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Hedgehog signalling in cancer

R. Toftgård

Karolinska Institutet, Department of Biosciences and Center for Nutrition and Toxicology, Novum, SE-141 57 Huddinge (Sweden), Fax + 46 8 6081501, e-mail: rune.toftgard@cnt.ki.se

Abstract. Hedgehog signalling is a key regulator of embryonic development controlling proliferation and/or cell fate determination. With identification of the Hedgehog receptor PTCH1 as a tumour suppressor gene that underlies the human nevoid basal cell carcinoma syndrome (NBCCS), the Hedgehog signalling pathway was firmly linked to cancer. It now appears that constitutive activation of Hedgehog signalling, by inactivating mutations in PTCH1 or activating mutations in the coreceptor SMOH, is required and possibly sufficient for basal cell carcinoma development and also contributes to the formation of a variety of other tumour types, including medulloblastoma and rhabdomyosarcoma. Several lines of evidence, including transgenic mice experiments, suggest that the critical cellular effect is stimulation of proliferation mediated by the transcriptional effector GLI1. Additional components of the signal transduction machinery as well as essential target genes remain to be identified, and involvement of the Hedgehog signalling pathway in other tumour types and/or hereditary cancer predisposition syndromes is to be expected.

Key words. Hedgehog signalling; PTCH tumour suppressor; GLI1; suppressor of fused; basal cell cancer; medulloblastoma; cancer.

Introduction

The Hedgehog signalling pathway is known to play a very important role during development, regulating both cell fate and proliferation (for recent reviews see [1, 2] and this issue). In fact, both the *hedgehog* and *patched* genes were identified by analysing mutants obtained in the early genetic screen in *Drosophila* by Nüsslein-Vollhard and Wieschaus [3]. During recent years it has also become increasingly clear that in many instances the signalling and molecular players that control development are the same, and when inappropriately regulated, drive tumorigenesis and cancer development. This is perhaps not so surprising given that hallmarks of a tumour cell are excessive cell growth and defects in the interaction with neighbouring cells and matrix. Two of the best examples of this connection are the much studied WNT/APC/β-CATENIN pathway linked to colorectal cancer and several other tumour types, and the sonic hedghehog/patched (SHH/PTCH) pathway, which is the subject of the present review.

Signal transduction

Important knowledge about the genes and molecules involved in Hedgehog signalling has been obtained by genetic analysis in *Drosophila* implicating at least eight major components, including *hedgehog* itself, *dispatched*, *patched*, *smoothened*, *fused*, *suppressor of fused*, *costal-2* and *cubitus interruptus* [4, 5]. Hedgehog is encoded by a single gene and is an unusual secreted molecule undergoing autocatalytic cleavage and covalent lipid modification (see Incardona and Roelink, this issue). The release of lipid-modified, but not non-modified, Hedgehog variants is determined by the newly discovered putative multipass transmembrane protein Dispatched [5], and on the receiving cell Hedgehog binds to the receptor protein Patched. In addition to *dispatched*, another gene called *tout-velu* that controls the synthesis of cell surface heparan sulfate glycosaminoglycans appears to be involved in regulating Hedgehog diffusion [6]. In contrast to Dispatched, Tout-velu is required in the receiving cell, but in a similar manner only affects diffusion of lipid-modified

Hedgehog [7]. Interestingly, Patched and Dispatched appear to be related, and both contain putative sterol sensing domains (SSDs), implying that both Hedgehog release and transduction of the signal is subject to regulation by lipids. It has been well documented, however, that a nonmodified 19-kDa N-terminal fragment of Hedgehog is fully capable of interacting with Patched and of eliciting a response in competent cells.

Patched interacts with and normally represses signalling by the 7-transmembrane (TM) domain protein Smoothened. When Hedgehog binds to Patched, the repression of Smoothened is relieved [4], and in a so far uncharacterised manner, a signal is transduced to a microtubule (MT)-associated complex containing Costal-2, a protein with a kinesin-like motif [8, 9], the serine/threonine kinase Fused [10], Suppressor of fused [11] and the Zn-finger-containing transcription factor Cubitus interruptus [12]. Cubitus interruptus is capable of both repressing gene transcription by means of the N-terminal fragment localising to the nucleus in the absence of Hedgehog signalling and of activating gene transcription via the full-length and activated factor. Processing and activation [13] of Cubitus interruptus is not understood in its molecular details, although phosphorylation of Cubitus interruptus by protein kinase A is important in processing [14]. Genetically, *hedgehog*, *smoothened* and *fused* are positively acting components in the pathway, whereas *patched*, *costal-2* and *suppressor of fused* have a negative role.

