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Review

GLI transcription factors: Mediators of oncogenic Hedgehog signalling

*Maria Kasper, Gerhard Regl, Anna-Maria Frischauf, Fritz Aberger**

Department of Molecular Biology, University of Salzburg, Hellbrunnerstrasse 34, A-5020 Salzburg, Austria

ARTICLE INFO

Article history:
Received 22 June 2005
Received in revised form
8 August 2005
Accepted 10 August 2005
Available online 10 January 2006

Keywords:
GLI proteins
Oncogenes
Hedgehog proteins
Gene expression regulation
Signal transduction
Neoplasms

ABSTRACT

The current concept of tumourigenesis holds that cancer results from the progressive acquisition of mutations that endow affected cells with selective growth advantages by activating multiple processes including intrinsic mitogenic and pro-survival pathways. Constitutive activation of the Hedgehog (HH)/GLI signalling cascade has recently been implicated in the growth of a number of human malignancies ranging from semi-malignant tumours of the skin to highly aggressive cancers of the brain, lung, pancreas and prostate. This review focuses on the role of the GLI zinc finger transcription factors, which mediate Hedgehog signalling at the distal end of the pathway. We summarise recent data on the mechanisms by which latent GLI proteins are activated in response to stimulation of Hedgehog signalling. Based on the identification of a growing number of direct GLI target genes, we propose that HH-driven tumourigenesis relies on multiple cellular processes such as promotion of G1/S phase progression, enhancement of cell survival by providing anti-apoptotic cues, increase in metastatic potential of Hedgehog responsive cells, and activation of potential tumour stem cells. In view of the critical role of GLI genes in Hedgehog-associated cancers, strategies that aim at interfering with GLI function are likely to represent efficient approaches in future targeted cancer therapy.

1. Introduction

The Hedgehog (HH)/GLI signal transduction pathway controls a variety of developmental processes such as pattern formation, differentiation, proliferation, and organogenesis (for review see Ref. 1). Besides its crucial roles in embryogenesis, HH/GLI-signalling has recently attracted considerable interest in the field of cancer research, as a series of publications has implicated aberrant pathway activation in the growth and maintenance of common malignancies such as basal cell carcinoma (BCC), lung, esophageal and biliary cancer, as well as pancreatic and prostate cancer. Specific targeting of HH/GLI-signalling may therefore offer a highly effective therapeutic strategy for the treatment of a variety of lethal tumours (reviewed in Refs. 2 and 3).

In this review we focus on the mechanisms that regulate the activity of oncogenic GLI zinc finger transcription factors. These proteins act at the distal end of the HH pathway where they control transcriptional programs in response to pathway activation. Further, we discuss recent results on HH/GLI signalling in different tumour entities with emphasis on target gene expression profiles. Considering the biological activity of GLI proteins, their regulation in response to pathway activation and their target genes, we propose mechanisms by which aberrant activation of GLI oncogenes may cause cell transformation and tumourigenesis.

* *Corresponding author*: Tel.: +43 0 662 8044 5792; fax: +43 0 662 8044 183.
E-mail address: fritz.aberger@sbg.ac.at (F. Aberger).
0959-8049/$ - see front matter
doi:10.1016/j.ejca.2005.08.039

2. GLI proteins as nuclear executors of HH-signalling

HH-signal transduction is initiated by the binding of processed and lipid-modified HH-protein to its receptor Patched (Ptc), a 12 transmembrane protein, which in the absence of HH-protein represses signal transduction by inhibiting the seven-pass transmembrane protein Smoothened (Smo). Binding of HH-protein to the Ptc receptor abolishes the inhibitory effect of Ptc on Smo, allowing Smo to transduce the signal towards the nucleus, a process regulated by complex interactions and modifications of a large number of cytoplasmic proteins (for detailed reviews see Refs. 1,4,5). The net effect of pathway stimulation is the activation of members of the GLI family of zinc finger transcription factors, which translate the extra-cellular HH-stimulus into defined transcriptional programs in a context-dependent and cell-type specific manner (reviewed in Ref. 6).

