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The Hedgehog pathway: role in cell differentiation, polarity and proliferation

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Abstract

Hedgehog (Hh) is first described as a genetic mutation that has “spiked” phenotype in the cuticles of *Drosophila* in later 1970s. Since then, Hh signaling has been implicated in regulation of differentiation, proliferation, tissue polarity, stem cell population and carcinogenesis. The first link of Hh signaling to cancer was established through discovery of genetic mutations of Hh receptor gene PTCH1 being responsible for Gorlin syndrome in 1996. It was later shown that Hh signaling is associated with many types of cancer, including skin, leukemia, lung, brain and gastrointestinal cancers. Another important milestone for the Hh research field is the FDA approval for the clinical use of Hh inhibitor Erivedge/Vismodegib for treatment of locally advanced and metastatic basal cell carcinomas. However, recent clinical trials of Hh signaling inhibitors in pancreatic, colon and ovarian cancer all failed, indicating a real need for further understanding of Hh signaling in cancer. In this review, we will summarize recent progress in the Hh signaling mechanism and its role in human cancer.

Keywords

Hedgehog; Smoothened; PTCH1; Cancer; Signal transduction; Clinical trials; Animal model

Introduction

The general signaling mechanism for the Hh pathway is conserved from fly to mammal although more and distinct components are discovered in mammalian cells (Ingham and

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Placzek 2006). Hedgehog signaling molecules in mammal include three ligands (Sonic hedgehog—Shh, Indian hedgehog—Ihh and Desert hedgehog—Dhh), two receptors (PTCH1, PTCH2), a key signal transducer smoothened (SMO) and three transcription factors (Gli1, Gli2, Gli3) (see Fig. 1). When ligands are not present, SMO function is inhibited by another transmembrane protein Patched (PTCH1, PTCH2). Upon binding of an active Hh ligand, this inhibitory effect is lifted, allowing SMO to signal downstream, eventually leading to active transcription of Gli molecules through binding the specific consensus sequences located in the promoter region of the target genes (Kinzler and Vogelstein 1990; Sasaki et al. 1997).

In the last few years, there are significant progresses in our understanding of Hh signaling. This is reflected by the fact that nearly half of the publications on Hh signaling were produced in the last 5 years. The progress has been made in the following areas: (1) better understanding of Hh signal transduction, particularly on structures of smoothened and its small molecule regulators; (2) more reliable mouse models for Hh signaling associated carcinogenesis; (3) better understanding of Hh signaling activation mechanisms during cancer development and metastasis; (4) increasing number of clinical and preclinical studies on cancer treatment using Hh signaling inhibitors; (5) emerging evidence of Hh signaling activation in drug resistant cancer cells.

Signal transduction of the Hedgehog pathway

All Hh proteins are secreted molecules, functioning at short range on nearby cells or at long range to distant cells during development (Ingham and McMahon 2001; McMahon et al. 2003; Taipale and Beachy 2001). Hh protein precursors undergo post-translational modifications [auto-cleavage to release the N-terminal fragment (HhN), covalently binding to a cholesterol moiety at the C-terminal end and palmitoylation by a palmitoylacyltransferase at the N-terminus of HhN] (Buglino and Resh 2008; Lee et al. 1994; Porter et al. 1995, 1996). Recent data indicate that association of Hh with lipoprotein increases the Hh activity (Palm et al. 2013). Molecules involved in Hh protein transport and distribution include the transmembrane transporter-like protein dispatched (Disp) (Caspary et al. 2002; Kawakami et al. 2002; Ma et al. 2002), metalloproteinases (Dierker et al. 2009), the heparan sulfate proteoglycans Dally-like (Dlp) and Dally (Beckett et al. 2008; Lum et al. 2003) or their regulators (Baena-Lopez et al. 2008) as well as enzymes such as Sulfateless and Tout velu (Bellaiche et al. 1998; Koziel et al. 2004; Toyoda et al. 2000).

The mammalian Hh signaling pathway with major players is shown in the diagram of Fig. 1. Several molecules are engaged in reception of Hh ligands, with Patched (PTC, one PTC in fly and two PTCs in vertebrates—PTCH1 and PTCH2) as the major receptor (Stone et al. 1996). Studies from cultured cells indicate that PTC inhibits SMO at a substochiometric concentration (Taipale et al. 2002). Hhinteracting protein (HIP) can compete with PTC on Hh binding, resulting in negative regulation of Hh signaling (Chuang and McMahon 1999). On the other hand, interference hedgehog (Ihog) or its vertebrate homologues cell adhesion molecule-related/down-regulated by oncogenes (CDO) and BOC (brother of CDO), GAS1 and Glypican-3 (GPC3), serve as co-receptors of Hh (Allen et al. 2007; Capurro et al. 2008; Martinelli and Fan 2007; Okada et al. 2006; Seppala et al. 2007; Tenzen et al. 2006; Yao et

