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Role of Sonic hedgehog signaling pathway in neuroblastoma development

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Abstract

Malignant transformation of normal cells is a complex and accumulative process. Understanding this event gives insight into mechanisms of developmental biology and physical interaction of cellular machinery with surrounding ambient factors. However, the trend of embryonic malignancy is not interactive with ambient factors, rather a cause of deregulations of internal developmental process. In this review, we have attempted to explore the possibility of Sonic hedgehog role (Shh) in the development of neuroblastoma tumour. It is the major extra cranial tumour and develops in very early stage of childhood. Sonic hedgehog signaling is very well studied in another major childhood tumour i.e. medulloblastoma that contributes 20-25% of childhood tumours, and one-fourth of medulloblastoma is due to abnormality in the Shh signaling pathway. Therefore, we would consider whether Shh could also contribute to the development of neuroblastoma. Although scientists are coming up with the role of Shh in the neuroblastoma, the Sonic hedgehog signaling is very much one of the promising pathways because of its multi-dimensional role not only in CNS development but also in organogenesis and other major tumour development.

Keywords: Sonic hedgehog, Neuroblastoma, Cyclin D2, Methylation, Cyclopamine, GLI1, PTCH1.

Introduction

Sonic hedgehog signaling is functional in most of the developmental process and especially in the central nervous system patterning. The Sonic hedgehog protein ligand is mainly secreted from Purkinje cells in the cerebellum and then it acts as both autocrine and paracrine to target cells. This Shh ligand has two functional units, one is N-terminal 20 kd (Bumcrot et al, 1995; Porter et al, 1995) and the other is C-terminal 25kd (Porter et al., 1996). Shh-N possesses signaling activity and Shh-C possesses autocatalytic property. After autocatalytic activity of C-terminal, there is cholesterol modification in C-terminal and palmitoylation in the N-terminal. These lipid modifications facilitate the transport of Shh ligand from origin of cells to functional target (Fig.1A). A target cell of this Shh signaling has two receptors, one is twelve trans-membrane named PTCH1 and the other is smoothed (Agren et al., 2004), seven transmembrane receptor. When there is switch off of Shh signaling then this PTCH1 transmembrane receptor inhibits the activation of SMO, however,

the inhibition mechanism of PTCH1 on SMO is not very much clear. After the binding of Shh ligand on PTCH1, instantaneous conformational changes occur and SMO is relieved from the inhibition of PTCH1 (Fig.1B). Then, SMO enters into cytoplasm and activates other regulatory molecules viz, Fu, Cos-2, etc that assist GLI1 movement from cytosol to nucleus. This GLI1 is main zinc-finger transcriptional factor and induces the expression of many other target genes in the nucleus (Fig.1B) (Ruiz Altaba, 1999). In this signaling target, majority of genes are from cell cycle, apoptosis and other developmental genes. Besides the main transcriptional factor GLI1, there are two other GLIs in this signaling i.e. GLI2 and GLI3 transcriptional factors. GLI2 sometimes acts as activator of this pathway and at other times as repressor of this pathway, its action depending upon the presence of GLI1. Albeit, GLI3 always acts as repressor of this pathway, in the absence of Shh signaling, GLI3 enters into the nucleus with the help of SUFU and shows reversal as compared to GLI1 on Shh target genes. Interestingly, a few recent studies reveal that this GLI3 is also in two forms, one is

activator (GLI3A) and the other is repressor (GLI3R) (Fuccillo et al, 2006).