In contrast to *Drosophila*, where all transcriptional responses to HH-signalling are controlled by the Gli homologue Cubitus interruptus (Ci), which can both activate and repress HH-target genes, vertebrates have at least 3 distinct Gli genes, Gli1, Gli2 and Gli3. The highly conserved DNA binding domain of Gli proteins comprises 5 zinc finger domains of the C2-H2 class[7] and all Gli proteins were shown to bind to the consensus sequence GACCACCCA.[8] In addition, Gli proteins contain a C-terminal transactivation domain, but only Gli2 and Gli3 were shown to have an N-terminal repression domain.[9] Evidence suggests that Gli1 and also Gli2 represent the main activators of HH-target genes, while Gli3 acts mainly as repressor,[10–12] although some Gli3 activator function is also involved in induction of target gene transcription.[13–15]

The mechanisms by which Gli genes are regulated in response to HH-signalling are still not completely understood. Unlike Gli1, which represents a direct transcriptional HH-target gene,[13,14,16,17] Gli2 and also Gli3 are considered latent transcriptional regulators activated by HH signalling. But how does pathway stimulation lead to the formation of transcriptional activator forms of Gli2 and Gli3? Although this question cannot yet be answered in detail for vertebrate HH/GLI signalling, work on the molecular mechanisms controlling the activity of the *Drosophila* Gli homologue Ci has shown that regulation of Ci activity involves a number of factors that interact with and modify Ci protein (for detailed reviews see Refs. 4 and 5). In the absence of HH ligand, Ci is anchored in the cytoplasm via the kinesin-related protein Costal-2 (Cos-2). This complex also contains the Fused kinase (Fu) and Suppressor of Fused (Sufu), a protein without known sequence motifs. In this complex, a large fraction of full-length Ci is processed into a 75 kD Ci repressor form (CiR) that comprises the N-terminal repressor domain and the zinc finger DNA-binding domain but lacks nuclear export signals, cytoplasmic anchoring sequences and a transcriptional activation domain. CiR acts in the nucleus to shut off the expression of HH-targets.[18,19] CiR formation has been shown to involve phosphorylation of Ci by PKA, GSK3 and CK1 and the activity of the F-box proteins Supernumerary Limbs (Slmb).[20–23] Upon stimulation with HH protein, Smo protein accumulates at the cell membrane and the Cos-2 complex appears to be recruited at higher efficiency to Smo by directly interacting with the cytoplasmic tail of Smo that is phosphorylated in HH-responding cells. This recruitment step appears to be required for the release of Ci from the Cos-2 complex, probably by activation of Fu kinase and phosphorylation of SuFu. This allows the stabilisation of full-length Ci and the formation of the Ci activator form, of which a small fraction translocates to the nucleus to activate HH-target genes (see Fig. 1) (reviewed in Refs. 5 and 24).

In vertebrates, SUFU has been shown to play a key role in negatively regulating the activity of Gli proteins by sequestering them in the cytoplasm.[25] In addition, loss of SuFu function in man results in a phenotype that resembles, in some aspects, loss of the pathway repressor Patched, including congenital malformations and predisposition to medulloblastoma.[26] Consistent with the negative regulation of Gli by SuFu, mutant forms of SuFu are unable to retain GLI proteins in the cytoplasm.[26] The detailed mechanisms that control SuFu remain to be discovered and may diverge from *Drosophila*. In addition to its role in sequestering GLI proteins in the cytoplasm, SuFu has also been implicated in repressing GLI target genes by recruiting, a transcriptional silencing complex with histone deacetylase activity consisting of SAP18 and SIN3, to target promoters.[27]

Modification of the N-terminus of Gli2 and Gli3 may also be a critical step in the regulation of the transcriptional activity of Gli proteins. This region is likely to harbour a repressor domain, since removal of a large portion of the N-terminus results in constitutively active Gli2 and Gli3 proteins.[9] In an elegant genetic experiment Mill and co-workers analysed *Sonic hedgehog* (*SHH*) mutant mice expressing either full-length or N-terminally deleted Gli2 in the basal layer of the epidermis and found that the activation of full-length Gli2 relies on the presence of functional SHH protein. By contrast, the N-terminal deletion protein was highly active in *SHH* mutant epidermis.[28] This raises the question of whether latent Gli proteins are converted into an activator form by removal of the N-terminus either by proteolytic processing or perhaps by modification of the N-terminus such as phosphorylation.

