalling activity in a complex feedback loop[75], and the reciprocal regulation of Wnts and WLS is essential for the Wnt-dependent establishment of body axes during early embryogenesis[30]. For example, WLS has been proven to be not only an upstream regulator for the secretion and gradient formation of Wnts[76,77], but possibly also a direct target of Wnt signalling pathways activated by β-catenin and Lef/Tcf[27,29]. It has been reported that Notum, which is critical for BMD and dentine morphogenesis, can be induced by WLS-mediated Wnt signalling[78-80]. Notum functions as a lipase that inactivates WNTs by cleaving the palmitoleate moiety and inhibits upregulated Wnt signalling in turn[79]. Furthermore, studies have found that some noncanonical Wnts secreted by WLS can also regulate FGF, BMP, SHH, JNK and TGF-β signalling pathways[57,68]. WLS is also a positive regulator for the NF-kB signalling pathway which is a requisite in embryonic development, especially for bone[81] and teeth[82-84].

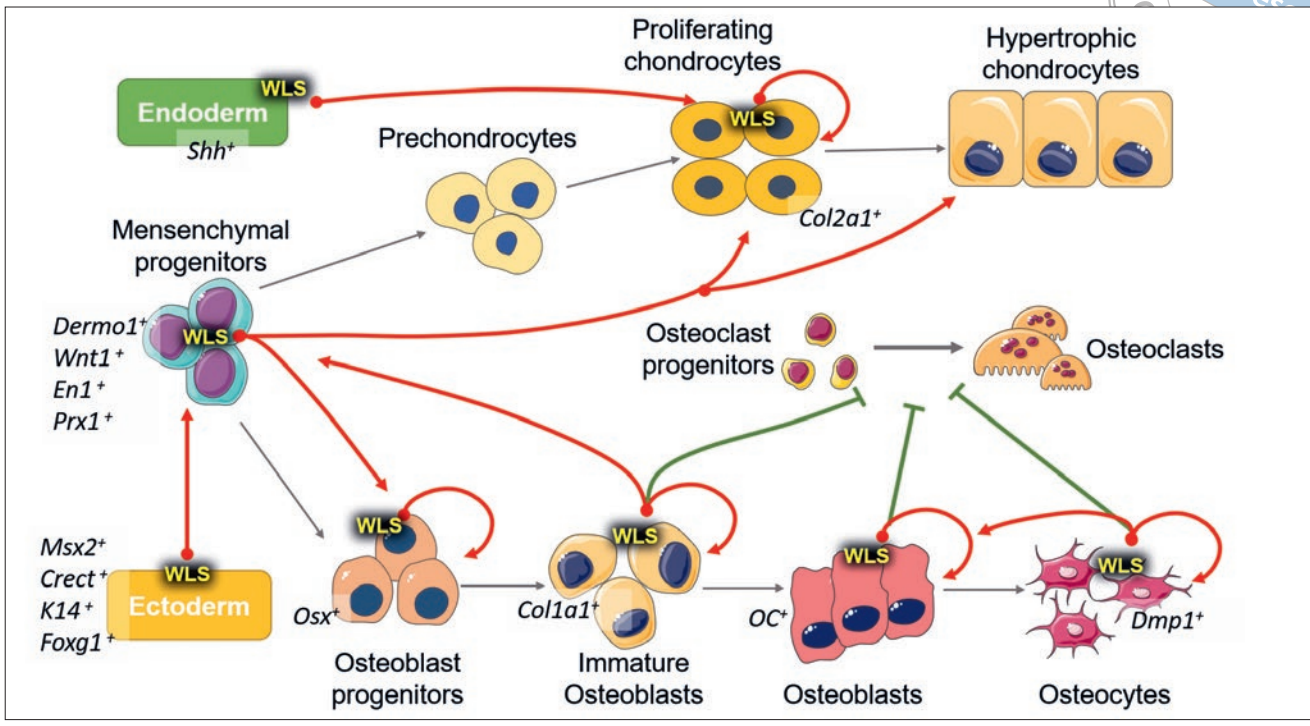

Fig 1 Regulatory roles of WLS on the various differentiation stages of osteolineage, chondrolineage, and osteoclast lineage cell. Mesenchymal WLS promote the expansion and differentiation of osteoblast precursor cells during intramembranous ossification, the expansion and maturation of the proliferating and prehypertrophic chondrocyte and the subsequent endochondral ossification. Cranial ectoderm WLS promote osteoblast progenitor specification and cell proliferation in the mesenchyme. WLS in the embryonic endoderm promote the proliferation of chondroblasts and tracheal cartilage patterning in mesenchyme. WLS in the Osx-expressing osteoprogenitors promotes the differentiation and proliferation of themselves. In Col1a1 positive osteoblasts, WLS can sustain osteoblast survival, proliferation and differentiation, suppress osteoclastogenesis and confer a niche for BMSC self-renewal. WLS in OC-expressing cells and Dmp1-expressing cells promote the osteoblastic differentiation and mineralization activity and inhibit osteoclastogenesis. WLS in Col2a1-expressing cells is indispensable for chondrocyte hypertrophy in the growth plate and endochondral ossification.