The signalling pathway has in its central details been very well conserved from insects to vertebrates including humans, despite a significant increase in complexity (see Capdevila and Johnson, this volume). In humans and mice there are three *hedgehog*-related genes, *Sonic hedgehog* (SHH), *Indian hedgehog* (IHH) and *Desert hedgehog* (DHH), thus far two *patched* genes (PTCH1, PTCH2) and three *cubitus interruptus*-related genes (GLI1-3), see figure 1. All three Hedgehog proteins can bind Ptch1 [15, 16] and are believed to form heteromeric membrane-associated receptor complexes with the *smoothened* (SMOH) encoded 7-TM protein. The recently cloned PTCH2 gene [17–20] can also interact with Hedgehog proteins and Smoh, but its functional role in the pathway is currently unclear. Only a single

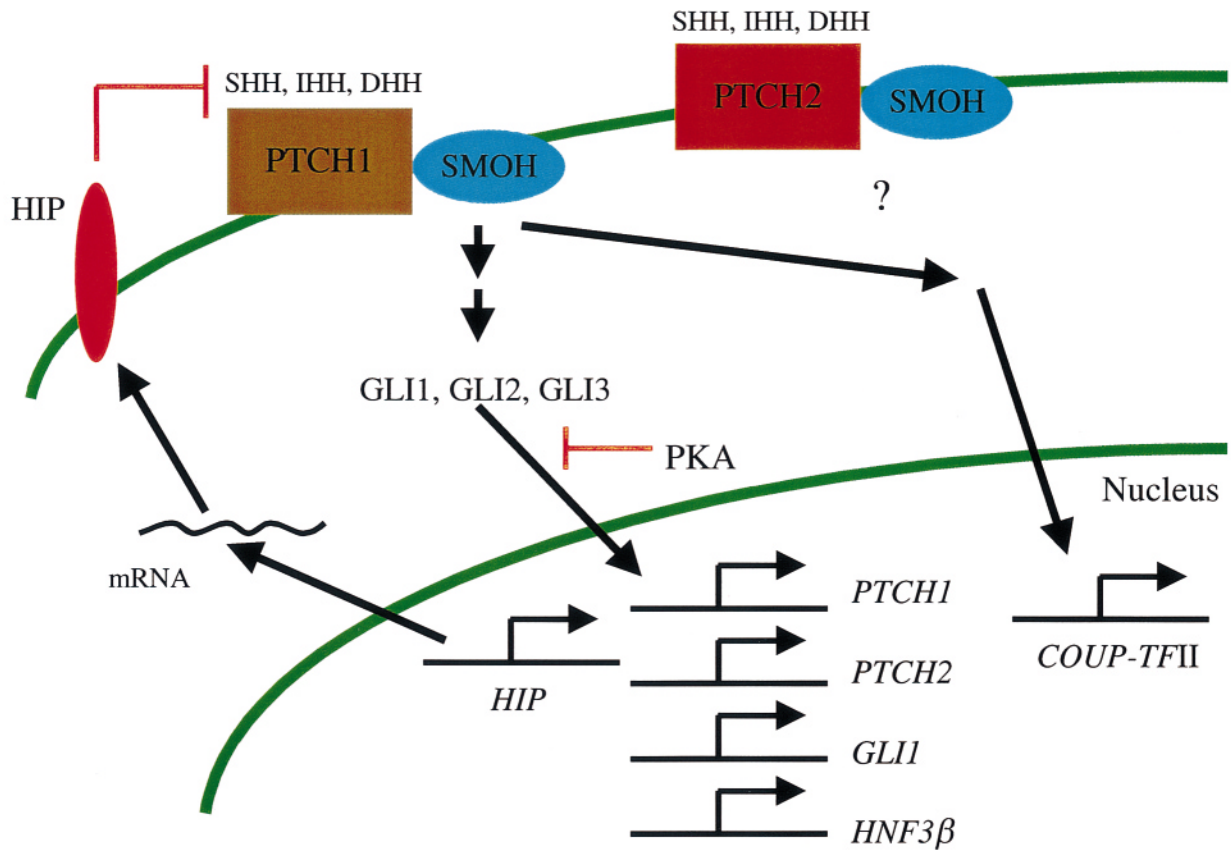

Figure 1. Scheme of major components in the vertebrate Hedgehog signalling pathway.

SMOH gene has been isolated, and recent evidence using a constitutive active variant suggests that Smoh can transduce all Shh responses [21]. The GLI proteins are transcriptional effectors in the vertebrate system that can be found both in the cytoplasm and nucleus depending on context [22], and there is evidence that at least GLI2 and GLI3 contain potent repressor domains and are proteolytically cleaved [22–24] to forms having either activator or repressor function, whereas GLI1 is mainly a transcriptional activator. Further illuminating the striking evolutionary conservation, a recent study showed that human GLI1 and GLI3 are functional in *Drosophila* and together can substitute for *cubitus interruptus* [25]. GLI1 functioned as an activator and GLI3 as a transcriptional repressor, and most interestingly, both human proteins were subject to regulation by Hedgehog.

An important difference from *Drosophila* is that in vertebrates GLI1 is always, and GLI2 in some instances, upregulated at the transcriptional level in response to Shh, whereas in the fly, Cubitus interruptus is only posttranslationally modified [26, 27]. The negative role of protein kinase A (PKA) in Hedgehog signal transduction has been conserved, as has the coactivator function of CBP, shown to interact with Gli2 and Gli3 but not Gli1 [1, 23].

An interesting question is whether all effects of Shh are mediated via Gli. That this may not be the case is suggested by the observation that Shh can directly induce transcription of COUP-TFII via a regulatory sequence lacking Gli binding sites [28], by analysis of mice lacking individual Gli genes or combinations of Gli genes [29] in comparison to mice devoid of Shh and finally by molecular analysis of signalling components in the chick mutant talpid^3 [30].