In contrast to Gli2 and Gli3, Gli1 is mainly regulated at the transcriptional level. In fact, induction of Gli1 mRNA expression by HH-signalling is a reliable marker for pathway activity. Transcriptional activation of Gli1 in response to HH protein does not require protein synthesis but is dependent on the presence of functional Gli2 and Gli3 protein.[14] Further, both human GLI2 and GLI3 were shown to activate the GLI1 promoter by directly binding to sequences with homology to the consensus Gli binding site.[16,17] This is also corroborated by genetic data showing that Gli2 mutant mice display dramatically reduced levels of Gli1 mRNA and that knock-in of Gli3 into the Gli2 locus can restore Gli1 expression.[13,29]

The ability of vertebrate GLI proteins to activate target genes is further controlled by interactions with additional factors. The dual specificity Yak1-related kinase Dyrk1 has been shown to increase Gli1-dependent target gene activation by retaining Gli1 in the nucleus and by enhancing its transcriptional activity.[30] The actin binding protein Missing in Metastasis (MIM) has recently been identified as SHH-responsive gene that modulates the activity of GLIs in skin development and tumourigenesis. Like Dyrk1, MIM enhances the transcriptional activity of Gli1 and Gli2. MIM interacts with the SuFu/

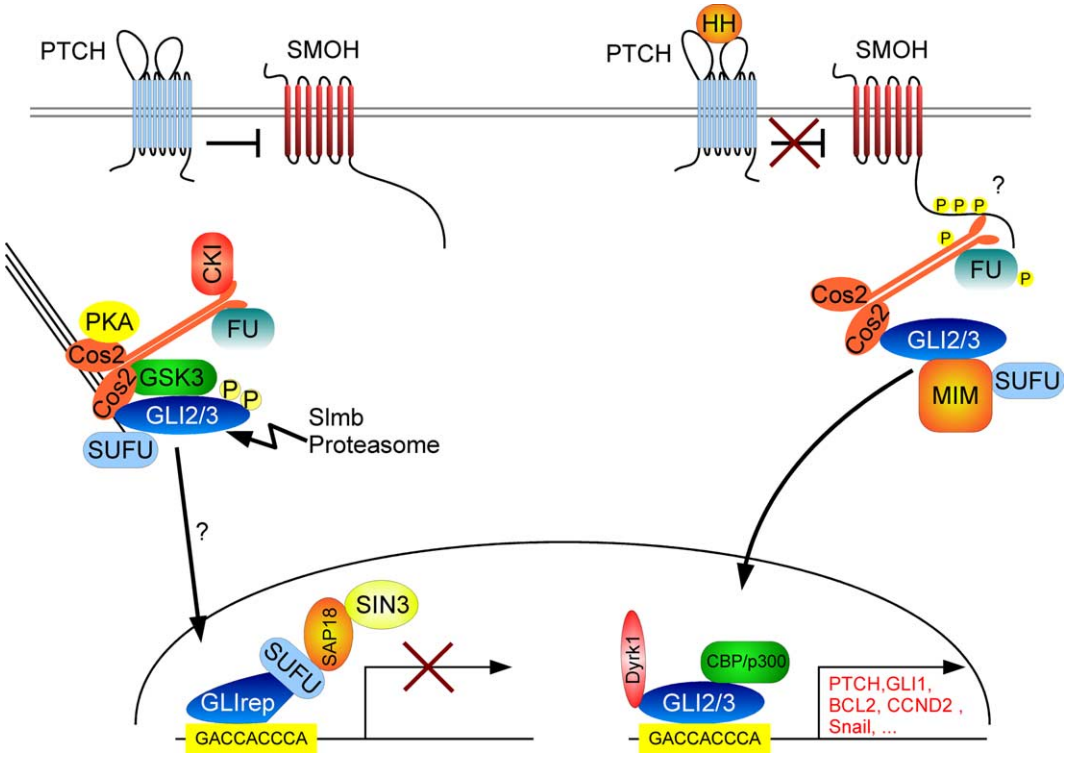

Fig. 1 – Possible regulatory mechanisms of GLI protein activity. Note that this model is largely based on results from studies of *Drosophila* HH-signalling. (Left) In the absence of HH-ligand, the latent GLI transcription factors GLI2/3 are bound to a multiprotein complex via interactions with a vertebrate functional homologue of *Drosophila* Cos2.[88] Phosphorylation of GLI2/3 by PKA, GSK3 and CK1 targets latent GLI proteins to proteasome-dependent repressor formation. Proteolytic processing requires functional Slmb protein. Target gene repression may involve the recruitment of a histone-deacetylase activity via interaction of GLI with SuFu-SAP18-SIN3. Alternatively, phosphorylation may simply inactivate latent GLI proteins. In either case, HH target genes are not expressed. (Right) On binding of HH protein to its receptor PTCH, phosphorylation of the cytoplasmic tail of SMOH may recruit the Cos2/GLI complex and disassemble the GLI-kinase complex, thereby allowing the activator forms of GLI2 and GLI3 to translocated to the nucleus and activate target gene transcription. The transcriptional activity of GLI proteins is further enhanced by interactions with MIM, Dyrk1 and CBP.

