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UNVEILING THE ROLE OF SHH FROM EMBRYOGENESIS TO ITS REACTIVATION IN TUMORIGENESIS: A MAXILLOFACIAL PERSPECTIVE

Dental Science

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ABSTRACT

Sonic Hedgehog gene (SHH) plays a vital role in embryogenesis through its secreted protein sonic hedgehog protein (Shh). During embryogenesis, Shh acts as a morphogen controlling proximal and distant signaling in the specific development of tissue lineages, patterning, regulation of cell proliferation and suppression of tissue apoptosis. Shh also exerts its role in odontogenesis by determining the site of tooth bud formation, in tooth morphogenesis and root formation. The difference in the specific development of a region by Shh can be explained by its [a] *'Spatial gradient* [b] *the 'form'* [c] *Concentration gradient and* [d] *Temporal gradient.* Shh signaling pathway has an extracellular and an intracellular component. A disruption of Shh pathway contributes to tumorigenesis of several cell types including those arising from odontogenic structures. This article reviews Shh from its formation in embryonic stages, its role in development and odontogenesis, to its reactivation in tumorigenesis and in specific to odontogenic pathologies.

KEYWORDS

Shh signaling, embryogenesis, Odontogenesis, tumorigenesis, Ptch mutation, odontogenic keratocyst, Nevoid Basal cell carcinoma.

INTRODUCTION

Sonic Hedgehog (Shh) is protein molecule encoded by SHH gene [Sonic Hedgehog], from the notochord. It plays a vital role in early development, bilateral organization of structures and dorsoventral neural patterning. Evidence from molecular studies suggest that all these processes are mediated by this single molecule, the secreted protein Shh (Siegel GJ, Agranoff BW, Albers RW, et al. (1999); Larsen, W. J. (1993); Lumsden, A., & Graham, A. (1995). This occurs mainly by a] proximal contact signaling resulting in *floor plate induction* and b] distant signaling resulting in *motor neuron induction.* At the ventral end of the neural tube, proximal contact signaling causes floor plate induction via Shh signaling, whereas distant signaling by Shh results in the same inductive effect throughout the midline tissues resulting in development of specific regions of the nervous system including motor neuron induction (Lumsden, A., & Graham, A. (1995); Simeone, A., et al (1995); Basler, K., Edlund, T., Jessell, T. and Yamada, T. (1993); Bumcrot, D. A., & Mcmahon, A. P. (1995)).

At the dorsal end of the neural tube, ectoderm encodes signals from the TGF- family expressing BMP's [BMP7and BMP4] along with related proteins dorsalin and activin, all of which are involved in *roof plate induction*. In the dorsoventral neural patterning, TGF- family signals plays an antagonistic role against the Shh.[Figure 1] This is one of the major factors which determines cell fates along the dorsoventral axis (Lumsden, A., & Graham, A. (1995); Simeone, A., et al (1995); Basler, K., Edlund, T., Jessell, T. and Yamada, T. (1993); Bumcrot, D. A., & Mcmahon, A. P. (1995). Shh has also shown to exert a key role in somite differentiation (Siegel GJ, Agranoff BW, Albers RW, et al. (1999); Lumsden, A., & Graham, A. (1995); Simeone, A., et al (1995); Basler, K., Edlund, T., Jessell, T. and Yamada, T. (1993). Thus Shh acts as a morphogen which has the potential to control proximal [short-range] and distant [long-range] signaling in the specific development of lineages of neural, endodermal, mesodermal stem cell, limb patterning. It also regulates cell proliferation and suppression of tissue apoptosis (Lumsden, A., & Graham, A. (1995); Simeone, A., et al (1995); Basler, K., Edlund, T., Jessell, T. and Yamada, T. (1993); Bumcrot, D. A., & Mcmahon, A. P. (1995); Gustafsson, M. (2002)).

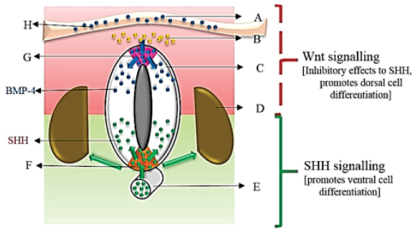

Figure 1: Role of SHH in Dorso-ventral neural patterning

A=Ectoderm, B= Neural Crest cells*, C=Neural Tube*, D=Somites***, E=Notochord, F=Floor Plate, G= Roof Plate, H=BMP-7; BMP-4;Dorsalin;Activin[TGF family].

forms components of PNS, ** Forms components of CNS , * derived from mesoderm and forms the precursors of axial skeleton and skeletal muscles]*

How is this possible by a same molecule to have both short-range and long-range signaling to develop specific regions in the nervous system?