A new addition to the pathway, Hedgehog interacting protein (Hip), not described in *Drosophila*, can bind and functionally inactivate all three Hedgehog proteins via its extracellular domain and is a general target gene of Hedgehog signalling along with PTCH1 and GLI1 [31]. Both PTCH1 and HIP serve in negative feedback loops of Hedgehog signalling, most likely reflecting the exquisite dose dependency of Shh signalling as best illustrated in specification of neuronal identity by a gradient of Shh signalling [32; Patten and Placzek, this issue] and by developmental defects due to PTCH1 haplo-insufficiency (see below). No mammalian counterparts to *costal-2* and *fused* have so far been described.

Nuclear-cytoplasmic shuttling of GLI

Assuming a role for the GLI proteins as major transcriptional effectors binding to DNA implicates access to the nuclear compartment; however, analysis of intact tissues such as hair follicles and basal cell carcinomas (BCCs) reveals them to be cytoplasmically localised [33, 34], similar to full-length Cubitus interruptus in *Drosophila* [13]. In cell culture, overexpressed Gli proteins may show either a cytoplasmic [35] or a nuclear localisation [22]. Taken together, these data indicate that there should be a mechanism to regulate nuclear entry/export and thereby signal intensity and duration. This hypothesis was confirmed last year when it was revealed that GLI1 contains a leucine-rich nuclear export signal (NES) and that removal or mutation of this NES results in nuclear localisation [35]. Inhibitor experiments indicated that export of GLI1 is mediated by the NES-receptor protein CRM1. Similar NES motifs are present in the other GLI proteins as well as Cubitus interuptus, suggesting that this is a general mechanism, which is also supported by data from *Drosophila* cells [36]. Subsequently, a bipartite nuclear localisation signal (NLS) in Cubitus interruptus and GLI proteins has been reported [37]. It is thus likely that the nuclear level of activator and repressor forms of GLI proteins is determined by the balance between import and export. Deciphering the molecular mechanisms by which Shh regulates this trafficking is now a very interesting and challenging problem.

It was also found that the vertebrate homolog to *Drosophila* Suppressor of fused, SUFUH, not only was an efficient inhibitor of GLI-induced transcription but also physically interacted with GLI1 and could sequester GLI1 in the cytoplasm [35, 38–40], providing another way to regulate GLI subcellular localisation. Moreover, SUFUH was found to be present in the nucleus and able to interact with GLI1 bound to DNA, revealing an additional potential inhibitory mechanism [35, 38]. A model of how GLI activity can be regulated by nuclear-cytoplasmic shuttling and interaction with SUFUH is given in figure 2.

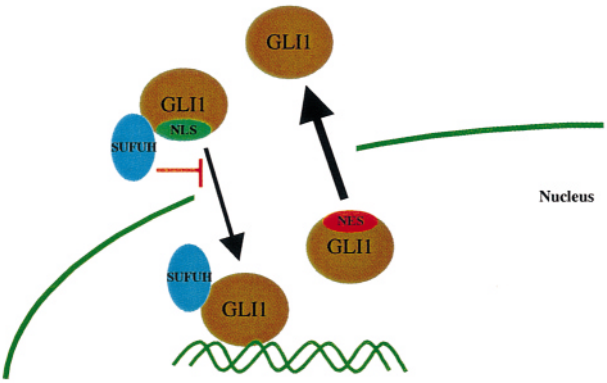

Figure 2. Model for the role of SUFUH and nuclear-cytoplasmic shuttling of GLI1 in the vertebrate Hedgehog signalling pathway.

Tumorigenesis and development

The SHH signalling pathway was firmly linked to tumorigenesis in 1996 when a human homolog of *Drosophila patched* (PTCH1) was found to be mutated in nevoid basal cell carcinoma syndrome (NBCCS) [41, 42]. In addition to predisposition to a wide variety of tumours, NBCCS is also associated with a number of developmental defects including skeletal abnormalities, macrocephaly, pitting of hands and feet, jaw cysts, eye anomalies, calcification of falx cerebri and polydactyly or syndactyly [43, 44]. A majority of germline mutations in PTCH1 (9q22.3) are truncating, making it reasonable to assume that haplo-insufficiency of PTCH1 underlies the developmental abnormalities. This view is also consistent with the requirement of dosage-dependent Shh signalling in cell fate determination [32] and the phenotype of Ptch1-deficient mice where Ptch1 $-/+$ animals recapitulate many features of NBCCS patients [45, 46]. To a very large extent there is an overlap between processes and tissues where Shh signalling is known to have a role during embryonic development [1, 2] and developmental abnormalities in NBCCS.

BCC is the most common tumour presenting in NBCCS patients, and it was rapidly shown that in both familial and sporadic BCC both alleles of PTCH1 are often inactivated [41, 42, 47, 48] demonstrating that PTCH1 is a tumour suppressor gene. Based on the tumour spectrum reported in NBCC patients, mutation studies and tumour spectrum in Ptch1 $-/+$ mice, defects in SHH-PTCH signalling are now implicated in a variety of different tumour types (see table 1).

