Review Article

Hedgehog Signalling Pathway: Carcinogenesis and Targeted Therapy

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Abstract

Hedgehog signalling pathway has not only a critical role in cell proliferation, differentiation and tissue polarity at embryonic period but also has a vital role in stem cell proliferation, tissue healing and carcinogenesis. Recent research has increased our understanding of this pathway and its relation to other signalling pathways. In addition, a large number of studies confirmed the alteration of Hh signalling pathway in various types of human malignancies including basal cell carcinomas, medulloblastomas, lung, gastrointestinal, ovarian, breast, prostate cancers and leukemia. More than 50 small biomolecules have been introduced which have inhibitory effects on Hh signalling pathway. Although, in many tumors some acceptable results have been showed in phase I clinical trial, closer studies are required to improve drug bioavailability, to decrease the side effects and to find the right small molecules for specific types of cancers, considering patients overall benefits as well.

Keywords: Hedgehog; Neoplasm; Molecular targeted therapy

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Introduction

For the first time, approximately 30 years ago, hedgehog was explained in Drosophila melanogaster by Eric Wieschaus and Christiane Nüsslein-Volhard who shared the 1995 Nobel Prize in physiology or medicine with E. B. Lewis. The Hedgehog signalling pathway genes are considered as essential components in cell proliferation, differentiation and tissue polarity during embryonic development. In adult, this pathway could have function in stem cell proliferation, tissue repair, regeneration and oncogenesis. In mammals, these genes programme the production of three specific extracellular Hh ligands (proteins) including DHH, (Desert Hedgehog), IHH, (Indian Hedgehog) and SHH (Sonic Hedgehog). Other components of the Hh signalling pathway include: Patched protein 1and 2, Smo FU, SUFU, KIF7, Gli1, Gli2 and Gli3.Various defect in these molecules is responsible for developmental abnormalities during embryonic period and postnatal malignant transformation as well[1, 2].

The hedgehog signalling pathway

In mammals, three hedgehog proteins including Sonic Hedgehog (SHH), Indian Hedgehog (IHH) and Desert Hedgehog (DHH) function as autocrine or remote-acting proteins in the target tissues. The proteins undergo autoprocessing by N-terminal signal sequence deletion and cholesterol modification (C-terminus) [2- 4]. Cholesterol modification is not only essential for the catalytic cleavage of Sonic hedgehog protein but also, the patched protein that binds the Sonic hedgehog protein also needs cholesterol to be functional [5]. Hh protein intracellular transport and secretion is regulated by a number of molecules for instance the transmembrane transporter-like protein Dispatched (Disp) and metalloproteases [6].In mammals, the ligands (mature Hh proteins) bind to the two membrane receptors, Patched1 and Patched2 [7]. PTCH-1 is not only vital for embryogenesis, but also is considered as a human tumor suppressor gene [8]. The presence and absence of Hh ligands can activate or inhibit transmembrane protein Patched (PTC) to allow or prevent transmembrane protein Smoothened (Smo) to the signal downstream respectively. Smo signal downstream leads to activation of the GLI transcription factors which regulate hyperexpression of genes related to the hedgehog pathway [9, 10]. Glioma-associated oncogene or GLI (transcription factor protein) has three different forms: GLI1 is an

activator of transcription, while GLI2 and GLI3 can have suppressive or activating function [2]. There are three various states of Smo:SmoA is inactive but internalised form, SmoB is also inactive form with the attachment to the cilium which can converts to the active form (SmoC) [11]. The interactions between various molecules of the Hh signalling pathway can occur in the cilium [12]. Cilia are tiny hair-like protrusions of the cell membrane with communicative functions which contribute to perception of the mechanical and chemical signals. In addition, it has a vital role in cell differentiation and polarity [13].

Significant numbers of research showed that in mammals cells the primary cilia have a crucial function in the hedgehog signalling pathway but with unknown mechanisms [11]. Not just localization of patched, Smo and GLI in the primary cilia but also mutation in the ciliary related genes with Shh-relevant phenotypes indicates the necessity of this crucial structure for developmental processes [14]. However, new research showed that just localization of Smo in cilium cannot activate hedgehog signalling pathway [11]. In addition, new finding showed that cilium is not required for Suppressor of Fused [(Su (Fu)] mediated negative regulation of GLI functions [15]. Furthermore, deletion of fused gene in mice which produce interacting protein named Suppressor of Fused (SUFU), can lead to hydrocephalus and death, however, without alternation in Hh signalling pathway, that could bring an argument about the role of Fused in Hh signalling pathway [16]. New research in mice showed that KIF7 as a small molecule is effective in downstream regulation of the hedgehog signalling pathway [17]. Ultimately, the production of tissues, regulation of internal environment, organ development and stem cell replenishment are complex functions that need interactions of many pathways such as Hh, Wnt/b-catenin, TGF-b/BMP, Notch and FGF signalling pathways [2]. Figure 1 shows the simplified model of hedgehog pathway and its relation to cancer development and progression.