Gli complex, showing that MIM encodes a novel component of the HH/Gli signalling pathway involved in enhancing the transcriptional response to pathway activation and perhaps also in controlling target gene specificity.[31] In *Drosophila*, activation of HH target genes by the Ci activator form requires the transcriptional co-activator Creb binding protein (dCBP), which directly binds to Ci via a domain that is conserved in vertebrate Gli3, and likely also in Gli2 but is lacking in Gli1[16,32,33] (our own unpublished observations). Similar to Ci, Gli3 directly interacts with CBP, which increases the transcriptional activity of Gli3[16] (see Fig. 1).

Genetic screens for recessive mutations affecting mouse embryonic development have led to the identification of several intraflagellar transport (IFT) proteins acting as regulators of Gli protein activity. IFT proteins play a critical role in the growth and maintenance of flagella and cilia. Intriguingly, loss of function mutations in *IFT172* and *IFT88* as well as in *Kif3a* and *Dnchc2*, two essential subunits of the anterograde and retrograde IFT motors, result in severe patterning defects of the neural tube similar to those observed in *SHH* or *Smo* mutant embryos.[34–36] Epistasis analysis has placed these genes downstream of *Ptc* and *Smo* but upstream of the *Glis*, suggesting a crucial role in the regulation of Gli activity in response to pathway activation. In the neural tube, where Gli activators are required for ventral cell specification, loss of IFT function causes a phenotype that is consistent with loss of HH-signalling. In contrast, analysis of Gli3 processing in IFT mutants suggests that IFT proteins also promote the formation of the Gli3 repressor form, as the absence of functional IFT172, IFT88, Kif3a and Dnchc2 leads to a significant decrease in the Gli3 repressor fragment.[35,36] These studies suggest that IFT controls both mammalian Gli activator and repressor activity, yet the detailed mechanisms by which this is achieved remain to be uncovered. As IFT proteins do not seem to play a role in *Drosophila* Hedgehog signalling, one might speculate that cilia are involved in the relay of the mammalian HH-signal and that IFT proteins organise a higher order structure that may facilitate the interaction of other HH-signalling components with latent Gli transcription factors.

The complexity of GLI regulation and function is further increased by the observation that GLI transcription factors may act as homo and/or heterodimers. A recent report by Nguyen and colleagues has shown that each Gli factor is re-

quired for neurogenesis in amphibians and that the neurogenic activity of Gli1 is lost if any of the three endogenous Glis is removed. Notably, the tumour-inducing activity of human GLI1 in frog tadpoles is abolished in the absence of endogenous Gli3 or Gli1 but not of Gli2, which may be explained in part by the specific formation of functional Gli heterodimers. This is corroborated by co-immunoprecipitation and co-localisation studies showing that protein–protein interactions within the Gli superfamily can occur via fingers 1 and 2 of the zinc finger DNA binding domain.[37] Intriguingly, Gli1-mediated induction of Hedgehog target genes in murine presomatic mesoderm depends on the presence of functional Gli2 und Gli3 proteins,[14] suggesting that mammalian Gli proteins may also interact with each other. Whether the transcriptional activity of mammalian Gli1 requires direct interaction with either Gli2 or Gli3 is unclear at present and further biochemical studies are needed to answer this question. The requirement for heterodimer formation of Gli proteins may also have important consequences for potential therapeutic approaches by inhibition of HH/GLI signalling in human cancers, as it increases the spectrum of molecules to be efficiently targeted. According to the heterodimer model, inhibition of HH-signalling at the level of ligand or SMOH may block tumour growth even in the presence of residual GLI1, as this interference is thought to efficiently prevent activation of the putative GLI1 heterodimer partners GLI2 and GLI3. Further more, targeted inhibition of either GLI protein for instance by RNAi may turn out a promising anti-tumour strategy by disrupting the formation of oncogenic heteromeric GLI complexes.

The analysis of the intricate regulatory processes controlling the transcriptional activity of Gli proteins, by their interactions with multiple modulating proteins, will certainly be a major step towards a more detailed understanding of the context-dependent biological functions and distinct target gene specificities of Gli transcription factors in development and cancer.