Since there is no evidence for genetic heterogeneity in NBCCS, it is likely that for the tumour types consistently linked to the syndrome (BCC, medulloblastoma, ovarian fibroma, cardiac fibroma, fetal rhabdomyoma, meningioma, and ameloblastoma), impaired PTCH1 function is a contributing factor. A variety of other tumours have occasionally been reported in NBCCS patients [43, 44, 49], and again there is a striking correlation with known roles/expression of Hedgehog signalling during development [1, 2, 50, 51]. Moreover, when a large number of heterozygous Ptch1-deficient mice are analysed, a broad and overlapping spectrum of tumours is apparent [52] (table 1), demonstrating that such mice represent an appropriate model of the human syndrome. To date, mutations in PTCH1 have been reported in low frequency in three tumour types, trichoepithelioma [53], bladder carcinoma [54] and esophageal squamous cell carcinoma (SCC) [55], not connected to NBCCS but derived from tissues, which during embryonic development are targets for Hedgehog signalling.

Table 1. Tumour types linked to defects in SHH-PTCH signalling.

Tumour	Mutations in PTCH1	Present in NBCCS	Mutations in sporadic tumours
BCC	+	+	+
Medulloblastoma	+	+	+
Ovarian fibroma	nd (LOH)	+	nd
Cardiac fibroma	nd (LOH)	+	nd
Fetal rhabdomyoma	nd	+	nd
Meningioma	?	+	nd
Ameloblastoma	nd	+	nd
Trichoepitheliomas	+	-	+
Bladder carcinoma	+	-	+
Esophageal SCC	+	-	+
PNET	+	-	+
Case reports in NBCC		Tumors in Ptch1+/− mice	
Rhabdomyosarcoma		medulloblastoma	
Leiomyoma		rhabdomyosarcoma	
Benign schwannoma		BCC (after irradiation)	
Endometrial adenocarcinoma		fibrosarcoma (after irradiation)	
Ovarian carcinoma		SCC (after irradiation)	
BCC in lung		hemangioma	
Thyroid polyadenoma		cystadenoma of the Harderian gland	
Osteochondroma		intestinal carcinoma	
Apocrine poroma		endometrial sarcoma	
SCC (after irradiation)		pnet	

Abbreviations: SCC, squamous cell carcinoma; PNET, primitive neuroectodermal tumour; LOH, loss of heterozygosity; nd, not done; ?, only a limited number of meninigiomas have been analysed with a single mutation also present in the germline found.

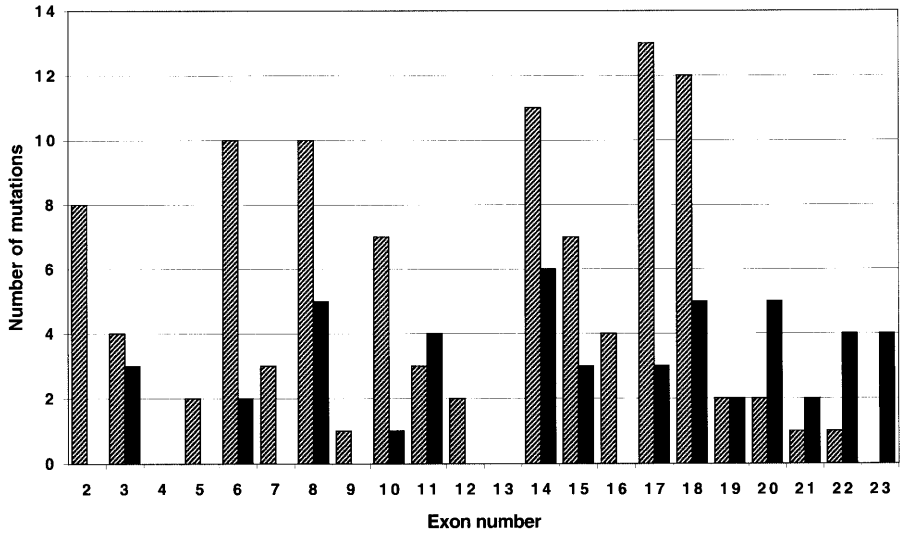

Figure 3. Distribution of PTCH1 mutations in relation to exon structure. Stippled bars denote truncating and splice mutations and filled bars missense mutations and in frame deletions and insertions. Data from the PTCH Mutation Database, (http://www.cybergene.se/PTCH/).

PTCH1 mutations

The PTCH1 protein has a predicted structure encompassing 12-transmembrane domains, two large extracellular loops presumed to be involved in ligand binding and intracellular N- and C-termini. At present, very little is known about functional domains and their relative importance or about genotype-phenotype correlations. In order to understand more about the protein and as a help to the research community, a locus-specific mutation database is being launched containing all published mutations (the database is called the PTCH Mutation Database and can be found at http://www.cybergene.se/PTCH/). Presently, a total of 152 disease-associated mutations (germline or somatic mutations in tumours) are described, and consistent with the notion that PTCH1 is a tumour suppressor gene, about two-thirds of these are truncating or splice mutations. The distribution of mutations in different PTCH1 exons is illustrated in figure 3. It is apparent that mutations, both truncating and missense, are spread over the gene from exon 2 to exon 23 with exons 6, 7, 14, 17 and 18 showing the highest number of mutations. These exons correspond to the predicted first extracellular loop, the third intracellular loop and the second extracellular loop, respectively. Worth noting is that missense mutations dominate towards the C-terminus and are also present in the putative SSD (exons 10 and 11). From previous biochemical studies it is known that the C-terminal end of Ptch1 is not required for binding to Shh or association with Smoh [15, 16], hence presence of mutations may suggest that the C-terminus has a regulatory role for Ptch1 function. In addition 15 polymorphisms within the coding sequence have been compiled.

