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Prevailing importance of the hedgehog signaling pathway and the potential for treatment advancement in sarcoma

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ABSTRACT

The hedgehog signaling pathway is important in embryogenesis and post natal development. Constitutive activation of the pathway due to mutation of pathway components occurs in ~25% of medulloblastomas and also in basal cell carcinomas. In many other malignancies the therapeutic role for hedgehog inhibition though intriguing, based on preclinical data, is far from assured. Hedgehog inhibition is not an established part of the treatment paradigm of sarcoma but the scientific rationale for a possible benefit is compelling. In chondrosarcoma there is evidence of hedgehog pathway activation and an ontologic comparison between growth plate chondrocyte differentiation and different chondrosarcoma subtypes. Immunostaining epiphyseal growth plate for Indian hedgehog is particularly positive in the zone of pre-hypertrophic chondrocytes which correlates ontologically with conventional chondrosarcoma. In Ewing sarcoma/PNET tumors the Gli1 transcription factor is a direct target of the EWS-FLI1 oncoprotein present in 85% of cases. In many cases of rhabdomyosarcomas there is increased expression of Gli1 (Ragazzini et al., 2004). Additionally, a third of embryonal rhabdomyosarcomas have loss of Chr.9q22 that encompasses the patched locus (Bridge et al., 2000). The potential to treat osteosarcoma by inhibition of Gli2 and the role of the pathway in ovarian fibromas and other connective tissue tumors is also discussed (Nagao et al., 2011; Hirotsu et al., 2010). Emergence of acquired secondary resistance to targeted therapeutics is an important issue that is also relevant to hedgehog inhibition. In this context secondary resistance of medulloblastomas to treatment with a smoothened antagonist in two tumor mouse models is examined.

Contents

1. Introduction

1.1. The hedgehog pathway

The developmental pathway hedgehog is activated by hedgehog ligands of which there are three homologs; Sonic, Indian and Desert. These ligands bind to patched (PTCH) a 12-transmembrane domain protein and membrane receptor. PTCH normally tonically represses a 7-transmembrane G-protein coupled like receptor, proto-oncogene, called smoothened (Smo). The binding of hedgehog ligand to PTCH relieves the repression of Smo and has a permissive effect on downstream intracellular signaling. Ultimately the pathway activates zinc finger transcription factors called Gli (Fig. 1). Ptch is itself regulated by Gli. An important intracellular component of the pathway is the product of the tumor suppressor gene, suppressor of fused (Sufu). *Sufu*$^{+/-}$ mice are not more liable to develop tumors in contrast to hererozygous germline loss of the other tumor suppressor gene *Ptch*. However, *Sufu*$^{+/-}$ *p53*$^{-/-}$ mice develop medulloblastomas and rhabdomyosarcomas (Lee et al., 2007).

Hedgehog signaling is important in development. A demonstrative example is that mutations in Smo cause the phenotypically diverse disorder holoprosencephalopathy (Roessler et al., 1996). The hedgehog

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pathway may also be inappropriately activated in some cancers. Intracellular abnormalities may arise distal to Smo such as with the aforementioned SUFU tumor suppressor or at the level of Gli transcription. The hedgehog pathway may be constitutively activated in tumor cells because of intrinsic mutations in the hedgehog pathway leading to 'oncogene addiction'. Alternatively the pathway may be up regulated by ligand-dependent activation of the hedgehog pathway in the stromal microenvironment with a paracrine requirement for hedgehog ligand (Yauch et al., 2008).

In addition to smoothened inhibitors, anticancer drugs that target the hedgehog pathway potentially include hedgehog ligand antagonists (e.g. 5E1 and robotnikinin) and inhibitors of Gli transcription activity (e.g. GANT58 and GANT61). It has been found that primary cilia are required for activation of the hedgehog pathway (Hassounah et al., 2012). There are two categories of cilia, primary or motile. The primary cilium is a microtubule based organelle that protrudes from the cell. Cells have only a single primary cilium, if present, that senses extracellular signals such as secreted hedgehog ligands. Positive and negative hedgehog pathway effectors are processed in this structure by post translational modification. Microtubules originate from a basal body and extend up the cilium. In some cancers primary cilia are lost. In the absence of cilia tumors are unresponsive to smoothened inhibitors and need to be treated with medications that inhibit Gli transcription activity. In a fascinating hypothesis of the importance of primary cilia, in the phase I clinical trial of GDC-0449 in basal cell carcinoma 66% of patients with Ptch1 or smoothened mutations responded to Vismodegib (GDC-0449) (Von Hoff et al., 2009). Hassounah and colleagues have proposed that these patients had cancer cells that possessed cilia as in another study ~63% of basal cell carcinomas had cilia when tested (Wong et al., 2009). Intraflaggelar transport is required for the local concentration and appropriate stochiometric proportions of hedgehog signaling pathway components (Rosenbaum & Witman, 2002; Scholey, 2003).