Biological function of WLS in osteogenesis

Recently, many studies have examined the essential biological mechanism of WLS in skeleton development, as shown in Fig 1. In the conventional Wls deletion mice model, WLS was shown to act downstream of WNT3 and regulate its signalling in early patterning of the A-P axis[29]. WLS mediated Wnt signals produced from each germinal layer may function distinctly during early embryo development. Mesenchymal WLS, including limb mesenchymal and cranial mesenchymal, plays an essential role in the expansion and differentiation of osteoblast precursor cells during intramembranous ossification, the expansion and maturation of the proliferating and prehypertrophic chondrocyte and the subsequent endochondral ossification, by mediating the secretion and function of both canonical and non-canonical Wnt ligands[50-54,56]. Cranial ectoderm WLS has been reported to be essential for osteoblast progenitor specification and mesenchymal canonical Wnt signalling response because its deletion in cranial ectoderm leads to diminished expression of *Lef1*, *Axin2*, nuclear *β-catenin* and a subset of mesenchymal Wnts, such as *Wnt5a, Wnt11*, *Wnt3a* and *Wnt16*[55]. More recently, studies have also shown that WLS in ectoderm, including facial, neural and limb, is important for promoting cell proliferation and inhibiting cell death both in the ectoderm and underneath the mesenchyme through modulation of the canonical Wnt signalling and BMP/FGF/JNK signalling axes[52,57,58]. Deletion of Wls in the embryonic endoderm using *Shh-Cre* could inhibit the proliferation of chondroblasts and the tracheal cartilage patterning in mesenchyme due to the alterative Wnt/β-catenin signalling activity[59].

Tan et al[62] reported that WLS in Osx-expressing osteoprogenitors is indispensable for regulating their differentiation and proliferation by inducing a canonical Wnt signalling response at the embryo and newborn stages, and concluded that the phenotype is caused by loss of WLS in undifferentiated osteolineage pro-

genitors instead of their differentiated derivatives and chondrocytes. In Col1a1-positive osteoblasts, WLS is required to sustain osteoblast survival, proliferation and differentiation through upregulation of canonical Wnt signalling activity[63,64]. On the other hand, it could also suppress osteoclastogenesis by modulating the expression levels of osteoblast-derived OPG, RANKL and M-CSF, and the secretion of osteoblastic Wnts (such as WNT5A) in a paracrine way, suggesting the WLS-mediated feedback loop is also necessary in osteoblast–osteoclast communication[63]. In addition, osteoblastic WLS is crucial for BMSC self-renewal and maintenance through its regulation of *Wnts*, such as *Wnt10b*[63]. However, Maruyama et al[54] reported that WLS in Osx-positive osteoprogenitors and Col1a1-positive osteoblasts is apparently dispensable during intramembranous and endochondral ossifications at the embryo and newborn stages. This is consistent with the fact that the immature osteoblast-specific Wls deletion by *Col1a1-Cre* did not show any change in the embryonic skeleton[54,63]. Like *Col1a1-Cre* induced Wls cKO mice, deletion of Wls in *OC-Cre* expressing osteoblasts (mature osteoblasts) also shows little effect on embryonic bone development[53,63,65]. Thus, it can be speculated that osteoblast-derived Wnts may be dispensable for embryonic skeletal development. However, Wls deficiency in mature osteoblasts seems to influence cell differentiation and mineralisation via canonical Wnt signaling, since these cells showed downregulated alkaline phosphatase activity and decreased expression levels of *Osx*, *OC* and *Axin2*[63,65,66]. It has also been shown that Wls mutation in *OC-Cre* expressing cells leads to an increased number and increased activity of osteoclasts even without a significant decrease in the OPG/RANKL ratio both *in vivo* and in vitro, suggesting that this might result from decreased exposure to Wnt ligands which exert complex, stage-dependent effects on osteoclast differentiation[65]. In a previous study, we also demonstrated that Wls cKO in the *Dmp1-Cre* mouse line, which are expressed in a subset of osteoblasts but mainly osteocytes, disrupted both perilacunar/canalicular remodelling mediated by osteocytes and the balance of osteogenesis and bone resorption at the bone surface mediated by osteoblasts and osteoclasts, at least partly through the canonical Wnt/β-catenin signalling pathway and the OPG/RANKL signalling pathway[67].

