REVIEW / *Derleme*

Palatogenesis: The Role of Sonic Hedgehog Signaling in the Secondary Palate Development

Palatogenez: Sekonder Damak Gelişiminde Sonic Hedgehog Sinyalinin Rolü

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Abstract

Palatogenesis is a complicated and precisely balanced process in which malfunctions induce congenital cleft palate, one of the most common embryonic developmental anomalies seen in newborns. Multiple signaling pathways and transcription factors have been implicated in palatal shelves development. The purpose of this article is to review one of the most important signaling pathways that plays a critical role in secondary palate development, namely Sonic hedgehog (Shh) signaling pathway. It includes an overview of the expression patterns of members of the Hedgehog signaling pathway and the role of Shh signaling in the reciprocal epithelial-mesenchymal interactions during secondary palate development.

Key words Sonic hedgehog (Shh); Hedgehog signaling pathway; palatal shelves; epithelial-mesenchymal interaction; cleft palate; cell proliferation.

Öz

Palatogenez, malfonksiyonları yeni doğanda en sık görülen embriyonik gelişimsel anomalilerden biri olan konjenital yarık damağa yol açan, karmaşık ve kusursuz olarak dengelenmiş bir süreçtir. Çok sayıda sinyal yolakları ve transkripsiyon faktörleri palatal çıkıntıların gelişimi ile ilişkilidir. Bu makalenin amacı, sekonder damak gelişiminde kritik rol oynayan en önemli sinyal yolaklarından biri olan Sonic hedgehog (Shh) sinyal yolağı hakkında derleme yapmaktır. Bu çalışma, sekonder damak gelişimi sırasında oluşan karşılıklı epitelyal-mezenkimal etkileşimlerde Hedgehog sinyal yolağı üyelerinin ekspresyon modellerini ve Shh sinyalinin rolünü genel olarak değerlendirmektedir.

Anahtar kelimeler *Sonic hedgehog (Shh); Hedgehog sinyal yolağı; palatal çıkıntılar; epitelyal-mezenkimal etkileşim; yarık damak; hücre çoğalması*

INTRODUCTION

In mammals, the oral cavity and the nasal cavity are separated by the palate which consists of a bony hard part anteriorly and a muscular soft part posteriorly. The anterior hard palate is critical for a normal speech and feeding, while the posterior movable muscular soft palate plays an important role during swallowing by closing off the nasal airway.[1] Cleft palate is one of the most common congenital malformations that can be caused by genetic or environmental perturbations during palatal development.[1] Palatogenesis depends on complex spatio-temporal networks of growth factors and transcription factors that are crucial for normal palate development.[1-3] The secondary palate begins to develop in mice at embryonic day 11.5 (E11.5) by the outgrowth of the palatal shelves (PS) from the oral side of the maxillary processes bilaterally. At first, the palatal shelves grow downwards on either side of the tongue (from E11.5 to E14.5), then they undergo a rapid elevation to a horizontal position and become oriented toward each other above the tongue (from E14.5 to E15). The elevated palatal shelves grow toward each other and adhere to form the median epithelial seam (MES) which then disappears to allow the fusion of the palatal shelves.[1,2] The palatal shelves consist mainly of neural crest-derived ectomesenchymal tissue core that is covered on the outside by stomodeum-derived epithelium.[1-3] Multiple signaling pathways and transcription factors regulate reciprocal epithelial-mesenchymal interactions that control the growth and patterning of the palatal shelves.[1-3] Sonic hedgehog (Shh) is a member of the Hedgehog (Hh) family of secreted proteins[4] and appears to be the key regulator of palatal shelves development.[3,5] The Hh signaling cascade is regulated by numerous factors at various stages, from modifying and releasing ligands to receiving and transducing signals.[6,7] Signaling is mediated by binding of Shh ligands to the twelve-pass transmembrane receptor patched 1 (Ptch1) in the recipient cells.[7] Cell Adhesion Molecule-Related/Downregulated by oncogenes (Cdo), Biregional Cdo-binding protein (Boc), and Growth arrest-specific 1 (Gas1) are co-receptors that are required for proper signaling.[8-11] In the absence of the Shh ligand, Ptch1 inhibits the pathway activity by acting as a ligand-independent inhibitor for the transmembrane protein Smoothened (Smo), which is required for the intracellular transduction of the Shh signaling.[7,12,13] This inhibition leads to the formation of the repressor forms of Glioma-associated oncogene (Gli) family of transcription factors, Gli3[6,7] However, binding of Shh ligand to Ptch1 leads to the activation of Smo that translocates to the cilium and allows pathway activation.[6,7] Ptch1 rapidly mediates sequestration and degradation of Shh to re-establish quiescence in recipient cells.[14,15] This process impacts the concentration and duration of signal activity in the determination of the cellular response.[14,16] Activation of Smo activates the intracellular signaling cascade through the modification of Gli protein transcriptional activity, where Gli2 functions as the principal activator and Gli1 enhances the transcriptional output, but is not essential for the development.[6,7]

