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The Journal of Biomedical Research, 2022 36(1): 1–9

JBR

Review Article

Role of aberrant Sonic hedgehog signaling pathway in cancers and developmental anomalies

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Abstract

Development is a sophisticated process maintained by various signal transduction pathways, including the Hedgehog (Hh) pathway. Several important functions are executed by the Hh signaling cascade such as organogenesis, tissue regeneration, and tissue homeostasis, among various others. Considering the multiple functions carried out by this pathway, any mutation causing aberrant Hh signaling may lead to myriad developmental abnormalities besides cancers. In the present review article, we explored a wide range of diseases caused by aberrant Hh signaling, including developmental defects and cancers. Finally, we concluded this mini-review with various treatment strategies for Hh-induced diseases.

Keywords: cancer, developmental anomaly, hedgehog signal transduction, drug, treatment

Introduction

The mammalian Hedgehog (Hh) signaling system is one of the most crucial cascades that control the growth of multicellular organisms[1–2]. In 1980, Eric F. Weischaus and Christiane Nusslein Volhard published groundbreaking discovery of the Hedgehog pathway[3]. In their study, they screened the mutations that affected the segmental patterning of *Drosophila melanogaster* larvae, known as the polarity mutants[3]. These mutant larvae exhibited poor posterior body segmentation giving rise to a spiky phenotype that looked like a hedgehog, thus named as Hedgehog (*Hh*) gene[3]. Myriad functional roles have been attributed to the Hh pathway which comprises cell survival, cell proliferation, patterning, tissue polarity[2], tissue regeneration, tissue repair[4], and cell fate[5]. Mutations in the principal elements of the Hh pathway leading to deregulated signaling cause several developmental disorders, which include polydactyly[6], holoprosencephaly (HPE)[7–8], microcephaly[9], cranio-facial defects like cyclopia, skeletal deformities[10], and nervous system abnormalities[11]. Rhabdo-myosarcoma (RMS), basal cell carcinoma (BCC), medulloblastoma (MB), leukemia, gastric, lung, ovarian, breast, colorectal, hepatic, and pancreatic malignancies are all linked to hyperactivation of Hh signaling[12–14]. Furthermore, research during the last

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Received: 23 August 2021; Revised: 09 September 2021; Accepted: 13 September 2021; Published online: 15 December 2021

CLC number: R730, Document code: A

The authors reported no conflict of interests.

https://doi.org/10.7555/JBR.35.20210139

two decades has illustrated the key roles played by the components of primary cilia and their importance in the relay of Hh signal transduction. Perturbations in these ciliary components lead to deregulation of multiple signal-transducing pathways including the Hh pathway and ultimately result in ciliopathies like cystic kidney disease[15], kartagener syndrome[16], retinal degeneration, and Joubert syndromes[15]. Considering the significant roles played by the Hh pathway in multiple diseases upon its deregulation, it is, therefore, essential to understand how the Hh pathway functions and how to target it to get therapeutic benefits.

The commencement of Hh pathway primarily requires the Hh ligands. The Hedgehog system encompasses three kinds of Hh ligands: Indian hedgehog (Ihh), Sonic hedgehog (Shh), and the Desert hedgehog (Dhh)[2,17–18]. Shh is the most studied one. The production, secretion, processing, and transport of these Hh ligands at sufficient levels are necessary for activating Hh signaling[2,13,18]. The initiation of Hh signaling pathway occurs when the Hh ligands are present. Binding of the ligand to the 12-span transmembrane receptor patched (Ptch) relieves Ptch induced repression of Smoothened (Smo)[2,13,18]. Further, phosphorylation of Smo by casein kinase 1 alpha (CK1α)[17] and protein kinase A (PKA) leads to binding of Smo to Suppressor of fused homolog (Sufu), which in turn leads to nuclear translocation of Gli factors, and thereby target genes are transcribed, including myc, Cyclin-D, Ptch1, and glioma-associated oncogene 1 (Gli1)[2,13,18]. When the ligands are absent, the Ptch suppresses the activity of Smo[2,13,18]. PKA, glycogen synthase kinase-3 (GSK3), and CK1α[17] phosphorylates the full-length glioma-associated oncogene (Gli-FL) subsequently leading to cleavage of Gli-FL into Gli-repressor (Gli-R)[2,13,18]. This Gli repressor, in turn, suppresses Hh target gene expression and thus negatively modulates the Hh signaling pathway (***Fig. 1***)[12–13,18]. Here, we explore some of the diseases/disorders caused by aberrant Hh signaling.

Medulloblastoma

Harvey Cushing and Percival Bailey coined the word "Medulloblastoma" in 1925 for the first time[12]. MB is a strikingly aggressive pediatric tumor with a cerebellar origin and counts for approximately 20% of central nervous system tumors[12]. The Hh pathway is indispensable for the normal development of the cerebellum[19]. Cerebellum formation takes place after