3. HH/GLI signalling in human cancer

First evidence for an involvement of HH/GLI-signalling in tumourigenesis has come from genetic analysis of Gorlin syndrome patients, who are predisposed to early onset of multiple Basal Cell Carcinomas (BCCs), a common non-melanoma skin cancer with low metastatic potential.[38] Gorlin patients are also at an increased risk of other tumours including medulloblastoma of the cerebellum and rhabdomyosarcoma (reviewed in Refs. 39–41). A series of studies identified the HH-receptor and pathway repressor *Patched* (*PTCH*) as the gene affected in Gorlin patients and showed that not only hereditary but also the majority of sporadic BCCs are characterised by loss of heterozygosity of *PTCH*, although about 10% of sporadic BCCs display activating mutations in the HH-effector *SMOH*.[42–45] Together with the analysis of transgenic mice with constitutive activation of HH-signalling in the epidermis, these results have led to the model of sustained ligand-independent activation of HH/GLI-signalling as an etiologic factor in the development of BCC.[45–51] More recent data have implicated HH/GLI-signalling in the progression and maintenance of common malignant tumours such as small cell lung carcinoma, pancreatic, stomach and prostate cancer. Unlike BCC and medulloblastoma, these tumours are ligand-dependent, as HH-neutralizing antibodies can block tumour growth in vitro and in vivo.[52–58] Given the broad spectrum of frequent and lethal malignancies involving HH/GLI-signalling, targeting the pathway should constitute an efficient anti-tumour strategy. In fact, when mice with HH-induced BCCs or medulloblastoma were treated with specific HH-pathway inhibitors[59,60] tumour load was dramatically reduced.[61–64] Furthermore, growth of xenografts of HH-dependent lung, pancreatic or metastatic prostate cancer cells was also significantly reduced and tumours were even eliminated upon treatment with pathway inhibitors such as cyclopamine, suggesting that interference with HH/GLI-signalling is a promising approach to targeted cancer therapies.[52–55,57]

4. Oncogenic properties of GLI transcription factors

A common property of HH-associated cancers is the elevated expression level of one or more GLI transcription factors. A series of gain and loss-of-function approaches has shed light on the requirement for individual GLI proteins in HH-associated tumours and on the processes by which GLIs induce tumourigenesis. Together with E1A, GLI1 is able to transform rodent cells and to promote G1-S phase progression in cultured keratinocytes in the absence of dermally derived mitogenic signals.[65,66] Keratin5-promoter driven expression of GLI1 in the epidermis of transgenic mice leads to development of epidermal tumours with characteristics of BCC.[49] Evidence that GLI1 is required for proliferation of tumour cells in man has come from RNAi-based loss-of-function studies showing that primary prostate carcinoma cells cease to proliferate when treated with GLI1 siRNA.[57] GLI1 also appears to control the metastatic properties of prostate cancer cells, since its overexpression is sufficient to convert low-metastatic prostate carcinoma cells into highly metastasizing lines.[53] Furthermore, spontaneous medulloblastoma development in *Ptc* heterozygous mice is reduced in the absence of functional Gli1,[67] suggesting that in vivo Gli1 is mediating critical downstream processes in HH-associated brain tumours, despite the fact that Gli1 is dispensable for mouse development.[68]

The role of GLI2 in mediating oncogenic HH-signalling has been best demonstrated in the context of BCC development. Similar to GLI1, GLI2 mRNA levels are elevated in BCC lesions compared to normal skin.[17,66] Its oncogenic potential has been shown by transgenic overexpression in the basal layer and in the Outer Root Sheath (ORS) of hair follicles. Keratin5-Gli2 transgenic mice develop tumours with histological and molecular characteristics of BCC.[50] In a recent report using a conditional tetracycline-regulated allele of mouse *Gli2* it was demonstrated that sustained expression of Gli2 is required not only for tumour initiation but also for tumour maintenance.[51] In this elegant study the authors showed that conditional activation of Gli2 expression in the epidermis and ORS of hair follicles leads to BCC-like tumours that are associated with hair follicle structures, suggesting that these tumours may derive from cells of the ORS. This may be due to