Comparing the cartilage and bone phenotypes of Wls cKO mouse models using a set of similar *Col2a1-Cre* lines, it was consistent that Wls cKO could cause delayed chondrocyte hypertrophy in the growth plate and impaired endochondral ossification by blocking canonical Wnts, such as *Wnt10b*[53,54,64]. Since *Col2a1-Cre* has also been proven active in osteoblasts and osteocytes, it cannot be concluded whether the decreased mineralisation of calcified cartilage in *Col2a1-Cre;Wls*$^{flox/flox}$ mutant mice is secondary to the chondrogenic defects or due to the diminished Wnts secretion by osteoblasts/osteocytes[53]. However, subtle differences were found among the examined mutant mice. Zhong et al[53] and Lu et al[64] reported an ectopic cartilage formation caused by the reduction of canonical Wnts and a disrupted orientation of proliferating chondrocytes resulting from downregulation of non-canonical Wnts, such as *Wnt5a* and *Wnt5b*; however, these were not observed in the study conducted by Maruyama et al[54], perhaps due to the timing of observations.

In summary, the above observations reveal the critical role of WLS in various cell types in osteogenesis and chondrogenesis.

Biological function of WLS in odontogenesis

Wls transcripts have been detected in both the dental epithelium and mesenchyme during early tooth development, implying WLS should be essential for odontogenesis[68]. Zhu et al[69] showed that WLS in the oral and dental epithelium is necessary for the activation of canonical Wnt signalling in the dental epithelium and the formation of a functional enamel knot without altering the odontogenic programme in the mesenchyme. The same team of authors also found that dental epithelial Wls cKO using the *Shh-Cre* line leads to aberrant cell proliferation in both the dental epithelium and mesenchyme with unaffected cell apoptosis at E16.5, and the downregulated canonical Wnt signalling activity in the inner enamel epithelium and mesenchyme at the early bell stage leads to defective differentiation of ameloblasts and odontoblasts, indicated by decreased transcriptional levels of *amelogenin*, *dentine sialophosphoprotein* (*Dspp*), *Col1a1*, *Osx* and *Nestin*[70].

Lim et al[66,71,73] demonstrated that WLS is also indispensable for the homeostasis of dental mineral tissue. In an *OC-Cre;Wls*$^{flox/flox}$ mouse model, the homeostasis of the periodontal complex and cementum was disturbed with spontaneous root resorption, thinner alveolar bone and an increased periodontal ligament space, perhaps due to a reduction in osteoblast function together with an increase in osteoclast activity[71,73]. A significant increase in dentine volume and density was also found at 3 months old, since the RUNX2-mediated repression of dentine sialoprotein (DSP) is relieved and odontoblast differentiation is accordingly enhanced because of the reduced activation of Wnt signalling pathways[66]. However, another study of the same mouse model

found a reduced dentine apposition during early postnatal development (P14, P28, P56), and suggested that Wls deletion in odontoblasts did not influence initial odontoblast differentiation but significantly inhibited its maturation, causing abnormalities in dentine apposition with downregulated *Wnt10a*, *Col1*, *DSP* and *β-catenin* in the odontoblasts[74]. These opposite phenotypes in dentine apposition may be due to mouse strains with different timing of expression of *OC-Cre* recombinase, and the different developmental biology between incisors and molars[74]. Yang et al[68] also showed that the interaction between WLS and TGF-β signalling is crucial in the mineral tissue homeostasis of the tooth as Wls cKO partially rescued the excessive Wnt signalling in *OC-Cre*;*Tgfbr2*$^{fl/fl}$ mutant mice. Therefore, although discrepancies exist, it can be concluded that WLS, either in the epithelium or mesenchyme, plays an indispensable role in tooth development and homeostasis.

Conclusion

WLS, a cargo receptor that mediates Wnts secretion, is one of the indispensable components of canonical or noncanonical Wnt signalling during bone and tooth development. Together with gene knockout strategies, WLS is a useful tool for studying the role of Wnts from each cell type during mineralised tissue development. This review hopes to provoke interest among researchers to elucidate the autocrine or paracrine function of Wnts in skeletal and dental development and use WLS as a therapeutic target for the treatment of bone or tooth disease in the future.

Conflicts of interest

The authors declare no conflicts of interest related to this study.

Author contribution

Dr Jia Hui DU performed the literature search and data analysis and wrote the draft; Drs Xin Quan JIANG and Shu Xian LIN devised the idea for the article. All authors revised the paper critically for intellectual content and approved the final version.

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