[Frontiers in Bioscience 16, 2259-2270, June 1, 2011]

RAS and Hedgehog - partners in crime

Matthias Lauth

Institute of Molecular Biology and Tumor Research (IMT), Philipps University, 35037 Marburg, Germany

TABLE OF CONTENTS

1. ABSTRACT

Both RAS and Hedgehog (HH) pathway activation can be found in approximately one third of all cancers. In many cases, this activation occurs in the same tumor types, suggesting a positive impact of a simultaneous activation of RAS and HH on tumor development. This review aims to summarize the current knowledge about the molecular and functional crosstalk of RAS and HH signaling in the development of hyperproliferative disease.

2. INTRODUCTION

While cellular signaling pathways were once viewed as a linear cascade of events during the transduction of an extracellular signal into the inside of a cell, it has become clear that this simplistic view has to be exchanged against intricate and densely interconnected signaling networks. Given this increase in complexity, it is the hope of cancer researchers to identify critical nodes within these networks which can be the target of therapeutic

interventions. This review focuses on the *RAS* oncogene as a key driver of malignant transformation and its interactions with the Hedghog signaling cascade.

Mutations in the three *RAS* genes (*HRAS*, *NRAS*, *KRAS*) can be found in approximately one third of all cancers, making them one of the most frequently mutated class of genes in humans (1). A high prevalence of *RAS* mutations can be found in e.g. pancreatic, lung and bladder cancers. These tumors also display features of an activated Hedgehog (HH) signaling pathway (2-4). Although HH signaling activation on its own does not lead to malignant cancer formation in the above organs (5), several reports indicate a positive functional interaction between RAS and HH during carcinogenesis.

3. RAS SIGNALING

RAS proteins are small GTPases which shuttle between a guanosine diphosphate (GDP)-bound inactive and a guanosine triphosphate (GTP)-bound active state (1). The cellular cytosol contains excess GTP and thus RAS is activated by GTP-binding once GDP is displaced from RAS with the help of guanine-nucleotide exchange factors (RAS-GEFs). RAS-GEFs (such as the SOS proteins) are under the control of upstream signaling components like growth factor receptors (6). RAS proteins are inactivated through their intrinsic GTPase activity which is further stimulated by GTPase-activating enzymes (RAS-GAPs). Mutations enhancing the activity of RAS are almost exclusively located at amino acids required for the intrinsic GTPase activity (amino acid positions 12 and 13) and alterations herein result in constitutive GTP binding (6).

Once activated by the binding of GTP, RAS proteins are farnesylated via a CAAX-motif in their C-terminus, which results in anchoring of the RAS protein in cellular membranes. Membrane anchoring is required for full RAS activity.

RAS proteins are a critical relay in the transmission of extracellular signals to intracellular effector molecules. Numerous effector molecules of RAS have been described, of which RAF, PI3K (and to a lesser extent TIAM1/RAL) are the best studied molecules so far (7, 8). Activation of the numerous effector molecules is responsible for the wide range of cellular responses upon RAS stimulation, such as induction of proliferation, enhanced cell survival or cell migration (6).

The human genome contains three *RAS* genes: *HRAS*, *NRAS* and *KRAS*. Germline mutations in these genes are associated with developmental syndromes such as Noonan (mutations in *KRAS* a.o.), Costello (mutations in *HRAS*) or Cardio-facio-cutaneous (CFC, mutations in *KRAS* a.o.) syndrome. Mostly, the mutations found in these syndromes result in moderate activation of the corresponding RAS protein (9).

3.1. RAS and cancer

Given the importance of RAS for the transduction of growth factor signals it is no surprise that activating mutations within *RAS* are associated with hyperproliferative disease. Interestingly, specific cancer types show a preference for the *RAS* gene which is mutated: *KRAS* mutations are the most frequent among the *RAS* family and are prevalent in e.g. pancreatic, lung, colorectal and cervical cancers; *NRAS* mutations can mostly be found in melanoma and *HRAS* mutations are associated with bladder cancer. This distribution was recently attributed to selective anti-differentiation effects of KRAS, but not NRAS or HRAS, on endodermal stem cells (10, 11). Hence, the propensity for *KRAS* mutations in the pancreas, the colon and the lung could be explained by the fact that these organs are endoderm-derived structures. Mutationally activated RAS proteins significantly endow cancer cells with a proliferation and survival advantage and an increased migration and invasion capability compared to normal cells. To some degree this is in contrast to the non-cancerous situation, in which RAS proteins are selectively responsible for proliferation and migration, but not for cell survival (12).