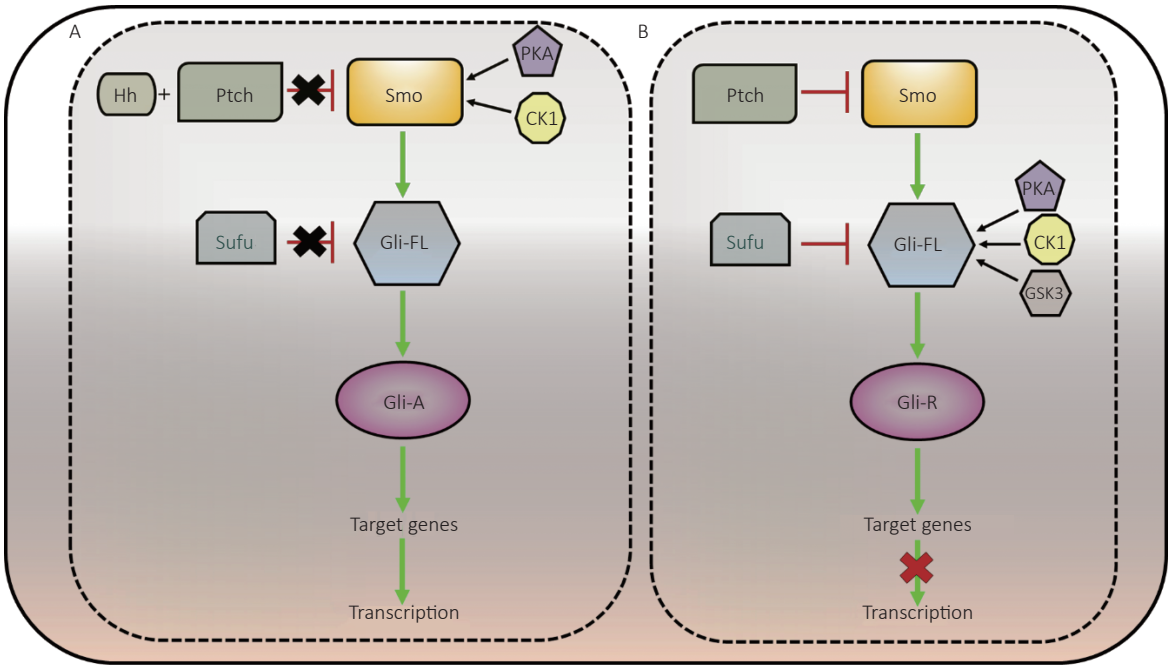

Fig. 1 **Graphical representation of active and inactive Hedgehog signaling pathway:** A: In the presence of Hh ligand, phosphorylation of Smoothened is carried out by CK1 and PKA which leads to removal of Sufu based inhibitory effect and leads to the formation of Gli activator that ultimately leads to induction of target gene transcription. B: When the Hh ligand is absent, PKA, GSK3, and CK1 phosphorylates the full-length Gli subsequently leading to proteolytic cleavage of full-length Gli into Gli repressor. The expression of Hh target genes is suppressed as a result of the Gli repressor. Hh: Hedgehog; Ptch: Patched; Smo: Smoothened; Sufu: Suppressor of fused homolog; Gli: glioma-associated oncogene; Gli-FL: full-length Gli; Gli-A: Gli activator; Gli-R: Gli repressor; PKA: protein kinase A; GSK3: glycogen synthase kinase-3; CK1: casein kinase 1. ⊣: inhibition; →: activation.

birth, the granule neuron precursors (GNP) cells multiply and gradually differentiate into neuronal granule cells which are the most common cell type in the brain[20]. Research has illustrated that the multiplication of GNP cells is primarily modulated by Shh produced by Purkinje cells[20]. In the dearth of Shh ligand, the GNPs do not replicate and move directly from the external granule cell layer (EGL) to the cerebellum's internal granule cell layer (IGL) midst differentiation into granule neurons, thus leading to a smaller cerebellum[20]. The crucial function of Shh signaling cascade was shown by using antibodies against Shh in chicks which led to a reduced cerebellar size[21–22]. The Hh pathway is downregulated in the brain and most tissues after birth, whereas MB is caused by persistent activation owing to mutations in Hh pathway components[23]. Hh signaling's functional relevance in MB was initially discovered in individuals with Gorlin syndrome[19]. Gorlin syndrome patients showed high susceptibility to MB due to germline mutations in *PTCH1* gene[19]. With the advent of the latest sequencing technologies, MB has been divided into Wnt subtype, Shh subtype, subtype-Ⅲ, and subtype-Ⅳ[12]. Among these four subtypes, the Shh subtype counts for almost 30% of all MB cases[12,24]. Shh-MB gets its name from the fact that the Shh pathway is always active. Both males and females are affected similarly in this subtype. Mutations in Smo, Ptch1/2, Gli-1/2, and Sufu are the leading causes of Shh-driven MBs[12]. Mutations in TP53 and PI3K pathway have also been demonstrated in Shh-MB[12,25]. Treatment of Shh-MB is critical and the treatment options include radiation, surgical resection, chemotherapy, and treatment with various inhibitors of the Hh pathway[12].

Basal cell carcinoma

Basal cell carcinoma is one among the most common skin cancers detected across the world[26]. Among the various signaling pathways that play a role in the pathophysiology of BCCs, the Hh pathway has emerged as the most important one[27]. The relevance of Hh pathway in the pathobiology of BCC was initially observed in patients with basal cell nevus syndrome (BCNS)[28]. BCNS is an autosomal disorder with high penetrance[29]. It can be distinguished by various developmental defects like odontogenic cysts, bifid ribs, palmoplantar pits. Its predisposition to basal cell carcinomas and multiple neoplasias[30] has been illustrated to be deregulated in most BCC patients[26]. For example, around 70% of patients with sporadic BCC exhibit mutations in *PTCH1* gene[26]. Mutations in *SMO* gene have been noted in 10% to 20% of sporadic BCC patients[27]. Apart from these, high expression levels of Gli due to inactivation of *PTCH1* in receiving cells and activating mutations in *SMO* have been shown to cause BCCs[26]. Also, mutations in *p53*[27] and *CDKN2A* have been reported in a minor group of patients with sporadic BCCs[31]. A majority of BCCs can be treated with chemotherapy, radiotherapy, and surgical resection[26]. However, advanced BCC is a substantial threat and prompt treatment is needed due to its ability to metastasize[26]. Recently, Hh pathway inhibitors like Sonidegib and Vismodegib have been approved for patients with recurrent BCCs and metastatic BCCs[26]. Other inhibitors targeting Gli1, PKC, and PI3K-mTOR pathways have also shown promising results[32]. However, adverse events (AE) have also been reported and addressing them should be the primary concern[32].