al. 2006; Zhang et al. 2006). In contrast to the inhibitory effect of GPC3, Glypican-5 (GPC5) and other HSPGs are shown to stimulate Hh signaling by promoting binding of sonic Hh to PTCH1 (Li et al. 2011b; Witt et al. 2013). The effect of GPC5 and Ihog homologues requires another secreted extracellular molecule—wnt inhibitory factor-1 (WIF1) (Avanesov et al. 2012; Sanchez-Hernandez et al. 2012). It is still not entirely clear how binding of Hh proteins results in the pathway activation. It is proposed that PTC limits SMO signaling via transporting endogenous small molecules specifically targeted to SMO. Candidates of these small molecules include PI4P, lipoproteins and pro-vitamin D3 (Bijlsma et al. 2006; Callejo et al. 2008; Khaliullina et al. 2009; Yavari et al. 2010). It is currently not very clear how these molecules regulate SMO signaling. Upon binding to Hh, PTCH1 undergoes Smurf1/2-mediated monoubiquitination at multiple sites and enters endocytosis pathway, resulting in protein degradation in lysozyme (Huang et al. 2013; Yue et al. 2014).

It is now known that glucocorticoid molecules can modulate SMO signaling through regulating its ciliary localization (Wang et al. 2012a). Several recent reports support SMO to G protein coupling (Molnar et al. 2007; Ogden et al. 2008; Philipp et al. 2008; Riobo et al. 2006) but the functional involvement of G protein coupling of SMO in carcinogenesis has not been convincingly demonstrated. Gα can also regulate Gli proteins independent of SMO (Douglas et al. 2011). It is quite clear that two important events occur during SMO signaling in mammalian cells. First, SMO protein undergoes conformational change to favor SMO signaling (Zhao et al. 2007) although the regulatory mechanism underlying this conformational change is not clear. Recent data suggest that SMO undergoes monoubiqutination, and the process is blocked by USP8 (Li et al. 2012a; Xia et al. 2012). Second, ciliary translocation of mammalian SMO protein is critical for Hh signaling (Corbit et al. 2005; Hoover et al. 2008; Huangfu and Anderson 2005; Huangfu et al. 2003; May et al. 2005; Zhang et al. 2005). Several reports now link neuropilin 1/2 (Nrp1/2) to SMO signaling (Cao et al. 2008; Hillman et al. 2011; Parra and Zou 2010; Snuderl et al. 2013).

Manipulation of SMO signaling is feasible because numerous small molecules can inhibit SMO signaling. Furthermore, a few small molecules can promote SMO signaling. Structural studies reveal the specific sites for SMO-small molecule interaction, with the cycsteine rich N-terminal domain binding to the cyclopamine and another pocket binding to LY2940680 (Bai et al. 2014; Duarte et al. 2013; Myers et al. 2013; Nachtergaele et al. 2013; Nedelcu et al. 2013; Rana et al. 2013; Wang et al. 2013; Weierstall et al. 2014). These studies indicate that not all SMO inhibitors bind to the same site, which may explain distinct clinical outcomes from different small molecule SMO inhibitors.

Several molecules are identified to be genetically downstream of SMO in *Drosophila*, including COS2, Suppressor of Fused (SuFu) and Fused. Recent study has identified two more molecules (p66beta and Mycbp) involved in regulation of Gli molecules (Lin et al. 2014). A COS2 homologue, kinesin like-protein KIF7, functions in the Hh pathway but is not directly associated with SMO (Cheung et al. 2009; Endoh-Yamagami et al. 2009; Hsu et al. 2011; Law et al. 2012; Li et al. 2012), suggesting that KIF7 does not contain all COS2 functions in vertebrates. Mutations of KIF7 have been detected in acrocallosal syndrome (Putoux et al. 2011, 2012; Speksnijder et al. 2013; Walsh et al. 2013). Gli3 gene mutations are also discovered in this syndrome (Speksnijder et al. 2013). In contrast, the phenotype of

Fused$^{-/-}$ mice is very different from *Shh* null mice (Chen et al. 2005; Merchant et al. 2005; Wilson et al. 2009), indicating that *Fused* is not critical for Hh signaling during early embryonic development in mice.

In addition to the *Drosophila* homologues, mammalian cells have several novel cytoplasmic regulators of Hh signaling, including Rab23 (Eggenschwiler et al. 2001) and tectonic (Reiter and Skarnes 2006). Rab23 and tectonic are all negative regulators downstream of SMO. We have shown that Rab23 is involved in Gli-SuFu interaction (Li et al. 2011a) (see Fig. 1). Unlike many Rab proteins, we found that Rab23 is localized both in the nucleus and in cytoplasm (Huang et al. 2010), suggesting that Rab23 may have other unrevealed functions apart from membrane trafficking. Tectonic 1/2, on the other hand, is involved in ciliary transportation of membrane-associated proteins, such as SMO (Garcia-Gonzalo et al. 2011). Mutations of tectonic1/2 genes have been found in several genetic disorders such as Meckel and Joubert syndromes (Garcia-Gonzalo et al. 2011; Huppke et al. 2014; Shaheen et al. 2011).