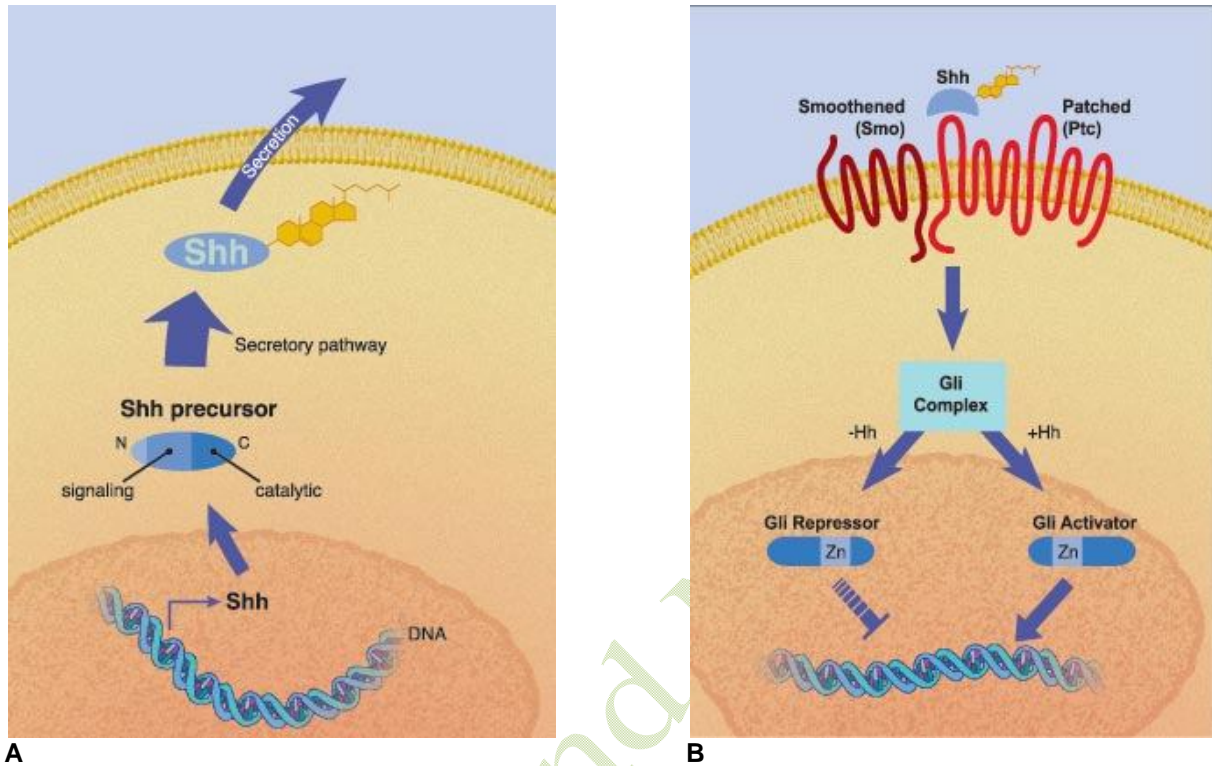


Fig. 1 A-B: Secretion of Shh protein and actions on target cell. **Fig.1A.** Shows autocatalytic activity of C-terminal of Shh and cholesterol modification on C-terminal of Shh ligand, which makes it functional on the target cell. **Fig.1B.** Active Shh protein binds to PTCH1 receptor and activates Shh signaling in the target cell with the help of Zinc finger main transcription factor GLI1 and in the absence of Shh signaling, another Zinc finger transcription factor GLI3 remains active. (Figures are adapted from Gilbert, 2002).