Linkage between hedgehog signalling pathway and human cancer

Diversity of defects and effects of the Hh signalling pathway on carcinogenesis

There are three basic types of cancer pathogenesis in terms of molecular dysregulation in the Hh signalling pathway. Type 1 is characterised by the Hh signalling activity in the absence of ligand that can be caused for instance by mutation in the PTCH 1 or SuFu or GLI genes. Therefore, in this type of cancer drugs must be effective at the downstream of the mutation. Gorlin syndrome is an excellent example of this type of cancer [2]. Recently, type 2 cancer was explained in colon cancer cell lines. This type is ligand-dependent and functions as an autocrine manner, which means the tumor cells secret high level of Hh ligands that activate Hh signalling pathway in the same tumor cells [18]. Type 3 cancer requires ligand. The activation of Hh signalling pathway affects the nearby cells including endothelial cells, immune cells, fibroblasts and epithelial cells. This paracrine manner of Hh ligand production is required for tumor growth. This type is the most common type of Hh signalling activation in malignant tumors which leads to the angiogenesis, tumor stem cell maintenance and activation of Insulin-like Growth Factor (IGF) and Wnt signalling pathways [19]. Finally, in reverse paracrine type (type 3 b), hh ligand is produced by the stromal cells which lead to overactivation of the Hh signalling pathway in tumor cells [2].

Basal cell carcinoma

Basal Cell Carcinoma (BCC) is considered as a high prevalent sporadic cancer in the white men with constant raise in frequency worldwide. However, many cases present as basal cell nevus syndrome (Gorlin syndrome). Gorlin syndrome is an autosomal dominant genetic syndrome which clinically present with multiple BCCs and extracutaneous cancer including medulloblastoma, and rhabdomyosarcoma. In the skin keratinocyte Hedgehog (Hh) pathway dysregulation is observed in both sporadic and hereditary basal cell carcinomas, which lead to the activation of Hedgehog (Hh) signalling pathway [20].There is a relationship between mutation in PTCH1 gene and Gorlin syndrome [21]. Approximately 50% of sporadic BCCs show mutation in the PTCH1 gene [22]. In addition, mutations of Smo are observed in approximately10% of sporadic BCCs [23]. Furthermore, SU (FU) mutation is found in a small percentage of BCCs [24]. However, still about 30% of basal cell skin cancer can occur without molecular evidence of dysregulation in the Hedgehog signalling pathway [22]. Because, nearly all BCCs show activation of Hh signalling, it seems that in 30% of sporadic BCCs alterations in other related molecules or Hh signalling components could explain Hh pathway activation [1].

Many candidate genes are considered as downstream effectors of the HH signalling including overexpressed Platelet-Derived Growth Factor Receptor-α (PDGFRα) gene[25] , overexpressed apoptosis inhibitors (BCL2) [26],and CASP8 and