Rhabdomyosarcoma

Rhabdomyosarcoma can be categorized as cancer of soft tissues that accounts for approximately 4.5 % of all known pediatric cancers[33]. Based on histopathological characteristics, RMS is classified as alveolar RMS (aRMS), embryonal RMS (eRMS), spindle cell, pleomorphic, and sclerosing RMS (ssRMS)[33]. Most cases of aRMS exhibit one or more chromosomal translocations: t (1;13) (p36; q14) or t (2;13) (q35; q14)[34], whereas eRMS is characterized by gain of chromosomes 13, 12, 11, 8, 7, and 2. Loss of short arm of chromosome 11 has also been reported[35]. The connection between RMS and Hh signaling was demonstrated by Hahn and colleagues in 1998. They showed that *Ptch1* heterozygous mice exhibited symptoms similar to those observed in Gorlin's syndrome[36]. Skeletal and neural abnormalities were also reported in these mice. Additionally, high levels of Ptch1 and Gli1, expression were noticed. The most distinctive feature of these mice was the high incidence rate of eRMS [36]. Although hyperactivation of the Hh pathway is an accepted view in RMS field, however, contradictory reports exist. For example, in one of the studies, the mRNA levels of *PTCH1*, as well as *GLI1* in eRMS tumors, did not correlate with analyzed clinical characteristics or survival[37]. While some researchers reported a loss of function of SUFU and PTCH in eRMS emphasizing the role of Hh signaling[38–40]. Furthermore, many studies on xenografted rhabdomyosarcoma models showed a reduction in tumor development upon treatment with Hh pathway inhibitors like GANT61, forskolin, and betulinic acid[35]. In a recent study, 18 RMS samples stained for

Gli1, Shh, and Ptch showed positive staining *via* immunohistochemistry[41]. In some reports, amplification of GLI1 has been shown in a subset of aRMS[42]. Furthermore, elevated levels of *Hh* genes namely *GLI1*, *GLI3*, and *PTCH1* were reported in eRMS[42]. In a very recent study, high expression levels of Ihh and Dhh ligands were shown in RMS[43]. ShRNA based silencing of these ligands led to a reduction in tumor size[43]. All these studies highlight the important role played by Hh signaling in RMS biology and also show that pharmacological targeting of Hh signaling in RMS could be used as a potential therapy.

Pancreatic cancer

Pancreatic cancer is one of the prevalent cancer-related deaths worldwide. Owing to the high metastatic potential of this cancer, the overall survival rate is below 5%. Hyperactivation of Hh signaling cascade has been demonstrated in pancreatic cancer stem cells (CSC) contributing to tumor initiation, maintenance, progression, and metastasis[44–46]. Aberrant expression of Shh and Hh ligands has been studied in invasive, pre-invasive pancreatic samples as well as in pancreatic cancer cell lines[47]. In pancreatic ductal adenocarcinoma (PDAC), two different modes of Hh signaling pathway activation occur in the tumor epithelial cells and stromal cells[48]. In the tumor epithelial cells, recognized PDAC oncogenic cascades, such as KRAS, TGF-β, and EGFR signaling, control its activity in a ligand-independent way[48]. Whereas, in stromal cells, the Hh pathway is canonically activated by Hh ligands. Aberrant Shh expression has been linked to oncogenic Kras expression in PDAC[48]. Furthermore, constitutive activation of NF-κB has been shown in pancreatic cancer and Shh is one of the target genes for the NF-κB[49–50]. NF-κB activation leads to an increase in transcriptional activity of Shh due to potential NF-κB binding sites in *SHH* promoter, which was demonstrated in cell-based experiments as well as in mouse models[50]. Kras is known to be a transcriptional activator for the NF-κB gene[51–52]. Oncogenic Kras may thereby increase Shh expression through NF-κB signaling[47]. However, new evidence challenges the Hh pathway's function in pancreatic cancer. Smo deletion in the pancreas had little effect on Kras-mediated pancreatic cancer formation, and removing stromal Hh signaling speeds up Kras-mediated tumor growth[47]. One of the characteristic features of PDAC is desmoplasia marked by fibroblast activation and proliferation as well as stromal cell synthesis of collagen, laminin, and fibronectin[46–47]. These fibroblasts known as cancer-associated fibroblasts play an important role in the development of PDAC[53]. The Shh ligand produced by cancer epithelial cells stimulates the neighboring stromal cells in a Smo-dependent way resulting in desmoplasia[46,54]. The use of fibroblasts derived from resected pancreatic adenocarcinoma tissues in a co-culture environment enhanced cancer cell proliferation, invasion, migration, and colony formation[53]. These findings point that epithelium-derived Shh is a key regulator of fibrosis in PDAC, and that the activated stroma promotes tumor development in PDAC[47]. Ma *et al* demonstrated that Sanguinarine (SNG), a naturally occurring isoquinoline compound, could inhibit the proliferation of pancreatic stem cells *via* induction of apoptosis[55]. They noticed high expression levels of Hh pathway components namely Ptch1, Ptch2, Gli1, Gli2, and Smo in pancreatic CSCs which could be inhibited by SNG[55]. Further, they also observed a decrease in the expression of Cyclin D1 and Bcl-2, target genes of Gli by SNG, indicating that SNG modulates cell cycle, cell survival, and apoptosis in pancreatic CSC. NANOG, a transcriptional factor, has been illustrated to be associated with various cancers and highly expressed in CSC[55]. In cancer patients, high NANOG expression is linked to poor prognosis[55]. *NANOG* is a direct target gene of Gli and treatment with SNG led to a reduction in its expression[55]. Thus, SNG could be used for Hh signaling-induced pancreatic cancer. Sulforaphane, a phytochemical modulates myriad cellular functions like cell cycle arrest, induction of apoptosis, angiogenesis, oxidative stress, carcinogen detoxification, and suppression of metastasis[56–57]. In a study by Li *et al*, sulforaphane greatly decreased tumor growth and self-renewal of both *in vitro* pancreatic CSCs and orthotopically implanted primary human pancreatic CSCs[58].