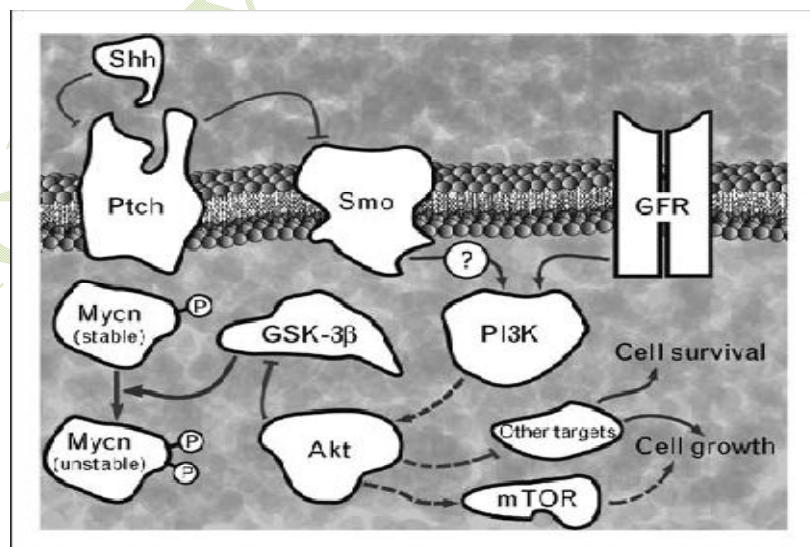


Fig. 2: Interaction of Shh and Myc in neuroblastoma development with the help of Phosphoinositide 3-kinase (PI3K). (Figure adapted from Mill et al., 2005)

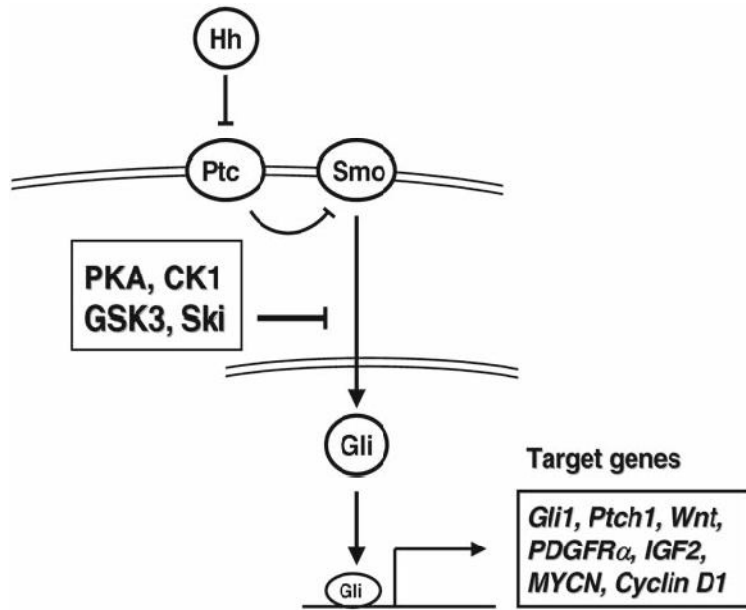
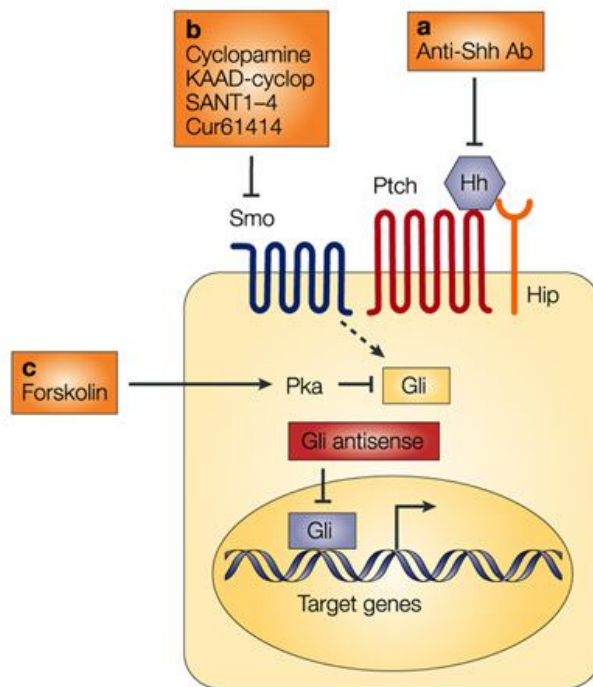


Fig. 3: Sonic hedgehog signaling downstream target genes. (Figure adapted from Altaba et al., 2004)



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Fig. 4: Possible therapeutic targets of Sonic hedgehog signaling pathway inhibition. (Figure adapted from Magliano and Hebrok, 2003)

The role of this pathway in the area of tumour development has been growing because this pathway, besides the other two major pathways viz, Notch and Wnt, support the renewable character of stem cells that are now considered as the cells mainly enforcing cancer progression. Many cases of recurrence of tumour have been reported after biopsy, chemotherapy, and radiotherapy and it has been suggested that these recurrences are due to the stem cells. In normal biopsy and traditional therapies, these stem cells usually escape because they are few in number, again divide due to their totipotent nature, and form the tumour. This totipotent character is supported by these pathways, including Shh signaling. This character and involvement in the early development process draws attention to this pathway and its understanding, for a better treatment of tumours.