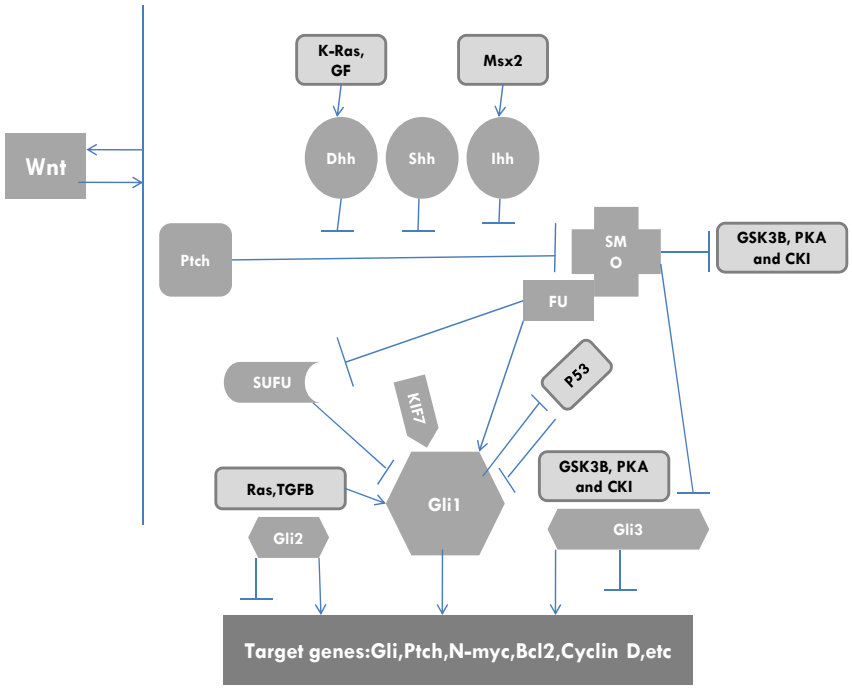

Figure 1. Hh signalling and cancer: Growth factors can increase the concentration of ligand which prevents the inhibitory effect of PTCH. Active Smo upregulates the GLI in cooperation with Fu. Finally GLI affects target genes. P53 and GLI antagonize one another, SUFU blocks the GLI, GLI2 and GLI3 could have activating or inhibitory effects whereas GLI1 is an activator of transcription. SmoC is actived Smo and inhibits GSK3β, PKA, and CKI. KIF7 is activator of GLI. Wnt and Hh signalling work together in a feedback loop manner.

FADD-like apoptosis regulator (CFLAR) genes [27] and down-regulation of the apoptosis-inducing factor orCD95 (FAS) [28]. However, the downstream pathway which is vital for HH-induced BCC tumorigenesis remains to be confirmed. In summary, SHH, Smo, GLI1 and GLI2 overexpression in transgenic mice are related to BCC carcinogenesis, whereas, PTCH1 knockout and Sufu genes (downregulation) have a linkage with BCC carcinogenesis in mice [29].

Medulloblastoma

Medulloblastoma is the most common and extremely aggressive childhood malignant childhood brain tumor. Approximately 15–20% of Central Nervous System (CNS) cancers are medulloblastoma. Its peak incidence is around age 5 and mainly occurs before age 10. Although, there are several therapeutic modalities, medulloblastoma is a poor prognostic tumor [30, 31]. In about 30% of medulloblastomas genetic changes indicate Hh signalling activation. However, just 50% of these cases showed loss of function mutations in PTCH and SuFu, and activating mutations in Smo. Therefore nearly 50% of tumors exhibit dysregulation of the Hh signalling in the absence of such mutations [32,33]. In a subset of tumors, there are evidence of both Wnt and Hh signalling pathways deregulation [34]. Finally, a group of medulloblastoma is a result of mutations in the Wnt signalling pathway [35].

Interaction between Wnt signalling and HH signalling in carcinogenesis

Previous research documents could not clearly confirm the role of Wnt signalling in BCC tumorigenesis [29]. However, a recent study supports the role of activated Wnt signalling in the downstream of the Hh signalling in BCC development, in both mice and humans[36].The crucial role of dysregulated Wnt signalling is evident

in a subset of medulloblastoma[34,35]. Depends on the type of tissue Hh and Wnt signalling pathways can have cross talk as a positive feedback or negative feedback activity. For example, Hh signalling has inhibitory effects on Wnt signalling in stomach adenocarcinoma[37].