Another natural compound, baicalein isolated from *Scutellaria baicalensis*, exhibits antitumor function against various cancers including hepatocellular, bladder, skin, myeloma, prostate, and breast cancer[59]. Baicalein has also been demonstrated to limit pancreatic CSC self-renewal by repressing the Hh signal transduction pathway[60]. Further, treatment of BxPC-3, a pancreatic cancer line, with baicalein led to a decrease in cell proliferation at micromolar concentrations[60]. In a separate research, pancreatic cancer cells resistant to Gemcitabine had higher amounts of Hh pathway components and CSC markers[61]. Cyclopamine treatment of these resistant cells reversed the chemoresistance and reduced CSC marker expression[44,61]. Taken together, inhibition of

Hh signaling in pancreatic cancer might be investigated as a viable approach to treat pancreatic cancer patients, based on the existing understanding.

Holoprosencephaly

HPE is a manifestation of aberrant developmental signaling pathways and comprises a wide range of neural and craniofacial defects[62]. It can be described as a condition in which the cerebral hemisphere is underdeveloped or completely fails to develop[63]. Insufficient or partial cleavage of prosencephalon during the 18th and 28th day of gestation leading to irregular facial as well as brain anatomy with cleft palate and lips are the general characteristics of HPE[63]. Anomalies like cebocephaly and ethmocephaly have also been observed in HPE patients[63]. Furthermore, severe cases of HPE exhibit cyclopia (single eye)[63]. Both males and females are affected equally in HPE. The rate of incidence of HPE is 1 in 16 000 live births and 1 in 250 during the early embryonic developmental period[63]. Multiple genes belonging to three different development pathways (sonic hedgehog, retinoic acid) and nodal pathway have been demonstrated to play role in the development of HPE[62]. One of the pathways that is needed for patterning of the forebrain is the Shh pathway and mutations in this pathway are the primary cause of HPE[64]. The Nodal signaling pathway is responsible for the beginning of Shh transcription in the notochord and prechordal plate. Shh released from the axial mesoderm initiates Shh transcription in the floor plate, medial, and ventral neural tube cells[65]. Diencephalon specification, ventral neuronal identification, and correct partitioning of the eye field into two domains need a graded hedgehog signal originating from the floor plate and axial mesoderm[62]. Research has shown that in zebrafish ptc1 and ptc2 mutants exhibit upregulation of Hh pathway contributing to mispatterning of somites and neural tube ventralization[62]. Furthermore, mutations in patched cause persistent suppression of the Hh pathway, resulting in abnormalities such as corpus callosum and midline deficits, both of which are HPE-related defects[66]. HPE is associated with 64 distinct mutations in the *SHH* gene, which include missense, frameshift, and nonsense mutations[67]. Perturbations in DISP, GAS1, CDO, GLI2, HHAT, ZIC2, SIX3, FGF8, and NODAL are prominent in HPE apart from other genes[68]. In HPE, Megalin, a Shh pathway lipoprotein receptor, plays an important role[69]. It is involved in the endocytosis of diverse ligands including Shh[69]. One of the characteristic aspects of HPE is the fusing of brain hemispheres, which is shown by megalin mutations in mouse embryos[69]. Treatment for HPE varies depending on the severity of the condition[63]. For HPE patients with cleft palate and lip, surgery is the principal treatment option[63].Few infants with HPE survive to adulthood and the survival usually correlates with the degree of brain malformation[63]. An integrative approach involving occupational and physical therapies are given to improve the quality of life[63]. The overall outcome for HPE patients is very bleak[63].

Ciliopathies

The primary cilia are fundamentally important structures and are responsible for receiving and relay of external signaling cues (both mechanical and chemical) to the internal environment of the cell[70]. Multiple receptors belonging to Wnt, Hippo, Hedgehog, and various other signaling pathways localize to the primary cilium[15]. Therefore, any kind of perturbations leading to defective formation of cilia, impairment of ciliary signaling, and ciliary trafficking results in abrogation of signals and a collection of diseases known as "ciliopathies"[71]. Several ciliopathies are linked to abnormal Hh signaling. A well-known example is Nephronophthisis (NPHP), a clinical condition characterized by cystic kidney, cerebellar ataxia, liver fibrosis, and retinal degeneration[72–73]. Mutations in genes encoding proteins found in centrosomes, basal bodies, and primary cilia cause NPHP[74]. Particularly mutations in nephrocystin-7 (*NPHP7*) also known as Gli-similar protein 2 (*GLIS2*) were noticed in this disease[75]. GLIS2 is a transcription factor and closely linked to GLI family of transcriptional modulators, thus connecting Nephronophthisis to Hh pathway[75–76]. Other examples connecting Hh signaling and ciliopathies are Meckel syndrome, oral- facial-digital syndrome and Joubert syndrome. In them, mutations in Jbts17 gene lead to decreased expression levels of *Shh* gene[77–78]. Besides, the *Jbts17* gene has been described to be important in ciliary trafficking and ciliogenesis[77–78]. Kinesin family member 3A (Kif3A) is an important component of cilia and plays a vital part in intraflagellar transport (IFT), and mutations in it lead to dysregulated Hh signaling, resulting in malformations during skeletogenesis[79], cerebellar development, and neural tube formation. Aside from Kif3A, mutations in IFT139 cause aberrant Gli3 repressor and activator ratios, leading to neural tube deformation[80]. Similarly, mice lacking IFT172 and IFT88 have aberrant ventral spinal cord patterning owing to defective Hh signaling[81]. All these examples