The expression patterns of members of the Hh signaling pathway during palate development

A study conducted by Rice et al. to describe how members of the Hh pathway are expressed during palate development, demonstrated that prior to palatal shelf elevation, Shh ligand is expressed in the thickened oral epithelium. Ptch1 and Ptch2 receptors are expressed in the palatal mesenchyme adjacent to the palatal oral epithelium with stronger expression for Ptch1. Ptch2 receptor is also expressed in the palatal oral epithelium. Smo receptor and Gli transcriptional effectors are expressed widely in the palatal mesenchyme. Gas1 regulator is expressed in the palatal mesenchyme with highest levels on the nasal side and adjacent to the medial edge epithelium (MEE). Hhip1 regulator is expressed in the condensed palatal mesenchyme surrounding the thickened palatal oral epithelium. Disp1 regulator is expressed in the palatal nasal epithelium. Rab23 regulator is expressed weakly in the palatal mesenchyme.[17] After palatal shelf elevation, Shh ligand is expressed in the developing palatal rugae in the oral epithelium. Ptch1 and Ptch2 receptors are expressed in the

palatal mesenchyme adjacent to the palatal oral epithelium. Smo receptor is expressed in the palatal mesenchyme surrounding the MEE. Gli transcriptional effectors are expressed in palatal mesenchyme adjacent to the palatal oral epithelium. Gas1 is expressed in the palatal mesenchyme surrounding the palatal oral, MEE and nasal oral epithelium. Hhip1 is expressed in the palatal mesenchyme adjacent to the palatal oral epithelium and its expression is almost identical to Ptch1 expression. Rab23 is expressed in the palatal nasal epithelium and is expressed weakly in the palatal mesenchyme adjacent to the palatal oral epithelium. Disp1 is expressed weakly in the palatal mesenchyme adjacent to both the palatal oral and nasal epithelium.[17] In addition, Ptch1 receptor, Gli transcriptional effectors, and Gas1, Hhip1 and Disp1 regulators are expressed in the developing palatine bone after palatal shelf elevation.[17]

Role of Shh signaling in the reciprocal epithelial-mesenchymal interactions and secondary palate development

Shh plays a crucial role in the development of the craniofacial complex.[18-20] Shh is expressed in the epithelium of facial primordia[21] and is one of most important molecules involved in the patterning of the facial mesenchyme.[20] Shh is a mitogen that induces the cell proliferation in many embryonic and adult tissues.[22] Dysfunction of Hh signaling causes a number of severe birth defects including holoprosencephaly (HPE), a defect characterized by the failure of the division of the embryonic forebrain into two cerebral hemispheres.[18-20] On the other hand, aberrant activation of the Hh signaling pathway has been implicated in the initiation and progression of various types of cancer.[22,23]

Several studies have been conducted to identify the role of Shh in the development of the craniofacial complex. One of those studies demonstrated that Hh signaling is not required for neural crest cells (NCCs) generation and migration, but is necessary for the later stages of craniofacial development.[24] Loss of Hh signaling in neural crest cells causes loss of many of the NCC-derived skeletal and non-skeletal components of the head except the NCC-derived neuronal cell types.[24] The study also indicated that Shh regulates facial development through Forkhead-box (Fox) genes, which showed changes in expression pattern in Wnt1-Cre;Smon/c embryos.[24]

The developing palatal shelves exhibit morphological and molecular heterogeneity along both the anteroposterior and mediolateral axes, with the medial side corresponding to the nasal side and the lateral corresponding to the oral side following palatal shelf elevation.[1] Initially, when the palatal growth begins, Shh is expressed throughout the palatal epithelium[17] and then its expression becomes restricted to small areas of thickened palatal oral epithelium that are corresponded to the developing rugae.[17,25-27] In mutant mouse studies, Shh expression pattern is used as a molecular marker for analysis of palatal shelf growth or patterning abnormalities.[26,28,29]