Hedgehog pathway dysregulation in extracutaneous and Non-BCCs skin cancers

Activation of Hh signalling could be seen in various noncutaneous malignancies, including brain cancer, gastrointestinal, prostate, lung and breast malignant tumors [22]. In addition, evidence support that Hh signalling is essential for carcinogenesis and spreading of malignant melanoma, ovarian cancer, leukemia and B-cell lymphomas [38-42]. New study showed that over 50% of childhood Embryonal Rhabdomyosarcoma (ERMS) could have Hh signalling activation [43].The majority of these extracutaneous cancers which have Hh signalling dysregulation show ligand dependent abnormalities [22]. Immunohistochemistry analysis of various component of the Hh signalling pathway including SHH, PTCH, SMO, GLI-1, GLI-2 and GLI-3 showed a dramatic overexpression , in skin and head and neck squamous cell carcinomas samples. SHH overexpression in particular was associated with poor prognosis and decreased overall survival. Therefore, the role of Hh signalling in SCC is clearly evident however, the mechanistic analysis is an essential research by using animal laboratory models [44].Overexpression of GLI1 [45] and GLI2 [46] have been shown in invasive and metastatic human melanoma cell lines. In addition, GLI2-mediated melanomas overexpression are more likely to develop bone metastasis [46].This signalling pathway has an important role in tumorigenesis because many research showed that its involvement in different stages of tumor development and progression. In pancreatic and esophageal cancers, for instance, this pathway is active not only in early stage, but also is active in metastatic tumor [47-49]. Recently, for the first time a study indicated that Patched, sonic hedgehog and GLI1 overexpression could be poor prognostic factors in patients suffering from colorectal adenocarcinoma [50]. Newly, an in vivo mice model study suggests that the Hedgehog signalling has a crucial role in converting normal prostatic cells into the cancer cells and also in metastatic clone production [51].The Hedgehog signalling dysregulation, the resultant cancer stem cell activation and EMT (Epithelial Mesanchymal Transition) phenomenon are contribute to tumorigenesis and tumor progression in several types of malignancies for instance, esophagus, gastric, colon and hepatic cancers [52]. There is a meaningful discrepancy in the role of Hh signalling in various human malignancies. This paradox could be explained by activation of Hh signalling pathway in specific tissues, cell types or using different standards to evaluate Hh signalling pathway [22]. For instance, several studies showed that Hh signalling is required just for cancer stem cell proliferation [53, 54], chemotherapy or radiotherapy resistance cancers support the effect of Hh signalling in cancer stem cell activity [55, 56]. Another reason for this discrepancy is that many scientists only analyze GLI1 expression for detecting the Hh signalling activation, whereas others evaluate expression of several Hh target genes, such as GLI1, PTCH1, sFRP1 and HIP and finally some groups evaluate Hh signalling activation just by immunohistochemistry. However, important research progress in this field can uncover this discrepancy in the near future [22].

Complex interactions of hedgehog signalling with other pathways in tumorigenesis

There are complex interactions of various signalling pathways with Hh signalling. Ras, NF-κB and Erα interact with Hh signalling in regulated manner by changing in SHH expression [57-59]. In addition, TGFβ, Ras, JUN, SCL/TAL1 and EWS-FLI1 oncoproteins can regulate GLI1 expression [60-64]. Furthermore, P53 and Hh signalling pathway have mutual antagonistic effect. P53 downregulates GLI1 expression and Hh signalling pathway block the activity of p53 by affecting MDM2 regulatory mechanism [65]. Finally, studies showed a substantial interaction between Wnt signalling and Hh signalling, for instance in gastric carcinoma Hh signalling has an inhibitory effect on Wnt signalling [37].However, our knowledge of the underlying molecular mechanism remains limited [22].

Hedgehog signalling and targeted therapy in cancer

Introduction

There are a huge number of small molecules with inhibitory functions on Hh signalling pathway. These compounds can affect various components of Hh signalling pathway mainly including ligand Hh molecules, Smo protein and GLI. In 2009, the first clinical trial on BCCs with acceptable result has been reported [66]. So far, four compounds including IPI-926 (natural Smo protein), GDC-0449, XL-139, LDE-225 (synthetic small molecules) and three small functional molecules (F-04449913, LEQ506 and TAK-441) are accepted to use in phase II and phase

I clinical trials respectively. Although, significant clinical responses occurred in human BCCs and Medulloblastomas in phasel clinical trial, the results of other types of tumors in clinical trials were not satisfying [22,33].

Cyclopamine and its derivatives as natural compounds

Cyclopamine is a natural steroidal alkaloid which obtained from the plant [67]. In 1998, for the first time the effect of Cyclopamine on Hh signalling became evident [1].This natural molecule to some extent has specific effect on Hh signalling in a small blood level (<10 µmol/L); however, higher amount of Cyclopamine can lead to cell death with no effect on Hh signalling [22, 68]. Cyclopamine not only has a therapeutic effect on mice model of BCCs, but has a preventive effect on BCC in mice as well. In addition, Cyclopamine has a preventive function in tumorigenesis (medulloblastoma) in mice with PTCH1 defect. However, it showed no therapeutic effect on squamous cell carcinoma or fibrosarcoma in animal laboratory models [28, 69].At present time for the new Cyclopamine derivative with dramatic acid stability and liquid solubility such as IPI-926, Phasell clinical trial for metastatic pancreatic ductal adenocarcinoma is underway [70]. A Study showed a significant role of Hh signalling in cancer stem cells survival in multiple myeloma and in chronic myeloid leukemia as well [71].Therefore, Hh signalling pathway inhibition is suggested as an effective targeted therapy in leukemias. In addition, many researches support the usefulness of Cyclopamine not only for Chronic Myeloid Leukaemia (CML) but also for imatinib-resistant mice and human leukemic cell lines [53,54].