SMOH mutations

According to the model for SHH signalling, loss of function of PTCH1 will derepress SMOH, leading to constitutive activation of the pathway. The same result would be obtained by gain-of-function mutations in SMOH. Confirming this view, such activating mutations have been found in BCC and medulloblastomas [56–58]. One of the mutations, Trp535Leu, resides in the seventh transmembrane region known to be important for signalling; another, Arg199Trp, in the extracellular N-terminus primarily involved in PTCH interaction; and the third, Arg562Gln, in the C-terminus. Two of the mutations (Trp535Leu, Arg562Gln) have been shown to result in receptors no longer sensitive to PTCH inhibition and thus constitutively active [59]. It should be noted that the Arg199Trp mutation was found in a tumour also having a PTCH1 mutation, so the biological significance of this alteration is not clear. In BCC a frequency of up to 20% of SMOH mutations is described [58].

BCC, trichoepitheliomas and SCC

BCC of the skin is the most prevalent tumour reported in NBCCS patients, which can develop from a few to

several hundreds. At the same time, sporadic BCC is the most common tumour in the Western world, with an estimated yearly incidence of about 1 million cases in the United States alone [60]. BCCs are keratinocyte-derived locally invasive tumours that almost never metastasise and have no identified precursor lesion. Mutations in PTCH1 have been detected at a high frequency (up to 40%) in both familial and sporadic BCC [41, 42, 47, 48, 57, 61, 62] and also demonstrated to result in inactivation of both PTCH1 alleles, consistent with PTCH1 being a classical tumour suppressor gene. Loss of heterozygosity (LOH) specifically at the PTCH1 locus in BCC occurs in 50–70% of the tumours [63–65] and SMOH mutations in up to 20% [58], suggesting that most, if not all, BCCs harbour mutations associated with activation of the SHH signalling pathway. This notion is further supported by the finding that, in line with the present view of the signalling pathway where PTCH1 and GLI1 are direct target genes, essentially all BCCs have an elevated level of PTCH1 messenger RNA (mRNA) [47, 61, 66]. Moreover, increased GLI1 mRNA and protein levels are consistently observed in BCC [33, 34]. Taken together, this leads to the conclusion that constitutive activation of SHH-PTCH signalling is required for BCC development, and that PTCH1 and SMOH are the targets for genetic alteration.

Trichoepithelioma is another hair follicle-derived skin tumour, which is similar to BCC and also occurs in both familial and sporadic variants. In fact, trichoepitheliomas and BCC often coexist in the multiple familial trichoepithelioma syndrome (MIM 60/606), tentatively linked to chromosome 9p, although trichoepitheliomas are apparently not part of the phenotype in NBCCS. Analysis of hereditary and sporadic trichoepitheliomas showed consistent upregulation of PTCH1 mRNA as well as PTCH1 mutations in sporadic tumours [53]. However, analysis of germline DNA from two nuclear families failed to reveal PTCH1 mutations, opening the possibility that the 9p gene encodes a novel component in the Hedgehog signalling pathway.

SCCs of the skin are, like BCCs, derived from keratinocytes of the skin, but NBBCS patients are in general not predisposed to SCC development [43, 44]. Of interest, another hereditary syndrome called multiple self-healing squamous epitheliomata (MSSE, MIM 132800), characterised by development of skin tumours with a morphology resembling well-differentiated SCC, has been mapped to chromosome 9q22.3 in a region overlapping with PTCH1. It was thus a possibility that MSSE and NBCCS were allelic syndromes, potentially caused by different types of mutations in PTCH1. Failure to detect PTCH1 mutations in MSSE patient DNA [67, 68] coupled with a lack of PTCH1 mRNA overexpression and PTCH1 mutations in sporadic skin SCC [69] makes this scenario less likely and implicates different pathogenetic mechanisms in the development of BCC and SCC. An interesting observation is that SCC development has been detected along with several other tumour types in an NBCCS patient following therapeutic irradiation [49]. Propensity to develop tumours after irradiation is an established feature in NBCC patients and has led to the suggestion that NBCCS is a genomic instability syndrome, but convincing evidence to substantiate this hypothesis is lacking. Now, a very recent study exposing Ptch $+/-$ mice to ultraviolet (UV) radiation also described increased frequency and size of SCC with tumours showing no wild-type allele inactivation or increase in Ptch1 transcription [70] and previously increased frequency of developmental abnormalities in Ptch1 $+/-$ embryos after exposure to ionising radiation have been reported [46]. A possible scenario could be that PTCH1 haplo-insufficiency in some thus far uncharacterised manner impairs the DNA-damage response and that keratinocytes able to serve as SCC precursors suffer an increased number of mutations in genes unrelated to PTCH1. Further mechanistic studies are clearly warranted to follow up on these interesting observations.