The ultimate effect of Hh signaling is activation of downstream Gli transcription factors, which regulate target genes in part by direct binding a consensus-binding site (5′-tgggtggtc-3′) in the promoter (Kinzler et al. 1988; Kinzler and Vogelstein 1990; Parker et al. 2011; Ruppert et al. 1988; Sasaki et al. 1997). The activity of Gli transcription factors can be regulated at several levels. First, nuclear-cytoplasmic shuttling of Gli molecules is tightly regulated (Barnfield et al. 2005; Kogerman et al. 1999; Sheng et al. 2006; Stecca et al. 2007). Protein kinase A can retain Gli1 protein in the cytoplasm via a PKA site in the nuclear localization signal domain (Sheng et al. 2006) whereas activated Ras signaling induces Gli nuclear localization (Stecca et al. 2007). Second, ubiquitination, acetylation and protein degradation of Gli molecules is regulated by several distinct mechanisms, including β-TRCP-, cul3/BTB- and numb/ Itch-mediated Gli ubiquitination, sumoylation and acetylation (Canettieri et al. 2010; Coni et al. 2013; Di Marcotullio et al. 2006; Han et al. 2012; Huntzicker et al. 2006; Jiang 2006; Pan et al. 2006; Wang and Li 2006). In addition, Gli3 (Gli2 to a less extent) can be processed into transcriptional repressors, which may be mediated by the β-TRCP E3 ligase (Wang and Li 2006; Zhang et al. 2009). SuFu not only prevents nuclear translocation of Gli molecules, but also inhibits Gli1-mediated transcriptional activity (Cheng and Bishop 2002; Li et al. 2013; Wang et al. 2010a). Other mechanisms to modify Gli functions include interaction with a negative regulator sucrose non-fermenting 5 (SNF5) (Jagani et al. 2010) and a positive regulator protein kinase C isoform τ/λ (PKτ/λ) (Atwood et al. 2013). It has been shown that BET bromodomain containing proteins, such as BRD4, occupy the promoters of Gli1 and Gli2, and inhibition of BRD4 by JQ1 can suppress Gli- mediated reporter gene activity (Tang et al. 2014). In fact, a novel BRD4 specific compound has been identified to inhibit Gli transcriptional activity (Long et al. 2014).

Several feedback regulatory loops exist in this pathway to maintain a certain level of Hh signaling in a given cell. PTC, HIP, GAS1, neuropilins (nrp) and Gli1 are both components as well as target genes of this pathway. PTC and HIP provide negative feedback regulation whereas Gli1 and Nrp1/2 form positive regulatory loops. On the other hand, GAS1 is down-regulated by the Hh pathway but it is a positive regulator for Hh signaling [reviewed in

(Yang et al. 2010)]. Alterations of these loops would lead to abnormal signaling of this pathway, such as inactivation of PTCH1 in BCCs.

Role of Hh signaling in differentiation, polarity and proliferation

Hedgehog was initially discovered as a segment polarity gene in *Drosophila* embryonic development. As an important morphogen, Hh forms a gradient to guide cells during embryonic development. In the developing spinal cord for example, Shh is secreted from the notochord and the floor plate, and spread to other parts of the spinal cord. It is demonstrated in vitro that Shh is a critical morphogen for motor neuron differentiation. This spatial and temporal gradient of Shh promotes the formation of different types neural subtypes, which has been nicely summarized elsewhere (Ribes and Briscoe 2009).

Hh signaling is also critical for cellular communications between the mesenchyme and the developing hair follicle. In the anagen phase of hair cycle in postnatal life, Shh is secreted by the dermal cells near the base of hair follicle to promote cell division/differentiation of bulge stem cells. Latter stages of hair cycle will be affected without Hh signaling. In catagen phase, Hh signaling is not detectable (Hsu et al. 2014).

The developing limb in vertebrates is one of the best model systems to study pattern formation and polarity. Shh is produced in the polarizing region, with Shh concentration high in the posterior region and low in the anterior region. In addition to Hh signaling, Wnt5a, FGFs and retinoic acid signaling all have gradients. The interactions of these morphogens give rise to the precise position for each digit. There are several excellent reviews on limb development (Benazet and Zeller 2009; Hui and Angers 2011; Johnson et al. 1994; Suzuki 2013).

The direct regulation of cell proliferation by Hh signaling is shown in cultured cerebella cells in the presence of recombinant Shh protein (Wallace 1999). There is a lot of in vivo evidence to show regulation of Hh signaling on cell proliferation. For example, numerous gene knockout mice show altered cell proliferation in neurons (Dahmane and Ruiz i Altaba 1999; Wechsler-Reya and Scott 1999) and hair follicles (Hsu et al. 2014). The exact molecular mechanism underlying cell proliferation regulation varies from cell type to cell type, but it is known that Hh signaling regulates gene expression of cell cycle- related molecules such as cyclin D2 and N-myc.