the fact that full-length Gli2 – as used in this study – requires HH-signalling to be converted into an activator form.[28] Since the sources of HH ligand reside in the matrix region of the hair follicle and in the sebaceous gland,[69,70] it is conceivable that Gli2 protein is only activated in the hair follicle region and ORS. Hutchin and colleagues also provide evidence that Gli2 activity is intimately associated with growth and maintenance of BCC, as conditional inactivation of Gli2 in BCC-like tumours induced rapid Gli target gene inactivation and persistent tumour regression. Tumours with inactivated Gli2 showed reduced proliferation and increased apoptosis. Intriguingly, regressed BCC cells could be re-induced to form BCC upon reactivation of Gli2, suggesting that a small portion of tumour cells is maintained in a quiescent state in the absence of Gli2. Regressed BCC cells displayed the remarkable ability to contribute to multiple epidermal lineages including hair follicle and sebaceous gland. These observations are in line with the concept that cancer may be the result of aberrant organ formation due to constitutive activation of developmental signalling pathways and that cancer may arise by aberrant activation of such signalling pathways in putative tumour stem cells (reviewed in Ref. 71). It is therefore tempting to speculate that aberrant HH/GLI signalling may provide proliferative cues to stem cells, which due to their longevity and capacity for self-renewal are likely targets for further oncogenic events. Constitutive pathway activity in stem cells may be achieved by various alterations including mutations in pathway repressors such as PTCH, continuous supply with ligand or unrestricted expression of essential signal transducers such as SMOH. Increased expression of SMOH has been shown to correlate with the metastatic potential of prostate tumour cells, probably by enabling tumour cells to continuously respond to environmental HH stimuli. In contrast, normal prostate cells do not express significant levels of SMOH but become responsive to HH upon forced expression of SMOH.[53] In view of the essential role of HH-signalling in tissue regeneration, tumour initiation may thus occur by keeping stem cells in a HH-dependent proliferative state. In prostate cancer, sustained expression of SMOH appears to be the limiting factor.

As tumour stem cells have been shown to drive tumour growth and are likely to be responsible for relapse and multi-drug resistance,[72,73] a detailed understanding of the role of HH/GLI in the control of stem cell behavior is critical for future therapeutic strategies.

5. GLI targets as molecular switches in HH-induced cancers

Although aberrant HH/GLI signaling, resulting either from mutations in pathway regulators or from constitutive ligand expression, has been shown to be an etiologic factor in a number of human cancers. The downstream mechanisms initiated by constitutive pathway activity are only poorly understood, partly because relatively little is known about the nature of those genes directly controlled by GLI proteins.

Understanding the downstream effects has also been hampered by context and cell-type specific effects of HH/GLI signalling, which implies that systematic analysis of transcriptional programs activated in response to pathway activation in different HH-associated tumour entities, will be a major requirement to define the molecular and cellular processes by which pathway activation can lead to tumourigenesis.

Recent experiments using a combination of global gene expression profiling, promoter assays and genetic studies of model organisms have led to the identification of direct HH/GLI target genes whose expression in response to pathway activation is likely to be a critical parameter in tumour initiation or maintenance.

In various cell types activation of HH-pathway activity increases the expression of key regulators of G1/S and G2/M phase progression of the cell cycle, thereby promoting the transition from a quiescent to a proliferative state.[74,75] D-type cyclins are consistently upregulated in a number of HH-responsive cells.[28,74–77] Induction of Cyclin D2 transcription is likely to be directly controlled by HH/GLI signalling. A GLI-binding site in the human Cyclin D2 promoter has been identified by electrophoretic mobility shift assays.[78] In addition, treatment of murine mesodermal cells with recombinant SHH-N protein induces Cyclin D2 mRNA expression even in the absence of protein synthesis.[14] Cyclin D2 induction depends on functional Gli2 and Gli3 protein, suggesting that activation of HH-signalling directly increases Cyclin D2 expression via the latent transcriptional activators Gli2 and Gli3.[14]

In *Drosophila*, HH-signalling directly controls the expression of Cyclin E,[79] though it is unclear whether this regulatory process is conserved in vertebrates. Another potential direct GLI target gene involved in G1/S phase progression is *E2F1*. Its mRNA rapidly increases in response to GLI expression, similar to the increase in the mRNA of the direct target gene *PTCH*.[74] Further promoter studies including chromatin immune precipitation will be required to characterise in detail the transcriptional regulation of cell cycle regulators by GLI proteins.

Besides direct activation of G1/S phase progression genes, HH-induced proliferation has been shown to involve the activity of the *Nmyc1* proto-oncogene. Treatment of cerebellar granule neuron precursor (CGNP) cultures with recombinant SHH protein results in G1/S phase entry and rapid upregulation of Nmyc1 expression, which is likely to be directly controlled by Gli, as Nmyc1 mRNA induction by SHH treatment is resistant to inhibition of protein synthesis.[80] In CGNPs, overexpression of Nmyc1 can mimic the proliferative effect of SHH, while interference with Myc function antagonises the effect of SHH on cell proliferation, suggesting that direct activation of Nmyc1 expression by SHH is a critical step in CGNP proliferation and HH-induced medulloblastoma development, respectively.[80]

Recent studies have also uncovered a role of GLI transcription factors in promoting cell survival. GLI1 and GLI2 have been shown to activate expression of the key anti-apoptotic factor BCL2 in epidermal cells.[81,82] Of note, although both proteins possess strong transcriptional activator function, GLI2 induced BCL2 expression about 10-fold more strongly than GLI1. By replacing the DNA binding domain of GLI1 with that of GLI2, it could be shown that the predominant activation of BCL2 promoter by GLI2 is likely to rely on preferential binding of the GLI2 zinc finger domain to a single GLI-binding

site located about 1.8 kb upstream of the transcriptional start site of BCL2.[81] These results provided evidence that subtle sequence variations in the highly conserved DNA-binding domain of GLI transcription factors may account for distinct quantitative transcriptional outputs and thus, for differences in the biological activities of GLI proteins.