Medulloblastomas and neuroectodermal tumours

In contrast to the high incidence of BCCs amongst NBCCS patients, less than 5% of such individuals develop medulloblastomas. This suggests that defects in Hedgehog signalling are less effective in driving formation of this tumour type, possibly depending on the influence of modifier genes. Such a view is partly supported by the high frequency of medulloblastomas seen in Ptch $+/-$ mice depending on genetic background [45, 46]. Alternatively, the progenitor cells may be less exposed to mutagens and hence less prone to lose the wild-type allele. Several studies have described PTCH1 mutations and less frequently SMOH mutations in sporadic medulloblastomas and related primitive neuroectodermal tumours (PNETs) [56–58, 61, 67, 71–73]. Mutations are more common in tumours with LOH of the PTCH1 region, indicating homozygous inactivation of the PTCH1 gene. Similar to BCC and trichoepithelioma, upregulation of PTCH1 mRNA using reverse-transcriptase polymerase chain reaction (RT-PCR) has been detected in 15/15 sporadic PNET [57], and an increased expression in tumour cells is frequently seen by in situ hybridisation analysis in both familial and sporadic tumours [A. B. Unden et al., unpublished observations]. Also, expression of GLI1 and SMOH was increased in the majority of sporadic PNET [57]. Similarly, murine medulloblastomas developing in

PTCH1 +/− mice show transcriptional activation of the Ptch1 and Gli1 genes [45]. It is therefore reasonable to assume that most human PNETs harbour a constitutively active SHH-PTCH signalling pathway even though mutations in PTCH1 or SMOH are only detected in a minor fraction of such tumours. Of interest, other genetic alterations involved in medulloblastoma development are found in the APC and *β-catenin* genes, which are part of the WNT/Wg signalling pathway, pointing towards a possible connection between the two pathways in the genesis of this tumour type [74, 75].

Significant further understanding of the role of deregulated SHH signalling in relation to medulloblastoma development has come from recent studies in mice, demonstrating that Shh is primarily expressed in the cerebellar Purkinje cells and serves as a mitogen for granule neuron precursor cells [76–78]. Such precursor cells with inappropriate activation of Hedgehog signalling, e.g. mutations in PTCH1, may thus be the origin of medulloblastomas.

Other tumour types

Fetal rhabdomyomas and rhabdomyosarcomas are observed in a low percentage of NBCCS patients. Mutational analysis of PTCH1 and SMOH is not yet reported in these tumour types, but a strong link to defects in Hedgehog signalling is supported by development of similar tumours in Ptch1 +/− mice on a CD1 background [46]. Also, in this case consistent upregulation of Gli1 mRNA expression is seen in the murine tumours, and elevated GLI1 mRNA levels are found in xenografts of human embryonal but not alveolar rhabdomyosarcoma [79]. Furthermore, an increase in GLI1 expression does not appear to be confined to rhabdomyosarcomas but to be a more general property of bone and soft tissue sarcomas [80]. A likely mechanistic clue is provided by the observation that Shh is a mitogen for chick skeletal myoblasts [81] and regulates muscle cell type determination [82].

A link between Hedgehog signalling and the observed GLI1 expression in chondrosarcomas and osteosarcomas [80] is provided by the now well-established role of IHH in regulating chondrocyte proliferation and maturation during development [83, 84]. Ihh is expressed in prehypertrophic chondrocytes and Ptch1 and Gli1 in adjacent proliferating chondrocytes, and constitutive activation of signalling in such cells would be expected to result in excessive proliferation. To date, no studies of PTCH1 or SMOH mutations in chondrosarcomas or osteosarcomas have been reported. However, an interesting twist is the recent finding that the *Drosophila* gene *tout-velu* is a homolog of the human genes EXT1–2, which are mutated in the disorder hereditary multiple exostoses (HME) [85]. Patients frequently develop benign tumours (exostoses or osteochondromas), which sometimes progress to chondrosarcomas or osteosarcomas, and based on loss of heterozygosity analysis, the EXT genes are regarded as putative tumour suppressor genes. Since EXT proteins like Tout-velu are involved in the synthesis of heparan-sulphate-containing molecules required for Hedgehog diffusion and signalling over a distance, it may be hypothesised that in the absence of EXT function, IHH producer cells and/or immediately adjacent cells only receive a proliferative signal but no stimulus to differentiate and become postmitotic, since such signals mediated by PTHrP [84] are dependent on long-range IHH signalling. The role of IHH signalling described above is probably also linked to the appearance of lytic bone cysts or hamartomas, which occur in 35–45% of NBCCS patients [43, 44].