Shh is a critical player in the reciprocal epithelial-mesenchymal interactions that are essential during the palate development.[1] Epithelium-specific inactivation of Shh or mesenchyme-specific inactivation of Smo in mice results in defects in palatal shelf growth. These studies in mutant mice have demonstrated that Shh signals from the epithelium to the underlying mesenchyme to promote cell proliferation and outgrowth of the palatal shelf.[30,31] Whilst inactivation of Shh expression in the epithelium causes cleft palate, inactivation of Smo expression in the epithelium does not cause cleft palate, implying that Shh exerts its effect on the adjacent mesenchyme.[30] However, Smo expression in the palatal mesenchyme affects the palatal epithelial cell proliferation, indicating the indirect action of Shh on the palatal epithelium via activating a mesenchymal signal that regulates cell proliferation in the palatal epithelium.[31,4] Administration of exogenous Shh protein induces palatal mesenchyme proliferation in palatal explant cultures.[30,32] This palatal mesenchyme proliferation is regulated, in part, by the cell cycle regulators cyclin D1 (Ccnd1) and

cyclin D2 (Ccnd2). In control embryos at E13.5, Ccnd1 expression is strong in the epithelium and mesenchyme of the palatal shelf whereas Ccnd2 expression is strong in the epithelium and weak throughout the mesenchyme of the palatal shelf. Osr2-IresCre;Smoc/c mutant embryos show downregulation in Ccnd1 and Ccnd2 in the developing palatal mesenchyme. These data indicated that Shh regulates, in part, cell proliferation through the maintenance of Ccnd1 and Ccnd2 expression.[31]

Shh signaling is required in the regulatory feedback loops between the epithelium and mesenchyme during palatal development.[32] Shh interacts with bone morphogenetic protein 4 (Bmp4) and muscle segment homeobox 1 (Msx1) to induce proliferation in the palatal mesenchyme. Msx1 is expressed in the anterior palatal mesenchyme of the developing palatal shelves.[32] The Msx1-/- mutant mouse embryos display complete cleft palate with reduced expression of both Bmp4 and Bmp2 in the anterior palatal mesenchyme and Shh expression in the anterior palatal epithelium.[32] In these mutant embryos, Transgenic Bmp4 expression is sufficient to restore the expression of Shh.[32] In addition, exogenous Shh protein in cultured palatal explants induces Bmp2 expression[32] and inactivation of Smo in the palatal mesenchyme down-regulates the expression of Bmp2 in the anterior palatal mesenchyme,[31] implying that Bmp2 expression is maintained by Shh signaling. Strikingly, Shh-soaked beads in palatal explant culture, do not induce cell proliferation after 8 hours in both Msx1-/- and wild type palatal mesenchyme, but cell proliferation can be detected around the beads after 24 hours. On the other hand, Bmp2 soaked beads induce a remarkable increase in the cell proliferation in the mesenchyme after 8 hours.[32] Furthermore, cell proliferation is repressed in palatal tissue explants contain beads soaked with an anti-Shh antibody. Interestingly, a bead soaked with both an anti-Shh antibody and Bmp2 is sufficient to induce cell proliferation, indicating that the mitogenic activity of Shh is mediated by Bmp2. These data suggesting that in the anterior palate Msx1 is required for Bmp4 expression in the palatal mesenchyme which in turn is required for the maintenance of Shh expression in the palatal epithelium that signals back to the palatal mesenchyme to promote cell proliferation by inducing the Bmp2 expression in the palatal mesenchyme.[32]

Shh also regulates the outgrowth of the palatal shelves through interaction with fibroblast growth factor 10 (Fgf10) signaling.[30,31] Fgf10 and Shh function in a positive-feedback loop,[30,31] mesenchymally expressed Fgf10 signals to the fibroblast growth factor receptor 2 (Fgfr2b) in the palatal epithelium to regulate the expression of Shh which signals back to the mesenchyme and induces the cell proliferation.[30] In the developing palatal shelves, Fgfr2b is expressed in the oral epithelium and at a much lower level in the mesenchyme of the nasal side at E13. Fgf10 is expressed in the palatal mesenchyme primarily underlying the MEE and the oral epithelial surface and directly adjacent to the epithelium in which Fgfr2b is expressed, suggesting epithelial-mesenchymal interactions.[30] Both Fgf10-/- and Fgfr2b-/- mutants exhibit downregulated Shh expression in the palatal epithelium and reduced cell proliferation in both palatal epithelium and mesenchyme.[30] On the other hand, exogenous Fgf10 protein induces proliferation and Shh expression in the palatal epithelium and it induces the Ptch1 in the adjacent mesenchyme.[30] According to these data, it is suggested that Shh acts downstream of Fgf10/Fgfr2b signaling and induces proliferation in the palatal mesenchyme, and indirectly induces the proliferation in the palatal epithelium through activation of Fgf10.[30]