The link of Hh signaling to cancer

The initial link between Hh signaling and human cancer was made from genetic studies of a rare and hereditary form of BCC- Basal Cell Nevus Syndrome (also named as Gorlin syndrome by the famous dentist Robert Gorlin), which often harbor genetic mutations of human *PTCH1* (Epstein 2001; Hahn et al. 1996; Johnson et al. 1996). Gorlin syndrome has two distinct sets of phenotypes: an increased risk to developing cancers such as BCCs, medul-loblastomas, rhabdomyosarcomas and meningiomas as well as developmental defects such as bifid ribs, ectopic calcification (Gorlin 1987).

Genetic mutations of the Hh signaling molecules are more frequently found in BCCs and some (∼30 %) medulloblastomas, with *PTCH1* and *SMO* frequently mutanted (Couve-Privat et al. 2002; Lam et al. 1999; Reifenberger et al. 1998, 2005; Xie et al. 1998). In addition, cancers associated with Gorlin syndrome, including rhabdomyosarcoma (Pressey et al. 2011; Tostar et al. 2006) and meningiomas (Aavikko et al. 2012; Clark et al. 2013; Kijima et al. 2012), are reported to have elevated Hh target gene expression, but genetic mutations of the Hh pathway are rare. Furthermore, activated Hh signaling has been detected in a variety of human cancer types, either in the tumor or in the stroma (Yang et al. 2010; Fei et al. 2012; Rodriguez-Blanco et al. 2013; Shin et al. 2011).

Genetically engineered mice with *Ptch1* and *Smo* genes have generated more convincing evidence for the critical role of Hh signaling in cancer. In addition to BCC and medulloblastomas, rhabdomyosarcomas develop in mice with expression of oncogenic *SmoM2* or knockout of *Ptch1* (Hahn et al. 1998; Hatley et al. 2012; Ignatius et al. 2012; Nitzki et al. 2011). One surprising finding from tissue specific *Ptch1* knockout is the development of gastrointestinal stromal-like tumors (GIST) (Pelczar et al. 2013), suggestive a role of Hh signaling in GIST. In the situation of small cell lung cancer (SCLC) mouse model, expression of oncogenic *SmoM2* increases the tumor number whereas *Smo* knockout reduces the tumor number (Park et al. 2011). Recent study of Barrett's esophagus indicates that Sonic Hh expression in the epithelium of esophagus can lead to stromal expression of Hh signaling target genes, which is similar to the human situation (Wang et al. 2010b; Yang et al. 2012). In contrast, tissue specific expression of oncogenic Smo molecule *SmoM2* has no effects on K-Ras-induced pancreatic cancer development (Tian et al. 2009) or on prostate cancer (Mao et al. 2006). The negative data, however, do not rule out the promoting effects of Hh signaling for tumor metastasis, a major factor for cancer mortality. Currently, there are only a limited number of mouse models for cancer metastasis. Even for the available animal models for cancer metastasis, several variable factors make cancer metastasis models less robust, such as mouse genetic backgrounds, low incidence and long duration to observe metastasis in mice.

Hh signaling in tumor initiation, promotion and metastases

It is likely that Hh signaling plays distinct roles in different types of cancer [reviewed in (Yang et al. 2010)]. Based on recent publications, we identified three major roles of Hh signaling during cancer development: a tumor development driver, a tumor promoter, or a regulator for residual cancer cells after therapy (see Fig. 2 for details). For example, activated Hh signaling can drive development of BCCs, medulloblastomas, rhabdomyosarcoma, GIST and Barrett's esophagus (Aszterbaum et al. 1999; Goodrich et al. 1997; Hahn et al. 1998; Hatley et al. 2012; Pelczar et al. 2013; Wang et al. 2010b). Hh signaling in these lesions serves as a tumor driver, at least in the mouse model. As to SCLC, Hh signaling can promote cancer development but is not sufficient to drive tumor formation (Park et al. 2011). Thus, Hh signaling activation is a tumor promoter for development of SCLC. In pancreatic cancer, on the other hand, inhibition of Hh signaling does not affect tumor formation but can promote tumor metastasis (Bailey et al. 2008, 2009; Chang et al. 2013; Feldmann et al. 2007, 2008; Gu et al. 2013; Olive et al. 2009; Tang et al. 2012). For other cancer types, Hh signaling may regulate the number of cancer stem cells or the tumor

microenvironment, such as leukemia and liver cancer (Boyd et al. 2013; Zhao et al. 2009). As more in vivo data are available, we predict more revelation of the tumor promoting role of Hh signaling. Tumor recurrence after therapy is a major issue in clinical care of cancer patients, such as chemotherapy or radiotherapy resistance, and will be discussed later in the review. For some cancer types, Hh signaling may not have any roles to play at all.