A contribution of defects in the Hedgehog signalling pathway to the pathogenesis of breast carcinomas was suggested by the description of two somatic missense mutations in PTCH1 [86]. In a subsequent study, a further presumed activating mutation in SHH was reported in a breast carcinoma and in one each of medulloblastoma and BCC [87]. In a larger study, however, no mutations in either PTCH1, SMOH or SHH were detected in breast carcinoma [88] nor were any SHH mutations detected when analysing a number of different tumour types, including breast [89]. These observations suggest that mutations in the analysed components of the Hedgehog signalling pathway are rare in breast carcinoma. Interestingly, it was recently reported that Ptch1 and Gli1–3 were all expressed during murine postnatal mammary gland development and that Ptch1 +/− heterozygous mice develop ductal hyperplasias and dysplasias, which are reversible during pregnancy and lactation [90]. In light of these observations, further studies on the expression of Hedgehog signalling components and a search for mutations in human breast carcinomas appear warranted. It would also be interesting to see what happens in mice with conditional homozygous inactivation of Ptch1 targeted to mammary cells.

Mouse models of skin cancer development

As discussed above, Ptch +/− mice recapitulate many of the NBCCS phenotypes including spontaneous development of medulloblastomas, rhabdomyosarcomas and a number of other internal tumours but no skin tumours [45, 46, 52]. Overexpression of either Shh or a mutant variant of SMOH, which cannot be efficiently repressed by Ptch1 [59], in the skin of transgenic mice, however, resulted in epidermal proliferations in late embryonic skin that by marker analysis and morpho-

logical criteria partially resembled BCC [56, 87]. The mice died perinatally, and in the case of Shh overexpression the epithelial outgrowths in transplanted transgenic skin eventually differentiated into hair follicles [87]. Furthermore, by retroviral transduction of the SHH gene to primary human keratinocytes followed by transplantation to immune-deficient mice, similar BCC-like epithelial proliferations developed [91].

More interestingly, careful analysis of the skin in aged Ptch1 + / − mice revealed the presence of small basaloid tumours, which were greatly increased in number and size when mice were exposed to UV or ionising radiation [70]. Histologically the tumours in exposed mice resembled BCC (superficial and nodular) and trichoblastomas (a hair follicle-derived tumour sometimes appearing together with BCC). The UV-exposed mice also developed fibrosarcomas, which like the BCC, demonstrated transcriptional activation of Ptch1, suggesting the presence of deregulated Hedgehog signalling.

GLI1 is a transcriptional effector in skin cancer development

Accumulating evidence that GLI1 is mainly a transcriptional activator and that it is overexpressed in tumours with genetic alterations in PTCH1 or SMOH strongly indicates GLI1 as an essential transcriptional effector. Direct support for this hypothesis now comes from a study where the human GLI1 gene has been overexpressed in mouse skin [92]. Such mice rapidly and spontaneously develop tumours classified as BCC (nodular and superficial), trichoepitheliomas, cylindromas and trichoblastomas. The tumours show increased Ptch1 expression as well as an increase in Sufuh mRNA levels (see fig. 4), suggesting that Sufuh may also be a Shh target gene. In humans, BCC and trichoepithelioma are known to have deregulated Hedgehog signalling, and all of these tumours can coexist in human patients in different combinations (compare also the development of both BCC and trichoblastoma in Ptch1 + / − mice). Worth noting is that hereditary syndromes predisposing to cylindromas (MIM 132700) and trichoepitheliomas (MIM 60/606) exist and have been mapped to chromosome 16q and 9p, respectively. Taken together, these findings are compatible with the hypothesis that the cell of origin is a pluripotent progenitor cell residing in the apocrine/follicular unit and which can differentiate into different cell types. The cellular phenotype may be dependent on the level of GLI1 expression and activation of target genes in analogy with cell fate determination by graded Shh signalling in the spinal cord (see Patten and Placzek, this volume). The latter hypothesis may be tested by generating transgenic mice that allow regulation of the GLI1 expression level.

Since, in mice, secretion of Shh by the epithelial placode is required for the proper formation of the dermal papilla [93–95; Chuong et al., this issue] the observed tumours may be regarded as failed attempts to make a hair follicle. In line with this interpretation, human BCC rarely express SHH, and the epithelial outgrowths in Shh overexpressing mice could differentiate into hair follicles [87].

Whether or not GLI1 is the only effector able to drive tumorigenesis is still an open question, and GLI2 also expressed during hair follicle development [94] and in adult human skin [A. B. Unden et al., unpublished observations] is an interesting candidate in this regard.