Whereas Fgf10 is mainly expressed on the oral side and induces Shh expression in the palatal epithelium, fibroblast growth factor 7 (Fgf7) is mainly expressed on the nasal side and represses Shh expression in the palatal epithelium.[30,33] Application of function neutralizing antibody against Fgf7 induces the expression of Shh in the palatal epithelium[20] whilst addition of exogenous Fgf7 protein in palatal explant culture inhibits Shh expression.[30,33] Interestingly, exogenous Shh protein in cultured palatal explants

inhibits Fgf7 expression in the palatal mesenchyme.[30]

Fgf10 and Fgf7, are both ligands for Fgfr2b.[30] Fgf10 induces rapid phosphorylation of the tyrosine (Y)-734 residue on Fgfr2b which causes receptor recycling and enhances and prolongs the Fgfr signaling whereas Fgf7 causes rapid degradation of the receptors.[34] As Fgfr2b function is critical for maintaining Shh expression in the palatal epithelium, it is suggested that inhibition of Shh expression by exogenous Fgf7 in the palatal explant could be due to Fgf7-induced Fgfr2b degradation.[30] The expression patterns of Fgf7 and Fgf10 in developing palatal mesenchyme complement each other and normally Fgf7 antagonizes Fgf10 function to repress the expression of Shh in the nasal side of the palatal epithelium.[30,33]

In Distal-less (Dlx5) mutant palate shelves Shh expression expands to more medial palatal epithelium as a result of the loss of Fgf7 in these mutant palate shelves.[33] Normally both Dlx5 and Fgf7 are expressed in the nasal mesenchyme of the palatal shelves and it is suggested that Dlx5-regulated Fgf7 signaling is crucial for the negative regulation of Shh signaling and Oro-nasal patterning of the palatal shelves.[33]

As mentioned above, expression of multiple members of the Forkhead-box (Fox) is regulated by Shh signaling, this includes the expression of Foxf1 and Foxf2 in the facial primordia.[24,35] A novel Shh-Foxf-Fgf18-Shh circuit mediates reciprocal epithelial-mesenchymal signaling interactions during palatogenesis.[35] During palate development, mesenchymally expressed Foxf1 and Foxf2 maintain the expression of Shh signaling by inhibiting the expression of Fgf18 in the palatal mesenchyme.[35] Shh expression in the palatal epithelium is inhibited by Fgf18 expression in the palatal mesenchyme.[35] In palatal explant culture assays, exogenous fgf18 protein inhibits the expression of Shh in the palatal epithelium.[35] Furthermore, Foxf2-/- and Foxf-1c/c Foxf2c/c Wnt1-Cre mutant embryos exhibit ectopic Fgf18 expression in the palatal mesenchyme and loss of Shh expression in the palatal epithelium.[35] This reduction in Shh expression is possibly due to rapid Fgfr2b degradation caused by the ectopically expressed Fgf18. In contrast, vitro studies and crystal structure prediction claim that Fgf18 lacks affinity for Fgfr2b.[36,37] Whilst Foxf1a-/- mutant embryos die before craniofacial morphogenesis, Foxf2-/- mutant embryos exhibit complete cleft palate.[38,39]

Osr2-IresCre;Smoc/c mutant embryos show downregulation of Foxf1a and Foxf2 expression in the palatal mesenchyme and also show downregulation in Osr2 mRNA expression in the palatal mesenchyme indicating that Shh signaling regulates Osr2 expression in the palatal mesenchyme.[31] Along the mediolateral axis, Osr1 and Osr2 exhibit graded expression in palatal mesenchyme.[40] The expression of Osr1 is restricted to the lateral side whilst Osr2 is expressed gradedly in palatal mesenchyme with the strongest expression in the lateral side and the weaker expression in the medial side.[41] Disruption of Osr2 causes cleft palate that correlates with reduction in cell proliferation in the medial side of the palatal shelves and with mediolateral patterning defect. Osr2 is required for cell proliferation on the medial but not the lateral side of the palatal shelves, possibly due to partial functional redundancy between Osr2 and Osr1.[41]