Within the primary cilium, kinesin-2 motor complex is involved in antegrade flow of proteins to the tip of the primary cilium and Dynenin 2 is involved in retrograde transport of proteins. Ultimately the cilium is responsible for local concentration of hedgehog pathway components and has a coordinating role in pathway efficacy. Cilia thus have an important role in sensitivity to therapeutic hedgehog pathway inhibitors. In medulloblastoma cilia are correlated with the desmoplastic subtype (good prognosis) while absence of cilia is correlated with the anaplastic subtype (poor prognosis). It is noteworthy that the desmoplastic variant is the subtype most sensitive to treatment with smoothened inhibitors. Overall mutation driven hedgehog pathway activation can be cilia independent or dependent. Examples of the former phenomenon include Sufu mutations in medulloblastoma, loss of heterozygosity mutations of Sufu in rhabdomyosarcoma or loss of REN (KCTD11; a protein that antagonizes Gli mediated transcription of hedgehog gene targets) (Tostar et al., 2006; Scales & de Sauvage, 2009; Ng & Curran, 2011). Cilia dependent mutation that drive Hh pathway activation involves cases of deactivation of Ptch1 and activation of smoothened. Lastly ligand driven hedgehog pathway activated tumors are cilia dependent.

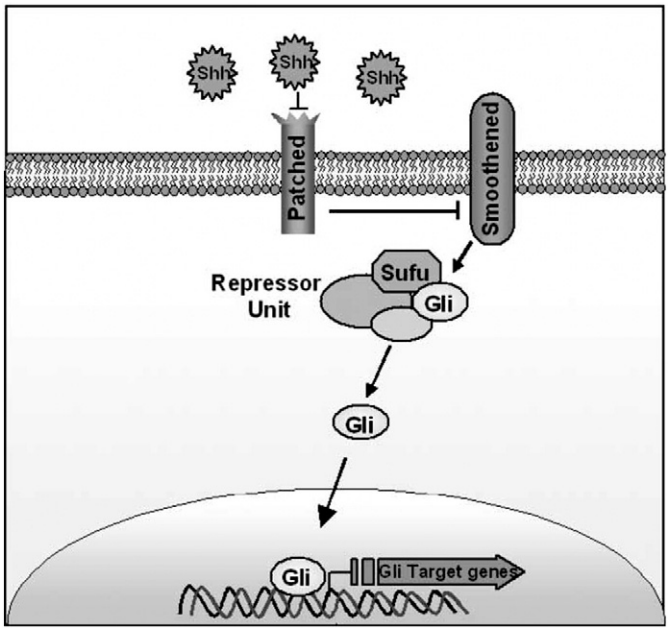

Fig. 1. The hedgehog signaling pathway.

1.2. The nevoid basal cell carcinoma syndrome (Gorlin syndrome)

The nevoid basal cell carcinoma syndrome is a phenotypic manifestation of a germline mutation of the tumor suppressor gene PTCH on chromosome 9q22.3 (Hahn et al., 1996). It is characterized by developmental defects and a predisposition to multiple basal cell carcinomas, odontogenic keratocysts of the jaws, cutaneous epidermal cysts, palmar and plantar pits, calcified dural folds and neoplasms or hamartomas, in particular ovarian fibromas, meningiomas and medulloblastoma (Gorlin, 1999). Cases of fetal rhabdomyomas, fetal rhabdomyosarcoma and rarely embryonal rhabdomyosarcoma have also been described in patients with Gorlin syndrome (Beddis et al., 1983; Cajaiba et al., 2006; Yang et al., 2011). Interestingly ~30% of sporadic cases of embryonal rhabdomyosarcomas have chromosome 9q22.3 locus molecular abnormalities (Calzada-Wack et al., 2002; Tostar et al., 2006).

Mice that are heterozygous for patched have phenotypic characteristics of Gorlin syndrome and on a CD1 background 10–15% develop rhabdomyosarcoma like tumors (Hahn et al., 1998). The predilection of PTCH1-mutant mice to form rhabdomyosarcomas has been linked with the ability of the malignant cells to resist apoptosis (Kappler et al., 2003).

The prevalence of the disorder is 1 in 56,000 and it had an autosomal dominant pattern of inheritance with 30–50% of cases attributable to new mutations. It has complete penetrance in affected individual (Kimonis et al., 1997). The frequency of basal cell carcinomas in patients with Gorlin syndrome varies with ethnicity with 90% of Caucasians and 40% of black patients developing BCCs (Lo Muzio et al., 1999). Medulloblastoma occurs in 1–2% of patients (Evans et al., 1991). It has been contended that PTCH is not a classic tumor suppressor gene as some components of the Gorlin syndrome phenotype such as cleft lip/palate, macrocephaly and bifid ribs required only 'one hit' in contradiction to the Knudson 'two hit' hypothesis (Cohen, 1999). Mutations in PTCH leading to nevoid basal cell carcinoma syndrome are distributed throughout the entire PTCH gene. Patients with Gorlin syndrome have an increased number of nucleoli after x-irradiation. This was first described by Dezawa in fibroblasts derived from 3 patients with this disorder (Dezawa et al., 1999). The increase in the number of nucleoli occurred concomitant with increased ribonucleoprotein immunoreactive aggregates within the nucleus after x-irradiation. Notably this increased was observed to decrease when the fibroblasts were cultured with actinomycin D a RNA synthesis inhibitor. A relevant clinical correlate is that patients with Gorlin syndrome that are treated for medulloblastoma with cranioaxial irradiation develop hundreds of basal cell carcinomas within the irradiation field. X-irradiation is now considered to be contraindicated for skin cancers arising in patients with Gorlin syndrome.

A randomized, double blind, placebo controlled trial of Vismodegib versus placebo that was published in 2012 was conducted on 41 patients with the nevoid basal-cell carcinoma syndrome. The patients were followed for a median of 8 months (Tang et al., 2012). The primary endpoint was a reduction in the incidence of new-basal cell carcinomas that were eligible for surgical resection at 3 months. The per-patient rate for the Vismodegib group versus the placebo group was 2 versus