The difference in the specific development of a region with this same molecule can be explained by the following factors. [a] *'Spatial gradient:* anterioposterior orientation of the responding tissue (Lumsden, A., & Graham, A. (1995)): The anterioposterior orientation of tissue during development is established by the synchronous roles of ectodermally derived anterior neural plate markers- follistatin, chordin and noggin by their inhibitory action on neural inhibitors BMP 2 and BMP 4 thereby establishing anterior neural domains; and the posterior domain is established by gradients of retinoic acid from Henson's node or basic fibroblast growth factor (bFGF) (Lumsden, A., & Graham, A. (1995); Gustafsson, M. (2002); Avantaggiato, V., Acampora, D., Tuorto, F. and Simeone, A. (1996)). *Lim-1* and *Otx-I* genes also exert a role in determining the most anterior portion during development (Lumsden, A., & Graham, A. (1995)). [b] *the 'form' of Shh:* Recent genetic revelation suggests that Shh may have [i] 'poorly diffusible' form and [ii] 'packaged' form (Gustafsson, M. (2002)). This difference in its form arises due to the covalent modification of Shh which affects its activities - contact signaling and distant signaling by Shh (Lumsden, A., & Graham, A. (1995); Zhu, A. (2004)). [c] *Concentration gradient of Shh*: i.e., the dose of Shh accumulated in a tissue. This can be explained by passive diffusion. As signal molecule moves from its point of origin to the effector tissue, it goes through dilution or degradation resulting in concentration gradient of the molecule. The role of receptor–ligand interaction, endocytosis and regulatory signaling also affect Shh protein gradient formation (Zhu, A. (2004)) and [d] *Temporal gradient of Shh*: i.e., the exposure time of Shh to these cells(Gustafsson, M. (2002);Zhu, A. (2004); Gilbert, S. F. (2000)). For example, the digits 3, 4 and 5 of limb are controlled by Shh and have different shapes. It was noted that all these digits have meagre difference in the dose of SHH, but has different exposure time to SHH. Thus, the effect of Shh is controlled by temporal as well as spatial gradient (Zhu, A. (2004)).

Another key factor during embryogenesis and development of an organism is the crosstalk of various signaling pathways. The hedgehog [Shh] signaling pathway and the Wnt signaling pathway are critical regulators of cell proliferation specifically during embryonic development and differentiation of various cell types. A disruption of these key interrelation between the two pathways plays a significant role in tumorigenesis of several cell types (Ding, M., & Wang, X. (2017); Wicking, C., Smyth, I. and Bale, A. (1999); Zaphiropoulos, P. G., Unde´n, A. B., Rahnama, F., Hollingsworth, R. E., & Toftgård, R. (1999); Bale, A. (2001)).

SHH SIGNALING PATHWAY

Shh pathway comprises of complex interplay of 7 important regulatory proteins. This includes (1)Shh, (2) Patched[Ptch] (3)Smoothened[Smo], (4) Costal-2 [cos-2], (5) Serine/threonine kinase fused [Fu], (6) the novel protein suppressor of fused [SuFu], and

(7) proteins from 3 Gli genes (Gli 1, Gli 2, Gli 3)(Wicking, C., Smyth, I. and Bale, A. (1999); Zaphiropoulos, P. G., Unde´n, A. B., Rahnama, F., Hollingsworth, R. E., & Toftgård, R. (1999); Bale, A. (2001)). In Drosophilia, there is only one hedgehog gene. However in vertebrates, research corroborates that there are three homologues of HH viz., Sonic (Shh), Desert (Dhh), and Indian hedgehog (Ihh). Shh is mainly responsible for dorsoventral patterning of the developing CNS; limb; lung; gut; teeth; skin and hair-follicles. Dhh is associated with the developing germline and is expressed mostly in the gonads and also in Schwann Cells of peripheral nervous system. Ihh are primarily involved in endochondral bone formation in the development of the skeletal system (Ding, M., & Wang, X. (2017); Wicking, C., Smyth, I. and Bale, A. (1999); Bale, A. (2001)). Each of these hh proteins are uniquely expressed but they share the common hedgehog signaling pathway, consequently mediating its effects on different tissues or organs (Wicking, C., Smyth, I. and Bale, A. (1999)).