demonstrate the important roles played by primary cilia and Hh signaling in various ciliopathies. Many more examples linking components of the primary cilia and Hh signaling have been demonstrated in the pathology of ciliopathies, however, they are out of the scope of this review.

Treatments

The spectrum of disorders caused by aberrant Hh signaling is very wide, hence the treatment strategies also vary depending upon the type of disease/disorder[17]. Mostly, for the Hh-induced developmental disorders, the overall survival is poor and no specific treatment strategy is available[62–63]. For example, patients with HPE suffer from multiple conditions, including seizures, genetic conditions, craniofacial abnormalities, cerebral palsy, and neurologic problem[82]. Diagnosis of HPE can be done by performing magnetic resonance imaging and ultrasound scanning during the first trimester[63]. For HPE patients with cleft palate and lip, surgery is the principal treatment option[63]. In HPE patients, intrauterine death is quite common[82] and the long-term outcomes for HPE survivors are often very poor, and occupational and physical therapies are given to improve the quality of life[83]. Multiple inhibitors against Hh cascade have been developed[32]. GLI antagonist 58 (GANT58), GLI antagonist 61 (GANT61), arsenic trioxide[43], and imiquimod are some of the inhibitors against Gli transcription factors[84]. Smoothened antagonist, cyclopamine, taladegib, sonidegib, vismodegib, saridegib, TAK-441, LEQ506, BMS-833923, PF-0444913, MK4101, itraconazole, and vitamin D3 are examples of Smo inhibitors[84]. Out of these Smo inhibitors, only GDC-0449 (vismodegib) and LDE225 (sonidegib) are FDA-approved[85]. Robotnikinin and 5E1 antibody are examples of the inhibitors that have been shown to inhibit Shh signaling[84].

Conclusion

In the domain of Hh signaling, tremendous development has been made over the last few decades. The multitude of functions carried out by this pathway and its disruption leading to a plethora of diseases puts this pathway in the scientific spotlight, and therefore, it is important for researchers to study this pathway and understand how to target it to yield therapeutic benefits. Modern research has helped to understand the functional aspects of various components of this pathway and widened our existing knowledge. However, there are numerous gaps and missing loopholes that must be addressed to unravel this enigmatic pathway. Recently, treatment strategies for Hh-induced cancers have been developed, but till now only three drugs have been approved by the FDA, which include Arsenic trioxide (ATO), Vismodegib (GDC-449), and Sonidegib (LDE225). These drugs have been shown to be effective although side effects like gastrointestinal problems, nausea, diarrhea, and alopecia were observed in treated patients. Combination therapies where the cumulative effect of dual or multiple drugs is higher than individual drug treatment are thus warranted as treatment modalities. Advances in genomics, proteomics, metabolomics, and drug discovery have opened new directions in the field of personalized (tailor-made) medicine to decrease the side effects of general drug treatment approaches. In conclusion, there is a need to develop safer as well as more specific drugs in the future. Our quest for scientific solutions has just begun and more needs to be done to expand the repertoire of our knowledge.

References

[1] Choudhry Z, Rikani AA, Choudhry AM, et al. Sonic hedgehog signalling pathway: a complex network[J]. *Ann Neurosci,* 2014, 21(1): 28–31.

[2] Varjosalo M, Taipale J. Hedgehog: functions and mechanisms[J]. *Genes Dev,* 2008, 22(18): 2454–2472.

[3] Nüsslein-Volhard C, Wieschaus E. Mutations affecting segment number and polarity in *Drosophila*[J]. *Nature,* 1980, 287(5785): 795–801.

[4] Petrova R, Joyner AL. Roles for Hedgehog signaling in adult organ homeostasis and repair[J]. *Development,* 2014, 141(18): 3445–3457.

[5] Franco HL, Yao HHC. Sex and hedgehog: roles of genes in the hedgehog signaling pathway in mammalian sexual differentiation[J]. *Chromosom Res,* 2012, 20(1): 247–258.

[6] Anderson E, Peluso S, Lettice LA, et al. Human limb abnormalities caused by disruption of hedgehog signaling[J]. *Trends Genet,* 2012, 2(8): 364–373.

[7] Singh S, Tokhunts R, Baubet V, et al. *Sonic hedgehog* mutations identified in holoprosencephaly patients can act in a dominant negative manner[J]. *Hum Genet,* 2009, 125(1): 95–103.

[8] Roessler E, Belloni E, Gaudenz K, et al. Mutations in the human *Sonic Hedgehog* gene cause holoprosencephaly[J]. *Nat Genet,* 1996, 14(3): 357–360.