HH/GLI signalling has also been implicated in the control of the metastatic behavior of prostate cancer cells. Invasive and metastatic growth of various prostate cancer cell lines can be significantly reduced by exposing cells to the HH-pathway antagonist cyclopamine, while overexpression of GLI1 can convert cells with low metastatic potential into highly malignant cells.[53] In this context it is noteworthy that GLI1 expression leads to upregulation of Snail, a transcription factor with a critical role in the transition from epithelial to mesenchymal character (EMT), which is associated with increased invasiveness and metastasis. Conversely, cyclopamine treatment of metastatic cells reduced the levels of Snail mRNA, suggesting that HH/GLI signalling may promote metastasis by inducing EMT via upregulation of Snail and consequently, repression of E-cadherin.[53] Another study using a conditional allele of *GLI1* provided evidence that activation of Snail transcription is directly mediated by GLI1, since GLI1 mediated induction of Snail mRNA expression occurred even in the absence of protein synthesis.[83]

Taken together, these results suggest that *GLI* oncogenes can directly induce a set of intrinsic tumourigenic processes: (1) stimulation of proliferation by transcriptional activation of key regulators of G1/S phase progression: (2) inhibition of apoptosis by direct induction of BCL2 expression and (3) enhancement of invasiveness and metastasis by direct activation of EMT-promoting factors such as Snail (Fig. 2). Given the