The role of Sonic hedgehog signaling is well studied in most common childhood tumour medulloblastoma, however, few investigations revealed its role in neuroblastoma development (Mao et al, 2009; Shahi et al, 2008).

Neuroblastoma arises from the deregulation in the development of neural crest stem cells, which differentiates into sympathetic nervous system. Neuroblastoma is malignant tumour of sympathetic nervous system in childhood and most common extra-cranial solid tumour. Our group has been checking the expression pattern of Shh signaling receptors SMO, PTCH1 and main transcription activator, GLI1 and repressor GLI1 in neuroblastoma cell lines (Shahi et al, 2008). Most of the cell lines expressed SMO, PTCH1 and GLI1, however, few cell lines expressed GLI3. This would support to certain extent Shh activation. Thereafter, we knocked down main transcriptional factor GLI1 with custom siRNA (Invitrogen, USA) and post-knockout gene expression strengthens the possibility of this pathway activation (Unpublished data). In another recent study, high expression of Shh ligands, viz, SHH, PTCH1, SMO and GLI2, was reported (Mao et al, 2009). This study also showed tumour inhibition in cell lines after Shh specific inhibitor cyclopamine treatment and knockdown of GLI2 inhibiting the colony formation. These results also suggest Shh activation in neuroblastoma. However, GLI2 is considered to be the major transcription factor to exploit for drug therapy that could affect this pathway in neuroblastoma

development, rather than GLI1. One of the previous studies also suggests some significant role of GLI2 and showed that GLI2 knockout mice die very early in the natal stage and also shows defect in neural tube including loss of floor plate and reduction in V3 interneuron (Matise et al., 1998). In another study, it is reported that mitogenic effect of Shh is responsible for cell-cycle progression, however, inhibition of this pathway with specific inhibitor cyclopamine decreases the concentration of CCND1 and increases the expression of p21 (Morton et al., 2007).

We know that granular cell progenitors (GCPs) proliferate, migrate and differentiate into neuronal cells and this cascade process continues with the help of Shh which is secreted by Purkinje cells in the cerebellum. This Shh is highly conserved mitogenic glycoprotein. The action of Shh is in gradient pattern on the target cells and cell fate (Hooper, 2005; Kolpak, 2005; Corrales, 2008). Aberrant activation of Shh signaling causes medulloblastoma development which originates from deregulated GCPs (Pomeroy, 2002). Very much analogous to Shh signaling role in the development of cerebellum, there is another signaling pathway operating in the development of neural crest i.e. N-myc (Wakamatsu, 1997). Myc shows high expression in one-third of neuroblastoma tumours (Vasudevan, 2005; Maris, 2005). There has been report of link between Myc expression and Shh activation in desmoplastic medulloblastoma (Pomeroy, 2002). Another study reveals that N-Myc is main conveyor of Shh mitogenic activity in GCPs (Kenney, 2003; Oliver, 2003). Shh signaling also influences high expression of N-myc in brain and skin (Mill et al, 2005; Rao, 2004). This also illustrates that Shh signaling causes stability in the Mycn protein with the help of high expression of phosphoinositide 3-kinase (PI3K), which destabilizes phosphorylation of Mycn (at Thr-50) (Fig. 2). Similarly, inactivation of Shh activates GSK-3 β , which phosphorylates N-myc and finally degrades it. These results suggest an interaction of Shh and Myc in neuroblastoma development and another significant therapeutic target kinase inhibitor like LY294002 for PI3K for the suppression of neuroblastoma formation.