[9] Garcia ADR, Han YG, Triplett JW, et al. The elegance of sonic hedgehog: emerging novel functions for a classic morphogen[J]. *J Neurosci,* 2018, 38(44): 9338–9345.

[10] Pan A, Chang L, Nguyen A, et al. A review of hedgehog signaling in cranial bone development[J]. *Front Physiol,* 2013, 4: 61.

[11] Dellovade T, Romer JT, Curran T, et al. The hedgehog pathway and neurological disorders[J]. *Annu Rev Neurosci,* 2006, 29: 539–563.

[12] Dhanyamraju PK, Patel TN, Dovat S. Medulloblastoma: "onset of the molecular era"[J]. *Mol Biol Rep,* 2020, 47(12): 9931–9937.

[13] Skoda AM, Simovic D, Karin V, et al. The role of the Hedgehog signaling pathway in cancer: a comprehensive review[J]. *Bosn J Basic Med Sci,* 2018, 18(1): 8–20.

[14] Doheny D, Manore SG, Wong GL, et al. Hedgehog signaling and truncated GLI1 in cancer[J]. *Cells,* 2020, 9(9): 2114.

[15] Wheway G, Nazlamova L, Hancock JT. Signaling through the primary cilium[J]. *Front Cell Dev Biol,* 2018, 6: 8.

[16] Goetz SC, Ocbina PJR, Anderson KV. The primary cilium as a Hedgehog signal transduction machine[J]. *Methods Cell Biol,* 2009, 94: 199–222.

[17] Sasai N, Toriyama M, Kondo T. Hedgehog signal and genetic disorders[J]. *Front Genet,* 2019, 10: 1103.

[18] Briscoe J, Thérond PP. The mechanisms of Hedgehog signalling and its roles in development and disease[J]. *Nat Rev Mol Cell Biol,* 2013, 14(7): 416–429.

[19] Gupta S, Takebe N, LoRusso P. Review: targeting the Hedgehog pathway in cancer[J]. *Ther Adv Med Oncol,* 2010, 2(4): 237–250.

[20] Vaillant C, Monard D. SHH pathway and cerebellar development[J]. *Cerebellum,* 2009, 8(3): 291–301.

[21] Dahmane N, Altaba ARI. Sonic hedgehog regulates the growth and patterning of the cerebellum[J]. *Development,* 1999, 126(14): 3089–3100.

[22] Huang SY, Yang JY. Targeting the hedgehog pathway in pediatric medulloblastoma[J]. *Cancers (Basel),* 2015, 7(4): 2110–2123.

[23] Romer JT, Kimura H, Magdaleno S, et al. Suppression of the Shh pathway using a small molecule inhibitor eliminates medulloblastoma in *Ptc1*$^{+/-}$*p53*$^{-/-}$ mice[J]. *Cancer Cell,* 2004, 6(3): 229–240.

[24] Millard NE, De Braganca KC. Medulloblastoma[J]. *J Child Neurol,* 2016, 31(12): 1341–1353.

[25] Northcott PA, Shih DJH, Peacock J, et al. Subgroup-specific structural variation across 1,000 medulloblastoma genomes[J]. *Nature,* 2012, 488(7409): 49–56.

[26] Dika E, Scarfì F, Ferracin M, et al. Basal cell carcinoma: a comprehensive review[J]. *Int J Mol Sci,* 2020, 21: 5572.

[27] Pellegrini C, Maturo MG, Di Nardo L, et al. Understanding the molecular genetics of basal cell carcinoma[J]. *Int J Mol Sci,* 2017, 18: 2485.

[28] Sari IN, Phi LTH, Jun N, et al. Hedgehog signaling in cancer: a prospective therapeutic target for eradicating cancer stem cells[J]. *Cells,* 2018, 7(11): 208.

[29] Takahashi C, Kanazawa N, Yoshikawa Y, et al. Germline *PTCH1* mutations in Japanese basal cell nevus syndrome patients[J]. *J Hum Genet,* 2009, 54(7): 403–408.

[30] Patankar AP, Kshirsagar RA, Dugal A, et al. Gorlin-Goltz syndrome: a series of three cases[J]. *Natl J Maxillofac Surg,* 2014, 5: 209–212.

[31] Saridaki Z, Liloglou T, Zafiropoulos A, et al. Mutational analysis of *CDKN2A* genes in patients with squamous cell carcinoma of the skin[J]. *Br J Dermatol,* 2003, 148(4): 638–648.

[32] Peer E, Tesanovic S, Aberger F. Next-generation hedgehog/GLI pathway inhibitors for cancer therapy[J]. *Cancers (Basel),* 2019, 11(4): 538.

[33] Chen C, Garcia HD, Scheer M, et al. Current and future treatment strategies for rhabdomyosarcoma[J]. *Front Oncol,* 2019, 9: 1458.

[34] Gleditsch K, Peñas J, Mercer D, et al. Intratumoral translocation positive heterogeneity in pediatric alveolar rhabdomyosarcoma tumors correlates to patient survival prognosis[J]. *Front Cell Dev Biol,* 2020, 8: 564136.

[35] Roma J, Almazán-Moga A, de Toledo JS, et al. *Notch, Wnt*, and *Hedgehog* pathways in rhabdomyosarcoma: from single pathways to an integrated network[J]. *Sarcoma,* 2012, 2012: 695603.

[36] Hahn H, Wojnowski L, Zimmer AM, et al. Rhabdomyosarcomas and radiation hypersensitivity in a mouse model of Gorlin syndrome[J]. *Nat Med,* 1998, 4(5): 619–622.

[37] MacDonald TJ. Hedgehog pathway in pediatric cancers: they're not just for brain tumors anymore[J]. *Am Soc Clin Oncol Educ B,* 2012, 32: 605–609.