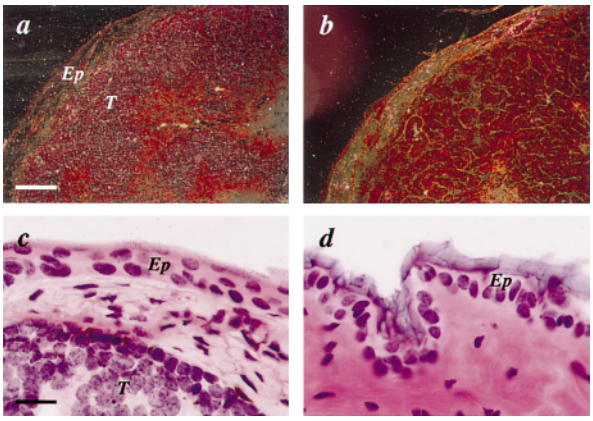

Figure 4. Overexpression of Sufuh mRNA detected by in situ hybridization in a BCC taken from a transgenic mouse expressing human GLI1 in the epidermal basal cells and in keratinocytes of the outer root sheath [92]. (*a*) Dark-field photomicrophotograph of a BCC showing abundant autoradiographic signal in the tumour cells (T), whereas no signal is observed in normal epidermis (Ep). (*b*) A section from the same tumour hybridised with the sense probe showed no signal. (*c*) Bright-field of the same tumour in higher magnification demonstrates specific signal in tumour cells (T), with no signal in adjacent epidermis (Ep). (*d*) Epidermis (Ep) from a nontransgenic sibling hybridised with the same antisense probe showed no postive signal. Scale bars (*a*,*b*) 135 μm and (*c*,*d*) 13 μm. A fragment from a mouse Sufuh complementary DNA clone was used as the probe under conditions as described elsewhere [92].

Is deregulated SHH signalling sufficient for BCC development?

Most tumours are believed to develop in stages and require a number of genetic alterations. BCC may, however, be an unusual case since no precursor lesion has been identified and the tumours are genetically quite stable. In spite of this, p53 mutations are very common in human BCCs as well as in patches of morphologically normal skin in UV-exposed areas [96], and it has

been speculated that p53 mutations are an early and important event in the pathogenesis of BCC. Analysis of tumours from GLI1 transgenic mice did not reveal any p53 mutations or mutations in the H-ras oncogene [92], and a recent analysis of human BCC also found tumours lacking p53 mutations in patients routinely using sunscreens [97]. In light of these findings and the very frequent tumour formation in GLI1 transgenic mice, it appears possible that a single genetic event, i.e. a sufficiently elevated GLI1 expression level in a responding cell caused by homozygous inactivation of PTCH1 or an activating mutation in SMOH, is the only thing required to drive tumour formation. Further studies looking for mutations in other oncogenes and tumour suppressor genes are required to substantiate the single-hit hypothesis.

Mechanisms of tumour formation

It is striking that in the best-established cases, where deregulated Hedgehog signalling drives tumorigenesis, i.e. BCC, medulloblastoma and rhabdomyosarcoma, Shh is a mitogen for the presumed progenitor cells [76–78, 81, 82, 93, 94, 98]. The details of how cellular proliferation is brought about is not known, but in one study, analysing cultured human keratinocytes overexpressing SHH, it was demonstrated that SHH caused a block in the response to differentiating signals and proliferation inhibitors such as p21 as well as allowed long-term proliferation [98]. At least for keratinocytes alteration of apoptosis does not appear to be important as judged by the lack of increase in the number of apoptotic cells after UV exposure of Ptch1 $+/-$ mice [70].

Thus, in the most likely scenario, constitutive activation of Hedgehog signalling, resulting in elevated activity of GLI1 (or GLI2), leads to cellular proliferation at the expense of maturation of the target cell into a postmitotic state. If this is true, an intervention to block GLI1 function may allow differentiation signals to act and drive tumour cells into a nonproliferative mature state. An eventual defect in the DNA-damage response caused by PTCH1 haplo-insufficiency could be relevant as a mechanism contributing to tumour formation, but judged from the appearance of skin SCC may only be important at very high exposures to UV or ionising radiation [49, 70].

Other genes in the pathway such as PTCH2, GLI2 or SUFUH have so far not been demonstrated as targets for genetic alterations. In the case of PTCH2, the mRNA level is elevated in BCC [19], but the functional role of PTCH2 in Hedgehog signalling is not yet known. Since homozygous Ptch1 inactivation is embryo lethal in mice and no NBCCS families have been mapped to the PTCH2 locus, PTCH2 may have functions distinct from PTCH1. Interestingly, in Pallister-Hall syndrome, caused by mutations in GLI3 leading to formation of a potent transcriptional repressor [24], hypothalamic hamartomas are frequently formed [99]. Whether this is related to a function dependent on or independent of SHH signalling is not known.

Questions for the future

A number of central and important questions need to be answered, building on the very rapid advance in knowledge over the recent years. Some of these are:

1) Is deregulated Hedgehog signalling important for additional tumour types—specifically, tumours developing in tissues where Hedgehog signalling plays a role during development? Analysis of PTCH1/GLI1 upregulation in tumour cells appears to be a good signal response.
2) Are established tumours like BCC and medulloblastomas still dependent on GLI1 for continued proliferation, or have they become autonomous? Here, relevant mouse models may give important answers.
3) What are the molecular mechanisms determining subcellular GLI trafficking, and which are the important target genes causing cell cycle activation?
4) What are the additional components in the vertebrate Hedgehog signal transduction pathway? Phenotypes of new mutations in *zebrafish* as well as human hereditary syndromes predisposing to adnexal skin tumours strongly suggest the presence of novel players. In addition, important links may exist to other signalling pathways, such as the Wnt pathway, which is also deregulated in a subset of medulloblastomas and hair-follicle-derived tumours.
5) Which are the modifier genes determining susceptibility to medulloblastoma and rhabdomyosarcoma? Again mouse genetics may be a central tool.
6) Which are the progenitor cells that upon constitutive activation of Hedgehog signalling can develop into tumours?

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