Hedgehog signals are acquired and transduced across the membrane by a simultaneous role of ptch and Smo receptors. Ptch is a putative trans membrane protein encoded by Patched gene mapped to chromosome 9q22.3-q-3 (Ling, G., et al (2001); Barreto, D., Gomez, R., Bale, A., Boson, W. and De Marco, L. (2000)). It is made of 1500 amino acids and consists of 2 extracellular loops with 12 membrane spanning domains. Ptch has the ability to be bound directly to hh which make its functions more superior and hence much research had been diverted to scrutinize its functions. Ptch also exerts a strong influence in the functions of Smo, belonging to the group of serpentine G coupled protein receptors. Albeit different homologues of Ptch exists, Ptch 1 is the most commonly associated receptor molecule for all forms of hh and its mutation is associated with birth defects. Ptch 2 is similar to Ptch1 but its functions are not precisely discovered (Wicking, C., Smyth, I. and Bale, A. (1999); Bale, A. (2001); Couvé-Privat, S, et al (2004); Borycki, A. G., Brown, A. M. C., & Jr Emerson, C. P. (2000)). In the Shh signaling pathway, there are two components; extracellular and intracellular. [Figure 2].

Extracellular Shh signaling:
when Shh is absent, Ptch-Smo acts as an inactive complex. Ptch inhibits the functions of Smo through the cell membrane rendering it inactive. On the contrary, when Shh molecule is present, Ptch-Smo becomes active complex and Shh binds to the two extracellular loops of Ptch emanating as release of inhibition on Smo. Shh binding to Ptch does not physically release Ptch-Smo complex. The inhibition on Smo is thought to be modulated via the conformational change in N terminus of extracellular component of Smo (Ding, M., & Wang, X. (2017); Wicking, C., Smyth, I. and Bale, A. (1999); Zaphiropoulos, P. G., Unde´n, A. B., Rahnama, F., Hollingsworth, R. E., & Toftgård, R. (1999); Bale, A. (2001)). This activates a cascade of intracellular signaling resulting in transcriptional up regulation of downstream target genes. Recent research also explores the likelihood that other genes can also exert its effects on the signaling pathway despite their unclear roles. Hedgehog interacting protein [hip] is one among them thought to be activated by hedgehog signaling. Hip binds all the three variants of hh proteins with comparable affinity to Ptch. Thus the can act as repressors of Ptch function (Wicking, C., Smyth, I. and Bale, A. (1999); Zaphiropoulos, P. G., Unde´n, A. B., Rahnama, F., Hollingsworth, R. E., & Toftgård, R. (1999); Bale, A. (2001)).

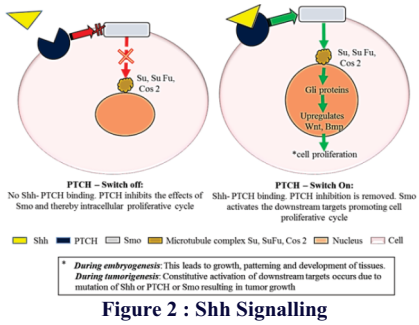

Figure 2 : Shh Signalling

***Intracellular Shh signaling*:**
It is a more intricate and diverse pathway. Once the Smo is activated, it activates the microtubule complex such as Fu, SuFu and Cos-2. This results in dissociation of microtubule complex which is required to activate the intranuclear Gli genes. Gli 2 and Gli 3 is considered to play major roles in Shh signaling and has dual functions in transcription such as activation and inhibition. When in its active form, Gli 2 and Gli 3 activates Gli 1(Wicking, C., Smyth, I. and Bale, A. (1999); Bale, A. (2001)). The synergistic role of these three Gli proteins is required to activate the downstream target genes including Wnt genes, genes encoding Bone morphogenic proteins [Bmps] and the Patched gene as well (Wicking, C., Smyth, I. and Bale, A. (1999); Bale, A. (2001); Couvé-Privat, S, et al (2004)). There are possibilities of involvement of other unidentified target genes in this signaling pathway. Also the target genes can vary and can be cell specific or organ specific. Thus the distinctiveness and regulatory mechanisms involved in consecutive activation of these different downstream genes has detrimental effect on the tissue specific tumorigenesis mediated by deregulated hedgehog signaling (Wicking, C., Smyth, I. and Bale, A. (1999); Bale, A. (2001)).