[38] Bridge JA, Liu J, Qualman SJ, et al. Genomic gains and losses are similar in genetic and histologic subsets of rhabdomyosarcoma, whereas amplification predominates in embryonal with anaplasia and alveolar subtypes[J]. *Genes Chromosom Cancer,* 2002, 33(3): 310–321.

[39] Bridge JA, Liu J, Weibolt V, et al. Novel genomic imbalances in embryonal rhabdomyosarcoma revealed by comparative genomic hybridization and fluorescence in situ hybridization: an intergroup rhabdomyosarcoma study[J]. *Genes Chromosom Cancer,* 2000, 27(4): 337–344.

[40] Calzada-Wack J, Kappler R, Schnitzbauer U, et al. Unbalanced overexpression of the mutant allele in murine *Patched* mutants[J]. *Carcinogenesis,* 2002, 23(5): 727–733.

[41] Oue T, Yoneda A, Uehara S, et al. Increased expression of the hedgehog signaling pathway in pediatric solid malignancies[J]. *J Pediatr Surg,* 2010, 45(2): 387–392.

[42] Zibat A, Missiaglia E, Rosenberger A, et al. Activation of the hedgehog pathway confers a poor prognosis in embryonal and fusion gene-negative alveolar rhabdomyosarcoma[J]. *Oncogene,* 2010, 29(48): 6323–6330.

[43] Almazán-Moga A, Zarzosa P, Molist C, et al. Ligand-dependent hedgehog pathway activation in rhabdomyosarcoma: the oncogenic role of the ligands[J]. *Br J Cancer,* 2017, 117(9): 1314–1325.

[44] Huang F, Zhuan-Sun Y, Zhuang Y, et al. Inhibition of hedgehog signaling depresses self-renewal of pancreatic cancer stem cells and reverses chemoresistance[J]. *Int J Oncol,* 2012, 41(5): 1707–1714.

[45] Jones S, Zhang X, Parsons DW, et al. Core signaling pathways in human pancreatic cancers revealed by global

genomic analyses[J]. *Science,* 2008, 321(5897): 1801–1806.

[46] Feldmann G, Dhara S, Fendrich V, et al. Blockade of hedgehog signaling inhibits pancreatic cancer invasion and metastases: a new paradigm for combination therapy in solid cancers[J]. *Cancer Res,* 2007, 67(5): 2187–2196.

[47] Gu D, Schlotman KE, Xie J. Deciphering the role of hedgehog signaling in pancreatic cancer[J]. *J Biomed Res,* 2016, 30(5): 353–360.

[48] Hogenson TL, Olson RLO, Fernandez-Zapico ME. Hedgehog signaling plays a dual role in pancreatic carcinogenesis[M]//Neoptolemos JP, Urrutia R, Abbruzzese JL, et al. Pancreatic Cancer. New York: Springer, 2018: 409–430.

[49] Rhim AD, Oberstein PE, Thomas DH, et al. Stromal elements act to restrain, rather than support, pancreatic ductal adenocarcinoma[J]. *Cancer Cell,* 2015, 25(6): 735–747.

[50] Bailey JM, Swanson BJ, Hamada T, et al. Sonic hedgehog promotes desmoplasia in pancreatic cancer[J]. *Clin Cancer Res,* 2008, 14(19): 5995–6004.

[51] Yauch RL, Gould SE, Scales SJ, et al. A paracrine requirement for hedgehog signalling in cancer[J]. *Nature,* 2008, 455(7211): 406–410.

[52] Hwang RF, Moore T, Arumugam T, et al. Cancer-associated stromal fibroblasts promote pancreatic tumor progression[J]. *Cancer Res,* 2008, 68(3): 918–926.

[53] Lee JJ, Perera RM, Wang H, et al. Stromal response to Hedgehog signaling restrains pancreatic cancer progression[J]. *Proc Natl Acad Sci U S A,* 2014, 111(30): E3091–E3100.

[54] Bailey JM, Mohr AM, Hollingsworth MA. Sonic hedgehog paracrine signaling regulates metastasis and lymphangiogenesis in pancreatic cancer[J]. *Oncogene,* 2009, 28(40): 3513–3525.

[55] Ma Y, Yu W, Shrivastava A, et al. Sanguinarine inhibits pancreatic cancer stem cell characteristics by inducing oxidative stress and suppressing sonic hedgehog-Gli-Nanog pathway[J]. *Carcinogenesis,* 2017, 38(10): 1047–1056.

[56] Houghton CA. Sulforaphane: its "coming of age" as a clinically relevant nutraceutical in the prevention and treatment of chronic disease[J]. *Oxid Med Cell Longev,* 2019, 2019: 2716870.

[57] Juge N, Mithen RF, Traka M. Molecular basis for chemoprevention by sulforaphane: a comprehensive review[J]. *Cell Mol Life Sci,* 2007, 64(9): 1105–1127.

[58] Li SH, Fu J, Watkins DN, et al. Sulforaphane regulates self-renewal of pancreatic cancer stem cells through the modulation of Sonic hedgehog-GLI pathway[J]. *Mol Cell Biochem,* 2013, 373(1–2): 217–227.

[59] Liu H, Dong Y, Gao Y, et al. The fascinating effects of baicalein on cancer: a review[J]. *Int J Mol Sci,* 2016, 17(10): 1681.

[60] Song L, Chen X, Wang P, et al. Effects of baicalein on pancreatic cancer stem cells *via* modulation of sonic Hedgehog pathway[J]. *Acta Biochim Biophys Sin (Shanghai),* 2018, 50(6): 586–596.