Activation of Hh signaling does not work alone, cross-talks with other signaling pathways play critical roles during cancer development and metastasis. Earlier studies indicated that *Ptch1*$^{+/-}$ mice with *P53* knockout all developed medulloblastomas whereas <30 % of *Ptch1*$^{+/-}$ mice (with wild type *P53*) had this type of tumor (Romer et al. 2004). We have shown that skin specific knockout of *Stat3* or its upstream activator *Il11ra* significantly reduced Hh signaling-mediated BCC formation (Gu et al. 2012). Increasing data have indicated close collaboration between Hh signaling and growth factor signaling pathways. Our earlier work indicated that PDGFRα is regulated by Hh signaling and is responsible for cell proliferation in BCCs (Xie et al. 2001). Now more links are reported between Hh and other pathways, including EGF, IGF, TGFβ, mTOR/ S6K1, RACK1, Notch and PKC (Eberl et al. 2012; Fan et al. 2010; Fernandez et al. 2012; Hsieh et al. 2011; Johnson et al. 2011; Keysar et al. 2013; Mainwaring and Kenney 2011; Shi et al. 2012; Wang et al. 2012b; Yang et al. 2010). While some of these molecules are involved in regulation of tumor microenvironment such as TGFβ, others are known to regulate cancer stem cells, such as PDGFRα and Notch. We will have more discussion on cancer stem cells in “Hedgehog signaling, cancer stem cell and residual cancer cells” section.

Increasing evidence indicates that Hh signaling plays an important role during tumor metastasis in several types of cancer, such as pancreatic and breast cancers (Gu et al. 2013; Heller et al. 2012). Studies from many groups indicate activation of Hh signaling in the stromal as well as tumor compartments in metastatic pancreatic cancer [Abbruzzese and National Institutes of Health (US) 2007; Bailey et al. 2009; Feldmann et al. 2007, 2008; Gu et al. 2013; Olive et al. 2009; Tang et al. 2012]. In fact, Hh signaling inhibitors are effective in suppressing tumor metastases of pancreatic cancer (Gu et al. 2013). Hh signaling also regulates bone homeostasis as well as bone metastasis in breast cancer, which is independent of the Hh ligands (Johnson et al. 2011). During tumor metastasis, Hh signaling activation is observed both in the tumor compartment and in the stroma (Gu et al. 2013). The molecules mediated Hh’s metastatic functions remain largely untested, but there are reports to indicate the following molecules: snail, TGFβ, wnt, HGF and muc5AC (Gu et al. 2013; Inaguma et al. 2010; Javelaud et al. 2012; Joost et al. 2012; Li et al. 2007b). Further studies will be needed to understand the molecular basis by which Hh signaling mediates cancer metastases.

Hedgehog signaling, cancer stem cell and residual cancer cells

Increasing evidence indicates that Hh signaling is critical for cancer stem cell maintenance and function (Dierks et al. 2008; Read et al. 2009; Zhao et al. 2009). For example, leukemia stem cell maintenance and expansion is dependent on Hh signaling (Dierks et al. 2008; Zhao et al. 2009). The effect of Hh signaling on normal hematopoietic stem cell (HSC) population, however, is still quite controversial, with some showing some effects but others

with no effects (Gao et al. 2009; Hofmann et al. 2009; Merchant et al. 2010; Siggins et al. 2009; Zhao et al. 2009). Based on cancer stem cell theory, it is anticipated that Hh signaling activation exerts resistance to cancer chemotherapy and radiotherapy (Reya et al. 2001). Several studies indeed have shown that Hh signaling activation is associated with chemotherapy or radiotherapy resistance (Sims-Mourtada et al. 2006; Yoshikawa et al. 2008). Hh signaling inhibitor IPI-926 enhances delivery of chemotherapeutical drug gemcitabine in a mouse model of pancreatic cancer (Olive et al. 2009). However, more recent data indicate that genetic depletion of Shh actually promotes tumor development, suggesting that the role of Hh signaling in pancreatic cancer development is not simply positive or negative role (Ozdemir et al. 2014; Rhim et al. 2014; Shin et al 2014).

Based on the published data, we propose that Hh signaling may help regulate cancer stem cells, which are generally insensitive to chemotherapy and radiotherapy. There is evidence to indicate that Hh signaling regulates expression of cancer stem cell-related markers: such as ALDH1, Bmi1, snail, Wnt2, PDGFRα, jagged-1, CD44 and c-MET (Gu et al. 2013; Joost et al. 2012; Liu et al. 2006; Song et al. 2011; Takahashi et al. 2011; Takebe et al. 2011; Tanaka et al. 2009). The level of Hh expression is often higher in the cancer stem cell population in several cancer types (Bar et al. 2007; Clement et al. 2007; Li et al. 2007a; Su et al. 2012; Visbal et al. 2011). Thus, we have reasons to believe that inhibition of Hh signaling may be effective in reducing the number of cancer stem cells, which may play an important role in chemotherapy and radiotherapy resistance.