Shh in Oral Epithelium- determination of tooth form and number

During development, the precise site of each tooth to be formed in the dental arch is initiated by focal concentration gradients of Shh in the oral epithelium and the activity of Wnt7b which is expressed complimentary to Shh in the sites of epithelium where there is no tooth formation. These focal areas of Shh later proliferate, become thickened and causes invagination into the ectomesenchyme marking the initiation stage of tooth development followed by budding. In contrast to the above, if there is absence of early signaling of Shh or expression of Wnt7b in early stages of tooth formation, there will be a lack of focal concentration gradient of Shh in the site, arresting the initiation stage of tooth formation. After the stage of budding, Shh mainly acts as growth factors through ecto-mesenchymal and intra epithelial signaling. Shh helps in cell polarization and to pattern tissues in early stages during formation of cusp. In later stages during root formation, Shh is focused to the Hertwig's epithelial root sheath cells whereas Smo, Ptch1, and Gli exert its effect on the mesenchyme of the dental papilla and follicle. Thus it acts as an important factor in morphodifferentiation and and cytodifferentiation of the tooth. [Figure 3] This points out to the balance established between the upregulators [Shh] and downregulators [WNtb, Das1] both through negative and positive interactions for optimal odontogenesis (Fisher, C. (2008); Seppala, M., Fraser, G., Birjandi, A., Xavier, G. and Cobourne, M. (2017); Khan, M., Seppala, M., Zoupa, M. and Cobourne, M. (2007))

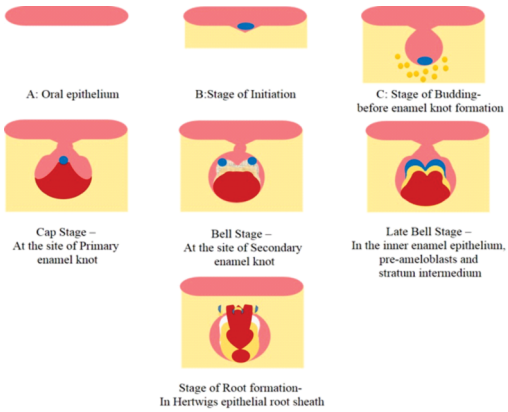

Figure 3: Sites of Shh Expression during odontogenesis (marked with blue)

Shh in tumorigenesis

The most remarkable role of Shh in human tumorigenesis and diseases evolved after the discovery of role of mutation of Ptch in both familial and sporadic forms of basal cell carcinoma. Different components involving the Shh signaling pathway both extracellulary and intracellulary have been attributed to the development of dysmorphic syndromes and tumorigenesis. Heterozygous mutation of Shh gene results in the developmental defects of forebrain and midface (holoprosencephaly). While Gli 1 has been associated with glioma formation, Gli 3 causes limb deformities associated with 3 different syndromes (Wicking, C., Smyth, I. and Bale, A. (1999); Zaphiropoulos, P. G., Unde´n, A. B., Rahnama, F., Hollingsworth, R. E., & Toftgård, R. (1999); Borycki, A. G., Brown, A. M. C., & Jr Emerson, C. P. (2000)).

The role of Ptch gained much attention as soon as its mutation was identified in Nevoid basal cell nevus syndrome (NBCCS) or Basal cell nevus syndrome or Gorlin- Goltz syndrome (Wicking, C., Smyth, I. and Bale, A. (1999); Zaphiropoulos, P. G., Unde´n, A. B., Rahnama, F.,

Hollingsworth, R. E., & Toftgård, R. (1999)). This syndrome can predispose an individual to a variety of tumors due to inactivation of both the alleles of [two hit hypothesis] Patched gene [tumor suppressor gene] in a given cell type such as medulloblastoma, meningioma, fibrosarcoma, rhabdomyosarcoma, cardiac fibroma and ovarian fibroma despite the most common one is basal cell carcinoma (Wicking, C., Smyth, I. and Bale, A. (1999); Zaphiropoulos, P. G., Unde´n, A. B., Rahnama, F., Hollingsworth, R. E., & Toftgård, R. (1999); Bale, A. (2001); Couvé-Privat, S, et al (2004); Borycki, A. G., Brown, A. M. C., & Jr Emerson, C. P. (2000); Mangum, R., et al (2016)). Ptch deregulation is also found in basal cell carcinoma and squamous cell carcinoma in patients with Xeroderma Pigmentosum (XP) (Couvé-Privat, S, et al (2004)). Ptch mutations are also associated with the development of tricoepitheliomas esophageal squamous cell carcinomas and transitional cell carcinomas of the bladder. The haploinsufficency of patched protein can also be recognized in most of the developmental defects of dorsoventral patterning during embryogenesis associated with NBCCS. NBCCS includes craniofacial anomalies such as development of multiple odontogenic keratocysts (OKC) [renamed as OKC from keratocystic odontogenic tumor in 2017] or dentigerous cysts of the jaw, calcified falx cerebri, skeletal defects such as fused, bifid or splayed ribs, spina bifida; pitting of hands and feet; limb deformities such as syndactyly and polydactyly (Wicking, C., Smyth, I. and Bale, A. (1999); Barreto, D., Gomez, R., Bale, A., Boson, W. and De Marco, L. (2000); Mangum, R., et al (2016); Pino LC, et al (2016); Athar, M., Li, C., Kim, A. L., Spiegelman, V. S., & Bickers, D. R. (2014); Gurgel, C. A., et al (2014); Lam, C., Ou, J. C., & Billingsley, E. M. (2013); Onodera, S., et al (2017)). [Figure4] Ptch mutation with disrupted Shh signaling also plays a role in the pathogenesis of Cleft lip and agnathia (Avril, L., et al (2013); Kurosaka, H., Iulianella, A., Williams, T., & Trainor, P. A. (2014)).