Pancreatic ductal adenocarcinoma is a relatively common cancer with high mortality, particularly in metastatic stages. There is no effective drug for metastatic pancreatic carcinoma [72]. Recent data showed an essential role of Hh signalling pathway in pancreatic cancer [73]. Prevention of Hh signalling with Cyclopamine in mice model of ductal pancreatic carcinoma supports the idea that Cyclopamine not only could increase the survival, but could prevent metastatic spread as well [74]. Hh signalling based phase II clinical trials for pancreatic and gastric cancers are underway [1].

Novel synthetic compounds with Hh signalling antagonistic effect

Studies showed that blocking of Hh signalling in mice models has minor adverse effects on bone marrow stem cells, which support the application of Hh signalling inhibitors in cancer prevention and therapy [75, 76].The majority of synthetic compounds are targeted to Smo; however, there are many other small molecules which are directed to SHH and GLI components of Hh signalling as well [66, 69, 77]. CUR61414 is an example of this type of compounds, which was used as topical drug to treat sporadic BCCs in a Phase I clinical trial. However, this study could not show the effect of this pharmaceutical agent on Hh target genes expression [78].GDC-0449 is another synthetic small molecule with hopeful responses in phase I clinical trial on BCCs and medulloblastoma, therefore, phase II clinical trial is underway. Medulloblastomas cases showed early dramatic response but drug resistance occurred due to Smo mutation [1, 77, 33]. GDC-0449 Phase II clinical trial in patients with advanced ovarian cancer, as a maintenance single drug could not prevent tumor relapse. In addition, GDC-0449 Phase II clinical trial of colon carcinoma, as a combination therapy with bevacizumab (Avastin; Genentech/Roche) showed no acceptable therapeutic effect [33]. Robotnikinin is a small antibody molecule with Hh signalling inhibitory effects not only in cell lines but also in human primary keratinocytes and in an artificial model of human skin. However, Ptc1 receptor is required for robotnikinin inhibitory effect and this antibody has no blocking effect on Hh signalling in the presence of purmorphamine and SAG (Smo agonists) [79]. Not only synthetic inhibitors of GLI1 function can prevent prostate cancer cell lines multiplication, but Cyclopamine derivativeswere effective in prostate cancer treatment in cell lines and xenograft tumor model as well [80, 81]. Furthermore, a new research on mice model supports that Hedgehog signalling targeted therapy may be a good therapeutic option for androgen-independent prostatic cancer [51].The proliferation of cancer cells in many types of tumors can be prevented by Cyclopamine or its analogs; therefore we can suggest that these Hh antagonists could have widespread application in cancer therapy [22].

Conclusion

In recent years, increasing knowledge regarding Hh signalling pathway and its interactions with various signalling pathways, oncogenes and tumor suppressor genes in tumorigenesis have led to the production of natural and synthetic small biomolecules which have inhibitory effects on Hh signalling pathway. In this review we showed that the new doors are opened into cancer targeted therapy in various types of cancers, however, a huge number

of research on cell lines, animal models and finally clinical trials is required to show that, these kinds of pharmaceutical agents are suitable in terms of bioavailability, effectiveness , acceptable side effects, to go from the bench to the bedside.

Acknowledgment

Due to limitation of space and the presence of huge number of articles about Hh signalling pathway, this manuscript just cited selected number of articles. Therefore, we appreciate all contributors in this field.

Conflict of Interest

The authors have no conflicts of interest and this article is for scientific propose only to distribute advanced knowledge of cancer molecular medicine to the readers.

Authors' Contribution

This work wrote in collaboration between all authors. The subject selection and article structure made and wrote by Abdolali Ebrahimi. Afshin Moradi rechecked the manuscript scientifically. Leila Larijani and Mohamad Reza Ebrahimi provided many useful original articles and also contributed to design the figure. Finally; all authors commented on the manuscript and approved it as well.

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