[61] Yao J, An Y, Wei J, et al. Cyclopamine reverts acquired chemoresistance and down-regulates cancer stem cell markers in pancreatic cancer cell lines[J]. *Swiss Med Wkly,* 2011, 141: w13208.

[62] Gongal PA, French CR, Waskiewicz AJ. Aberrant forebrain signaling during early development underlies the generation of holoprosencephaly and coloboma[J]. *Biochim Biophys Acta (BBA)-Mol Basis Dis,* 2011, 1812(3): 390–401.

[63] Dubourg C, Bendavid C, Pasquier L, et al. Holoprosencephaly[J]. *Orphanet J Rare Dis,* 2007, 2: 8.

[64] Bertrand N, Dahmane N. Sonic hedgehog signaling in forebrain development and its interactions with pathways that modify its effects[J]. *Trends Cell Biol,* 2006, 16(11): 597–605.

[65] Jeong Y, Epstein DJ. Distinct regulators of *Shh* transcription in the floor plate and notochord indicate separate origins for these tissues in the mouse node[J]. *Development,* 2003, 130(16): 3891–3902.

[66] Goodrich LV, Jung D, Higgins KM, et al. Overexpression of *ptc1* inhibits induction of *Shh* target genes and prevents normal patterning in the neural tube[J]. *Dev Biol,* 1999, 211(2): 323–334.

[67] Roessler E, El-Jaick KB, Dubourg C, et al. The mutational spectrum of holoprosencephaly-associated changes within the *SHH* gene in humans predicts loss-of-function through either key structural alterations of the ligand or its altered synthesis[J]. *Hum Mutat,* 2009, 30(10): E921–E935.

[68] Petryk A, Graf D, Marcucio R. Holoprosencephaly: signaling interactions between the brain and the face, the environment and the genes, and the phenotypic variability in animal models and humans[J]. *Wiley Interdiscip Rev Dev Biol,* 2015, 4(1): 17–32.

[69] McCarthy RA, Argraves WS. Megalin and the neurodevelopmental biology of sonic hedgehog and retinol[J]. *J Cell Sci,* 2003, 116(6): 955–960.

[70] Michaud EJ, Yoder BK. The primary cilium in cell signaling and cancer[J]. *Cancer Res,* 2006, 66(13): 6463–6467.

[71] Waters AM, Beales PL. Ciliopathies: an expanding disease spectrum[J]. *Pediatr Nephrol,* 2011, 26(7): 1039–1056.

[72] Hildebrandt F, Attanasio M, Otto E. Nephronophthisis: disease mechanisms of a ciliopathy[J]. *J Am Soc Nephrol,* 2009, 20(1): 23–35.

[73] Wolf MTF, Hildebrandt F. Nephronophthisis[J]. *Pediatr Nephrol,* 2011, 26(2): 181–194.

[74] Srivastava S, Molinari E, Raman S, et al. Many genes-one disease? Genetics of nephronophthisis (NPHP) and NPHP-associated disorders[J]. *Front Pediatr,* 2018, 5: 287.

[75] Attanasio M, Uhlenhaut NH, Sousa VH, et al. Loss of GLIS2 causes nephronophthisis in humans and mice by increased apoptosis and fibrosis[J]. *Nat Genet,* 2007, 39(8): 1018–1024.

[76] Adamiok-Ostrowska A, Piekiełko-Witkowska A. Ciliary genes in renal cystic diseases[J]. *Cells,* 2020, 9(4): 907.

[77] Damerla RR, Cui C, Gabriel GC, et al. Novel *Jbts17* mutant mouse model of Joubert syndrome with cilia transition zone defects and cerebellar and other ciliopathy related

anomalies[J]. *Hum Mol Genet,* 2015, 24(14): 3994–4005.

[78] Toriyama M, Lee C, Wallingford JB. The ciliopathy-associated CPLANE proteins direct basal body recruitment of intraflagellar transport machinery[J]. *Nat Genet,* 2016, 48(6): 648–656.

[79] Song B, Haycraft CJ, Seo HS, et al. Development of the post-natal growth plate requires intraflagellar transport proteins[J]. *Dev Biol,* 2007, 305(1): 202–216.

[80] Tran PV, Haycraft CJ, Besschetnova TY, et al. THM1 negatively modulates mouse sonic hedgehog signal transduction and affects retrograde intraflagellar transport in cilia[J]. *Nat Genet,* 2016, 40(4): 403–410.

[81] Liu A, Wang B, Niswander LA. Mouse intraflagellar transport proteins regulate both the activator and repressor functions of Gli transcription factors[J]. *Development,* 2005, 132(13): 3103–3111.

[82] Society for Maternal-Fetal Medicine (SMFM), Monteagudo A. Holoprosencephaly[J]. *Am J Obstet Gynecol,* 2020, 223(6): B13–B16.

[83] Pallangyo P, Lyimo F, Nicholaus P, et al. Semilobar holoprosencephaly in a 12-month-old baby boy born to a primigravida patient with type 1 diabetes mellitus: a case report[J]. *J Med Case Rep,* 2016, 10(1): 358.

[84] Tran BAP, Alexander T, Somani AK. Biochemical pathways and targeted therapies in basal cell carcinoma: a systematic review[J]. *J Surg Dermatol,* 2016, 2(1): 23–34.

[85] Jain S, Song R, Xie J. Sonidegib: mechanism of action, pharmacology, and clinical utility for advanced basal cell carcinomas[J]. *Onco Targets Ther,* 2017, 10: 1645–1653.