Role of Shh/Patch in the development of Odontogenic lesions.

The role of Shh deregulation and Ptch mutations in the development of Odontogenic cysts and tumors is a topic of much interest as more pathologies are observed to depict the mutations in addition to Odontogenic Keratocyst(Barreto, D., Gomez, R., Bale, A., Boson, W. and De Marco, L. (2000); Muzio, L. L., et al (1999); Levanat, S., et al (1996); Barreto, D., Gomez, R., Bale, A., Boson, W. and De Marco, L. (2000); Oniscu, A., et al (2004); Guo, Y., et al (2013); Song, Y., et al (2006).). These include dentigerous cysts, ameloblastoma, radicular cyst and calcifying epithelial odontogenic cyst (Gurgel, C. A., et al (2014); Kochaji, N., Goossens, A., Geerts, A., & Bottenberg, P. (2005); Peacock, Z. S., Cox, D., & Schmidt, B. L. (2010); Zhang, L., Chen, X., Sun, Z., Bian, Z., Fan, M., & Chen, Z. (2006); Mishra, P. (2015); Effiom, O., Ogundana, O., Akinshipo, A., & Akintoye, S. (2017); Bilodeau, E. A., Prasad, J. L., Alawi, F., & Seethala, R. R. (2014)). Association of Ptch has also been identified with Loss of heterozygosity of PTCH gene

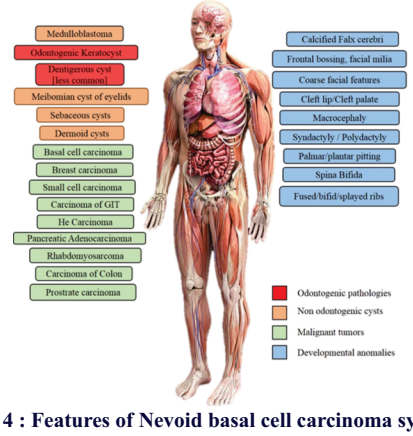

Figure 4 : Features of Nevoid basal cell carcinoma syndrome

has also been demonstrated in orthokeratinised keratocyst (Diniz, M. G., Galvão, C. F., Macedo, P. S., Gomes, C. C., & Gomez, R. S. (2010)). Few odontogenic lesions like adenomatoid odototogenic tumor, calcifying odontogenic cyst and odontogenic ghost cell tumor have also shown positive immunohistochemical localization to Shh, ptch, smo in their epithelial component (Bilodeau, E. A., Prasad, J. L., Alawi, F., & Seethala, R. R. (2014); Shimada, Y., et al (2013)). Association of Orthokeratinised Odontogenic Cyst (OOC) with Ptch has also been revealed in few cases (Diniz, M. G., Galvão, C. F., Macedo, P. S., Gomes, C. C., & Gomez, R. S. (2010)). Shh play a role in oncogenic changes in the odontogenic epithelium of ameloblastoma (Bilodeau, E. A., Prasad, J. L., Alawi, F., & Seethala, R. R. (2014)). The Ptch mutations in all such lesions are comparatively lower in occurrence in comparison to OKC. However, it is likely that Ptch expression in these odontogenic lesions predicts a factor of complication that may have to be dealt with, after the treatment or during the follow up period (Kochaji, N., Goossens, A., Geerts, A., & Bottenberg, P. (2005)).

CONCLUSION

Shh plays a crucial role from the early stages of the embryogenesis and development from neural patterning to odontogenic patterning by a constant interplay with various signaling molecules and pathways. This review was aimed to highlight the importance of Shh from the period of embryogenesis and odontogenesis to comprehend its role in tumorigenesis as an initiating factor and to understand its role in promoting oncogenic transformation in preexisting cysts or tumors.

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