Another study showed the possibility of Shh activation in the development of Olfactory Neuroblastoma (ONB). ONB is a rare malignant tumour, originating from the olfactory epithelium

of the nasal cavity (Finkelstein et al, 2000). This study indicated that the tumour cell line samples showed expression of PTCH1, GLI1 and GLI2 in 65-70% of specimens compared to non-tumour olfactory epithelium. The study checked the inhibition of this pathway by cyclopamine, which causes low expression of PTCH1, GLI1 and Cyclin D1, and up-regulation of p21, cell cycle arrest and apoptosis among tumour cells. The study also showed the differential role of Shh in different stages of ONB (Mao et al., 2009). Another regulatory molecule Pituitary Adenylate Cyclase Activity Peptide (PACAP) is responsible for the neuronal tumour formation. It has been shown that PACAP antagonizes Shh signaling in isolated cells of neural tube. It is also reported that early embryo (E10.5) showed decrease in expression of GLI1 in neural tube after the treatment with low amount of PACAP molecules. The interaction of Shh and PACAP is significant because mutation in both causes neural tube dependent holoprosencephaly HPE (type 3) (Roessler, 1996) and HPE (type 4) (Chang, 1993), respectively. Both of them are crucial for neural tube development, however, Shh proliferates neuronal tumour formation and PACAP acts as anti-proliferative to this tumour formation (Waschek et al., 2000).

The role of Shh signaling is wide in range and it covers ventral CNS to dorsal CNS, axon path finding and many other neuronal developments in early stage. Its action is gradient and spatial-temporal dependent. For this developmental process, other signaling and transcription factors are involved with Shh. The most important transcription factors are homeodomain viz., Pax, Nkx, Dbx and Irx families (Briscoe, 2000). Shh also modulates oligodendrocyte development in Ventral CNS (Davies and Miller, 2001). These oligodendrocytes originate from neuroepithelium. Shh also plays a role in the eye patterning, retinal-cell specification and axon pathfinder in ganglion cells.

It is well known that many Cyclins, viz Cyclin D1, Cyclin D2, Cyclin B1, apoptotic gene-like plakoglobin (γ catenin) and oncogene-like N-myc, are downstream target genes of Shh-GLI1 mediated signaling and some are up-regulated while others are down-regulated, which later promote cancerous tissue in proliferative stage (Altaba et al., 2004) (Fig. 3). Two major homeodomain transcriptional factors i.e. transcription factor I (PAX6) and transcription factor II (NKX2.2) are also downstream targets

of this signaling and it is reported that in normal development, NKX2.2 is up-regulated and PAX6 is down-regulated (Fuccillo et al, 2006). However, one report showed high expression of PAX6 in neuroblastoma (Pinson, 2006). There is no published report about the expression of NKX2.2 in neuroblastoma.

These studies reveal that there is not much investigation of Shh signaling and its downstream target genes in neuroblastoma tumour as compared to medulloblastoma. However, whatever literature is available for Shh signaling and its role in neuroblastoma development suggests few things that would make a milestone for further research into tumour biology, and specifically, in neuroblastoma. It is now clear that Shh signaling is active in neuroblastoma and it is one of the major signaling besides many others in the neuroblastoma development. It plays a key role because of supporting stem cells that are renewable and shows cross-talk with other signaling pathways and molecules like Myc, PACAP, Notch signaling, Cell-cycle genes, bone morphogenic pathway, fibroblast growth factor and Wnt signaling (9-11). The complex cross-talk of Sonic hedgehog signaling in the early development and tumour growth, giving a strong attention to control many tumours besides neuroblastoma.

In therapeutic aspect, we could think over many key points that could cover very efficient ways to cure these types of tumours. The most important molecule would be the specific inhibitor of this pathway, viz Cyclopamine and other kinase inhibitors like LY294002 for PI3K inhibition. We can also target main transcriptional activator GLI1 or GLI2 in some cases with the help siRNA mediated knockdown for suppressive effect on the growth of tumour (Fig. 4).

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