Chemotherapy and radiotherapy play an important role in the clinical care of cancer patients, but resistance to these treatments remains a major obstacle in cancer patient care. Increasing evidence shows higher Hh signaling in chemore-sistant cancer cells. Emerging evidence also indicates that Hh signaling may regulate cell's tolerance to anticancer agents. Smoothened antagonists thus have offered valuable therapeutic strategies against cancer, either alone or with chemotherapeutical agents. One Smo inhibitor, vismodegib (GDC-0449), is a FDA-approved drug for the treatment of locally advanced and metastatic BCC. Other four Smo inhibitors have progressed into phase II clinical trials (NVP-LDE225, IPI-926, XL-139, and LY2940680). In ovarian cancer, LDE225 increases paclitaxel sensitivity via regulation of MDR1 (Steg et al. 2012b). Vismodegib is sufficient to drive UDP glucuronosyltransferase (UGT1A)-dependent glucuronidation of ribavirin and Ara-C, leading to reduced drug resistance in acute myeloid leukaemia (AML) (Zahreddine et al. 2014).

Identification of the upstream regulators and downstream mediators of Hh pathway during chemotherapeutic drug response may be critical to understand how Hh signaling causes resistance to different chemotherapeutical agents. Overexpression of MAP3K10 promoted the proliferation and decreased the gemcitabine sensitivity of pancreatic cancer cells, through up-regulation of Gli1 and Gli2 (An et al. 2013). Crosstalk between PI3K/AKT signaling and the Hh pathway has been reported in a number of cancer, including prostate and breast cancers. A recent study reported an important effect of Hh signaling on docetaxel resistance in prostate cancer (Domingo-Domenech et al. 2012). Combination of Notch and Hh signaling inhibitors was able to reverse docetaxel resistance both in cultured cells and in xenografts. Activation of Hh signaling via PI3K is also reported in tamoxifen-resistance breast cancer (Ramaswamy et al. 2012), and combination of Hh signaling inhibitor

GDC-0449 with tamoxifen significantly reduced cell colony formation and tumor development in xenografts. Elevated Hh signaling increases expression of BCL2, enhances cell proliferation and protects cells against chemotherapy induced apoptosis (Das et al. 2013; Domingo-Domenech et al. 2012). These results suggest that the function of Hh signaling in drug resistance may vary based on tumor types and the type of chemotherapeutic agents. Therefore, it is necessary to consider the mechanisms of action for different drug treatments.

Activated Hh signaling is also shown to be responsible for drug resistance in ovarian cancer, cervical cancer and myeloid leukemic cells (Chaudary et al. 2012; Queiroz et al. 2010; Steg et al. 2012a). Recent study suggests that Hh signaling may be associated with anti-EGFR therapy (targeted therapy) resistance in head and neck cancer (Keysar et al. 2013). The exact mechanisms by which Hh signaling activation confers drug resistance are not entirely clear, but it is reported that Hh signaling can regulate expression of several drug resistance related genes such as ABCG2, MDR (Queiroz et al. 2010; Singh et al. 2011). The cancer stem cell theory can also explain some of the mechanisms.

Overcoming recurrence to radiotherapy is also very challenging, but recent studies suggest that inhibiting Hh signaling may help mitigate radiotherapy resistance in pancreatic and head/neck cancer. For pancreatic cancer, we found that combination of Hh signaling inhibitor BMS833932 (Yang et al. 2010) and radiation could significantly reduce the number of lymph node metastasis (Gu et al. 2013). Similarly, high expression of Gli1 is reported to be associated with lymph node metastases and tumor progression after radiotherapy in squamous cell carcinomas of head/ neck (Lin et al. 2013).

Summary and future perspectives

In summary, the link of the Hh signaling pathway to a variety of human cancer implies the high relevance of studying Hh signaling to human health. Despite of the impressive progress in Hh signaling in cancer, there are several challenges ahead of us in order to achieve better understanding of signaling mechanisms as well as to achieve clinical benefits for the cancer patients. First, because elevated transcriptional activity of Gli molecules by other signaling pathways (such as PI3K or TGFβ signaling) (Javelaud et al. 2012; Ramaswamy et al. 2012) occurs frequently in several types of cancer, specific inhibitors to SMO will not be effective in many situations. Thus, discovery and synthesis of Gli specific inhibitors is in great need for effective suppression of non-canonical Hh signaling in cancer. Another area is to develop reliable, physiologically relevant and reproducible mouse models for cancer metastases, which will allow us to test and optimize drug dosages to minimize the side effects. Third, Hh signaling does not work alone, identifying the optimal combination between Hh signaling inhibitors, chemotherapeutical agents or other targeted therapeutic agents in a given tumor type will help us to reduce the medical burden of cancer metastases. It is anticipated that additional novel therapeutic strategies will be developed for cancer clinical trials using Hh signaling inhibitors in the next few years.