Paired-box gene-9 (Pax9) plays a role in palatogenesis by regulating a molecular network containing Bmp4, Fgf10, Shh signaling and the Osr2 transcription factor.[29] In Pax9-/- mutant mice, expression of Bmp4, Fgf10, Msx1and Osr2 genes in the palatal mesenchyme and expression of Shh in the palatal epithelium is downregulated.[29] As mentioned above, in the anterior region of the developing palate, mesenchymally expressed Msx1 regulates the proliferation of the anterior palatal mesenchyme by inducing the expression of Bmp4, which signals to the epithelium to maintain the expression of Shh.[32] Because the Msx1 expression is restricted to the anterior region,[32] Bmp4 expression in the posterior region is maintained by Pax9.[29] Therefore, it can be said that Shh expression in the anterior and in the pos-

terior palatal epithelium depends on the functions of Msx1 and Pax9, respectively, in the palatal mesenchyme.[29,32] Mice lacking Pax9 function exhibit cleft palate and palatal shelf growth defects, including palatal rugae malformation.[29] As it is known, Shh is a critical player in palatal rugae patterning and loss of shh signaling causes mispatterning of palatal rugae.[27] In wild-type control embryos, Shh is strongly expressed in the developing palatal rugae, which shows an increase in number from two pairs at E12.0 to seven pairs by E14.5 while in Pax9-/- mutant embryos, expression of Shh is much weaker and palatal rugae patterning is disrupted in a way that only five pairs of palatal rugae are formed by E14.5.[29]

Pax9 and Shh expression are altered in the transforming growth factor-beta 3 (TGF-b3) null embryos.[42] TGF-b3 is expressed in the MEE of the developing palatal shelves and plays a critical role in the adhesion and disappearance of the MEE during palatal shelf fusion. In the TGF-b3 null embryos, alteration in Shh expression at E12.5 to E13.5 is minimal while at E14.5 and E15.5 is remarkedly reduced.[42] It is suggested that Shh and Pax9 might play a role in the TGF-b3 regulation of normal palatal fusion.[42]

Recent studies have shown that Shh signaling is also downstream of Wnt signaling in the developing palate. Mice lacking Wnt signaling in the palate epithelium exhibit altered Shh expression and no palatal rugae formation.[43]

Prickle like 1 (Prickle1) also affects Shh expression in the palatal epithelium during palate development.[44] Although Prickle1 mutant palates show no change in Bmp4, Fgf10, and Wnt5a expression in the palatal mesenchyme, Shh expression in the palatal epithelium is delayed.[44] Before the palatal development, Prickle1 is expressed in the internal maxillary processes and then around E11.5, its expression extends medially into palatal shelves. From E12.5 to E13.5 Prickle1 is highly expressed in the posterior palate.[44] Prickle1 mutant embryos exhibit short unfused palatal shelves with normal number of rugae. There is no disruption in the development of the palatal rugae except for delay. It is also noticed that the medial edges of the anterior palatal shelves do not develop rugae.[44]

It is suggested that Prickle1 mutation affects the palatal development at an early stage, in part, by disrupting the maxillary development causing short maxilla processes which subsequently causes short palatal shelves.[44] The delay in Shh up-regulation and rugae formation in the mutant embryos might be a secondary effect from the short palate[44], as Shh up-regulation and rugae formation require separation by a minimal distance.[25]

Some studies have demonstrated that coordinated functions of Shh and retinoic acid (RA) signaling pathways are required for normal development.[45] Shh signaling enhances the expression of Cyp26 genes.[45] CYP26 enzymes protect cells from physiological RA activity.[46,47] Abrogation of Shh signaling causes loss of the expression of Cyp26 genes and subsequently, causes enhancement of RA signaling.[45] The loss of Shh signaling and the enhancement of RA signaling cause cleft palate and mispatterning of palatal rugae.[45] Rat fetuses exposed to excess RA exhibit palatal malformation including cleft palate and supernumerary rugae.[48] Since RA signaling promotes the formation of palatal rugae, it is suggested that one mechanism by which Shh inhibits rugae formation is through the attenuation of RA signaling.[45]

CONCLUSION

Shh signaling is believed to be the primary regulator of the growth and modeling of palatal shelves. Shh signals from the epithelium to the underlying mesenchyme which induces cell proliferation during palate development. Shh regulates the expression of several genes and coordinates the reciprocal epithelial-mesenchymal interactions. Disruption in Shh expression causes a number of birth defects including cleft palate.

Author contributions

RT conceptualized, design and coordinated the manuscript. RT and EŞ participated in writing and reading the final article.

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Conflict of interest

The Authors declare that they have no conflicts of interest to disclose

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