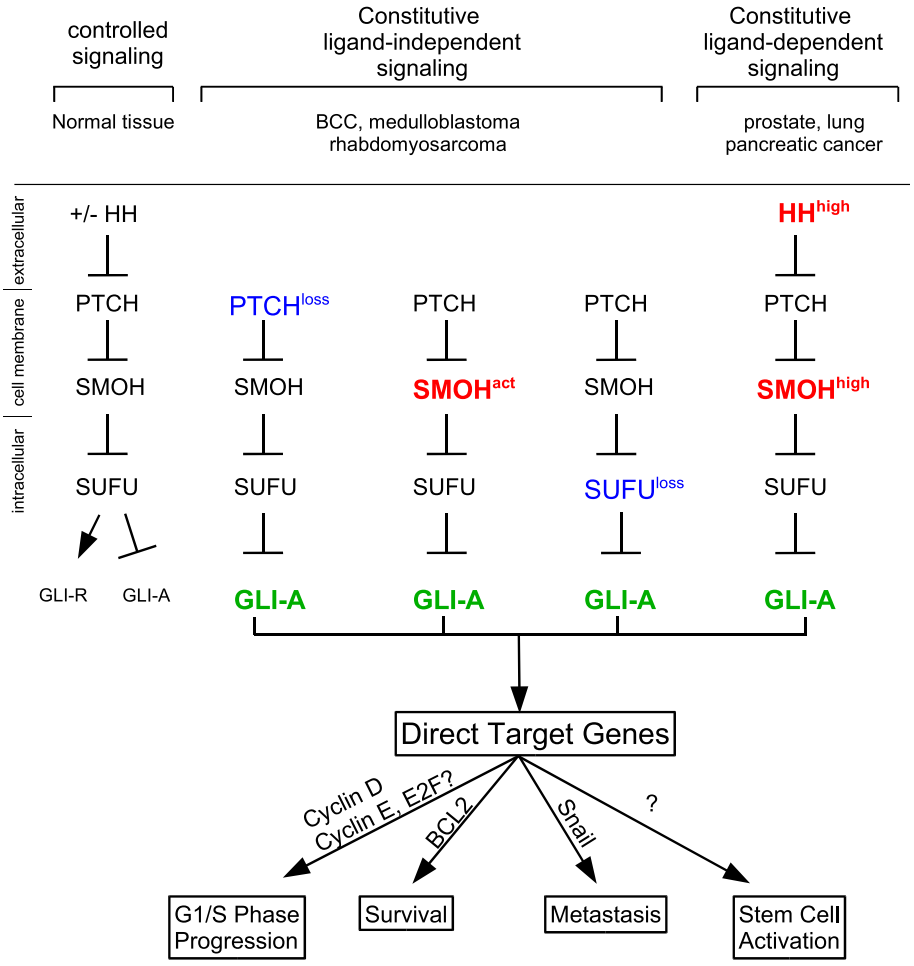

Fig. 2 – Model of tumourigenesis resulting from constitutive HH/GLI signalling in human cancers. In normal tissue, HH/GLI pathway activity is controlled by the availability of ligand and negative-feedback mechanisms such as increased expression of PTCH in response to pathway activation. A precise balance of GLI activator (GLI-A) and repressor (GLI-R) forms assures proper development and differentiation. Ligand-independent tumour growth has been shown to arise from loss of PTCH or SuFu repressor function ($PTCH^{loss}$, $SUFU^{loss}$) or activating mutations in the HH-effector SMOH ($SMOH^{act}$). By contrast, prostate, lung and pancreatic cancer have been shown to depend on the presence of HH ligand. These tumours display constitutively elevated pathway activity that is likely to be due to increased ligand (HH^{high}) and SMOH ($SMOHH^{high}$) expression. In this model, both ligand-dependent and ligand-independent tumours are characterised by an increase in GLI-A activity and GLI target gene expression. Possible oncogenic routes activated downstream of HH/GLI such as proliferation, survival, metastasis and stem cell activation are driven by (direct) transcriptional stimulation of key regulators of these processes.

broad spectrum of biological activities controlled by HH/GLI signalling it will not be a big surprise if future studies aiming at the genome-wide identification of direct GLI target genes will unravel a multitude of additional routes by which aberrant HH-signalling contributes to cancer in man.

6. Outlook and potential therapeutic strategies

Studies of Hedgehog-signalling in the past years can be considered a paradigm of how basic research addressing developmental processes in flies and vertebrates can promote and accelerate the development of novel targeted strategies to combat a variety of lethal cancers. It has become clear that constitutive HH/GLI signalling is an etiologic factor in common malignancies including cancers of the lung, prostate, brain, pancreas and skin. Sustained application of specific HH-pathway inhibitors has proven effective in preventing growth of many of these tumours in vitro and in xenografts, suggesting that targeted interference with HH/GLI signalling is a promising approach. Current strategies focus on the use of small molecule inhibitors of SMOH. Given the absolute requirement of SMOH function for HH-signal transduction,[84,85] this is a reasonable yet limited approach, since only tumours where pathway activation has occurred upstream or at the level of SMOH can be treated. Tumours with inactivating mutations in SuFu[26] or other downstream components will most likely not respond to SMOH antagonists. In the future it will be necessary to screen large numbers of tumour samples for mutations in all pathway components to get an estimate of the frequency of genetic events leading to aberrant pathway activity downstream of SMOH.

Keeping in mind the potentially limited efficacy of SMOH inhibition, blocking pathway activity at the distal end, i.e. at the level of the GLI transcription factors would be an ideal strategy for the majority of – if not all – HH-associated cancers. First results using RNAi against GLI1 have already shown that proliferation of prostate tumour cells is significantly reduced by targeted inhibition of GLI1 function.[57] Further, conditional inactivation of Gli2 in murine epidermis is sufficient to trigger complete regression of BCC-like tumours,[51] suggesting that selectively blocking GLI2 and GLI1 function may prove an efficient approach to stop HH-driven cancer growth. However, the molecular details of the regulation of GLI proteins themselves and of HH/GLI target genes are only beginning to emerge. It will require identification of all GLI-interacting proteins as well as sophisticated and thorough studies of how GLI proteins interact with regulatory proteins either in the cytoplasm or in the nucleus. Once a comprehensive picture of these regulatory steps has been established, strategies for selective interference with GLI function can be developed more efficiently. These may rely on blocking for instance the interaction of GLI proteins with critical co-factors or on interfering with nuclear import of latent GLI transcription factors. In the era of robotics and high-throughput screening, the chance to identify potent inhibitors of GLI function is certainly high. Perhaps the most direct and simplest strategy, however, would be to prevent the synthesis of oncogenic GLI proteins by therapeutic RNAi. Although such applications have not yet made their way into clinic, the rapid progress in the RNAi field, particularly in the development of efficient, targeted and sustained siRNA delivery strategies is highly encouraging.[86,87]

Conflict of interest statement

The authors declare that this work does not involve any conflict of interest.

Acknowledgements

Owing to space restrictions, we apologise to those whose work has not been referenced. Our work described in this article was funded by FWF Grants P15618-B14 and P14227-MOB. G.R. is supported by the Austrian Genome Research project GEN-AU.

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