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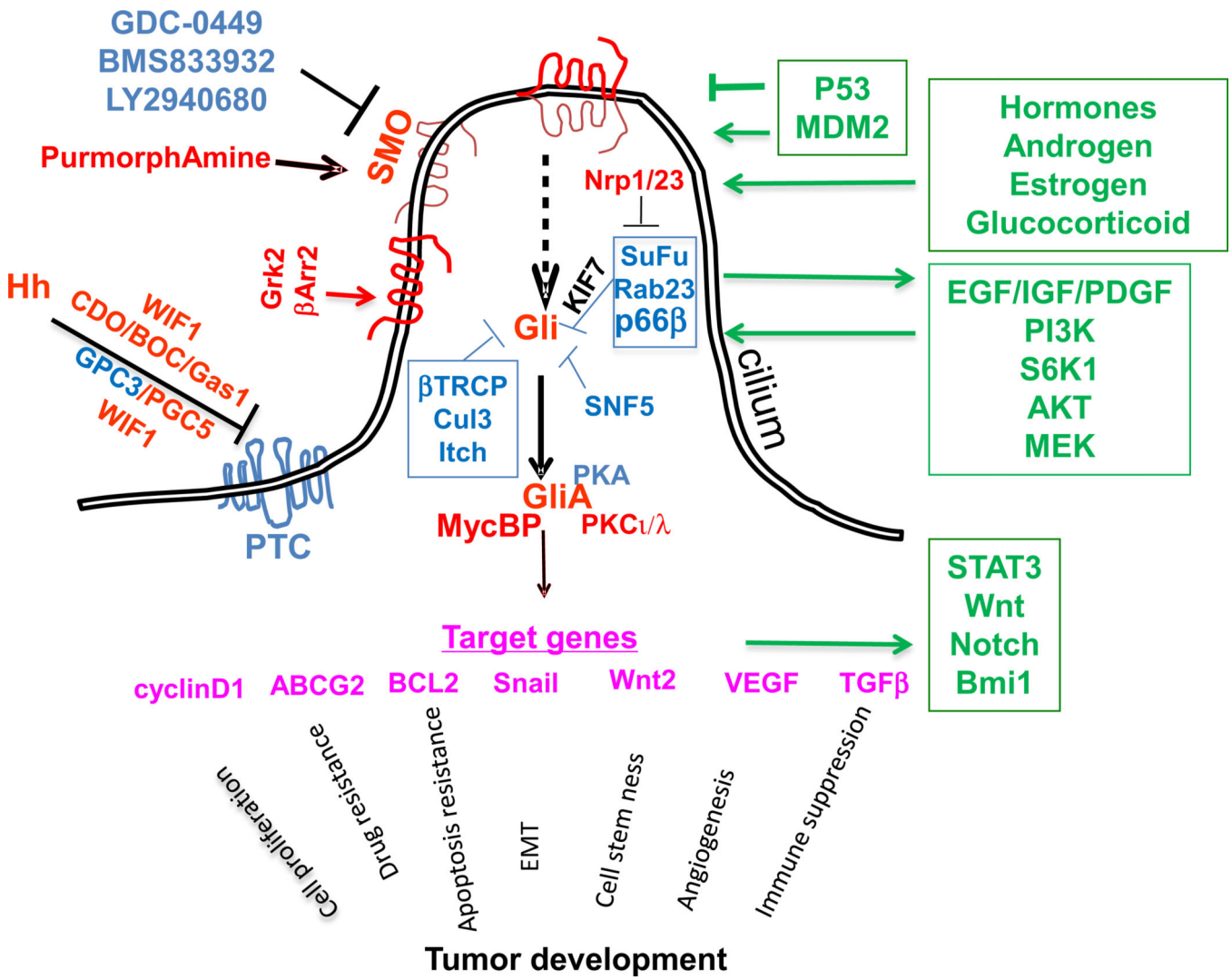

Fig. 1.

A diagram of Hh signaling in mammalian cells. SMO is the key signal transducer of the Hh pathway. In the absence of the Hh ligands, Hh receptor PTC is localized in the cilium to inhibit SMO signaling. Co-receptors of Hh include CDO (cell adhesion molecule-related/down-regulated by oncogenes), Brother of CDO (BOC), Gas1, Glypican 3 (GPC3) and GPC5. Wnt inhibitory factor-1 (WIF1) can also regulate Hh signaling through association with CDO, BOC or GPC5. Gli molecules are processed with help of Su (Fu)/KIF7, β-TRCP molecules into repressor forms, which turn off the Hh signaling pathway. Other negative regulators of Gli molecules include Rab23, Protein kinase A (PKA), Suppressor of Fused (SuFu), tumor suppressor SNF5, Culin 3 (Cul3), p66β and Itchy E3 ubiquitin ligase (Itch) through regulation Gli protein modifications, nuclear-cytoplasm shuttling as well as transcriptional activities. In the presence of Hh, PTC is shuttled out of cilium and is unable to inhibit SMO. The ciliary localization of SMO is thought to require β-arrestin 2 (βArr2) and G protein coupled receptor kinase 2 (GRK2). Hh reception promotes SMO conformational changes to form dimers/oligomers. Gli molecules are then processed to active forms (GliA), which will activate the Hh target genes. KIF and SuFu can inhibit this

process. Mycbp and protein kinase C isoform τ/λ (PKτ/λ) positively regulate Gli transcriptional activity. Positive regulators are in *red*, negative regulators are in *blue* and target genes are in *pink*. KIF7 can function (in *black*) as a negative regulator or a positive regulator. The interacting pathways with the Hh pathway are in *green*. Although the role of cilium for Hh signaling during embryonic development is well established, cancer cells generally lack cilia. It has been demonstrated that lack of cilia prevents development of BCCs in mice, it is not clear whether this is true for all other types of Hh signaling-associated cancer (color figure online)

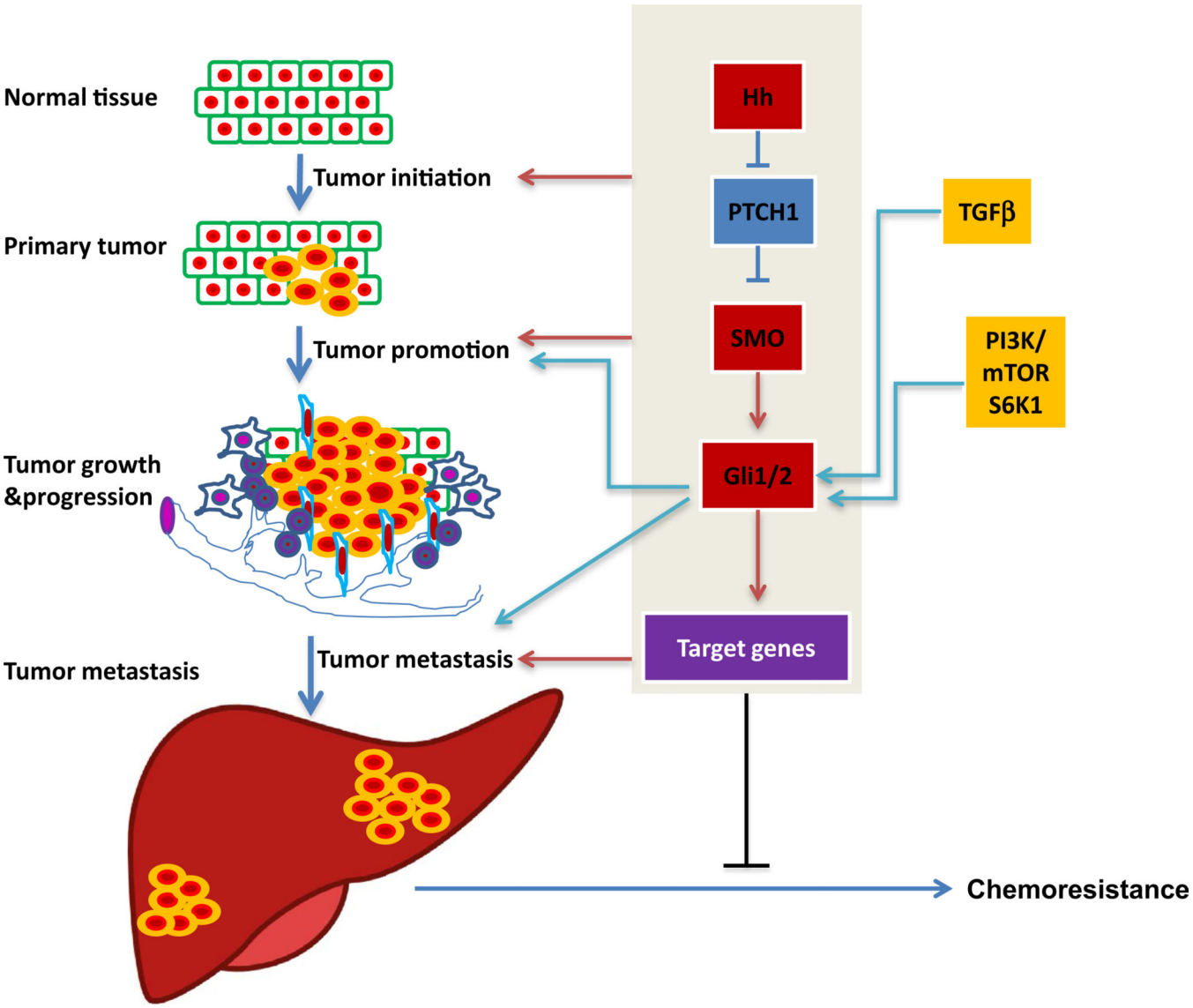

Fig. 2.

Different roles of Hh signaling in cancer. Hh signaling in cancer may function in three manners: (1) a tumor driver; (2) a tumor promoter; and (3) a treatment resistant factor. In mice, activation of Hh signaling, via mutations of PTCH1 or SMO, drives development of basal cell carcinomas, medulloblastoma, rhabdomyosarcomas and GISTs, indicating that Hh signaling is the tumor drive for these four types of cancer. In the humans, gene mutations occur frequently in these tumor types. For small cell lung cancer, activation of Hh signaling is not sufficient to drive tumor formation. However, expression of mutant SMO, SmoM2, accelerates development of SCLC derived from Rbl/p53 deletion and Smo deletion reduces the tumor development. Thus, Hh signaling is a tumor promoter for SCLC. In addition, Hh signaling is activated in chemoresistant cancer cells in prostate and ovarian cancer, and inhibition of Hh signaling can sensitize cancer cells to chemotherapy