RAS protein levels can be very high in tumors compared to normal tissue. However, frank overexpression of activated RAS in normal cells leads to oncogene-induced cellular senescence due to activation of senescence checkpoints such as INK4A and ARF (13, 14). Given that for some cancers such as mammary and pancreatic cancer, high RAS levels are required for tumor progression, these senescence checkpoints have to be overcome by the cancer cell during the course of the disease. Therefore, RAS expression levels increase over time during carcinogenesis with the parallel inactivation of check point genes such as *TP53 /INK4A/ARF* (13-15).

As mentioned before, RAS activates several downstream effector molecules and an increasing number of these effectors have been implicated in tumor development, such as the RAF-MEK-ERK cascade or the PI3K arm (16, 17). An emerging topic is the importance of the RAL proteins in tumorigenesis and metastasis (18, 19). Clinical attempts to target mutant (oncogenic) RAS by pharmacological inhibition of their farnesylation have been disappointing (20). One reason for the failure might be that KRAS can be geranyl-geranylated in the presence of farnesyltransferase inhibitors, thus suggesting the existence of a bypass mechanism. Therefore, understanding the crosstalk of RAS with other oncogenic pathways might open new avenues in the treatment of cancer.

4. THE HEDGEHOG SIGNALING PATHWAY

The Hedgehog (HH) signaling pathway belongs to one of the few key signal transduction systems critically required for proper embryo formation. Besides its function as neuronal guidance cue and regulator of cellular proliferation, HH signaling is prominently involved in patterning processes. This is best exemplified in the vertebrate neural tube, where a dorso-ventral gradient of HH ligand instructs the positions and identities of neurons to be formed (21).

During development, HH signaling functions as a molecular communicator between two adjacent cell

populations. Usually, the HH ligand (three HH proteins in mammals: Sonic (SHH), Indian (IHH), Desert (DHH) HH) is generated by the producing cell and is released into the extracellular space. Addition of a cholesterol moiety to the N-terminus (through an autoproteolytic activity of the HH C-terminal domain) and a subsequent palmitoylation of the C-terminus of HH ligands (by the acyltransferase HHAT) render them highly lipophilic preventing their wide range diffusion (22). The released HH protein subsequently binds to the PATCHED (PTCH1) receptor on neighboring cells. Binding of HH to PTCH1 initiates a cascade of derepression steps of which the first one is the release of SMOOTHENED (SMO) from PTCH1-mediated inhibition. The interaction of PTCH1 and SMO is complex and not fully understood. It is currently assumed that PTCH1 possesses catalytic activity and transports an endogenous small molecule inhibitor (potentially Vitamin D_3) across the cell membrane which blocks SMO function (23). Pathway regulation at the level of PTCH1 and SMO is confined to a specialized cellular compartment, the primary cilium. This solitary microtubule-containing protrusion of the cell membrane is crucial for HH signal reception and receptor-induced signal transmission. In the absence of HH ligand, PTCH1 resides within the primary cilium while SMO is excluded from it. Ligand binding leads to the exit of PTCH1 from the cilium allowing SMO to enter the cilium and initiate signaling to downstream elements. SMO entry into the primary cilium and SMO activation seem to be two independent steps (24). Signaling of the G-protein coupled receptor-like SMO to its effectors is not very well understood, but involves β-arrestin and G-protein-coupled receptor kinase 2 (GRK2) (25, 26). Ultimately, the downstream effectors GLI2 and GLI3 are activated. In the unliganded state of PTCH1, GLI2 and GLI3 proteins are subject to limited proteolysis giving rise to truncated repressor forms (it should be mentioned that GLI2 processing is very inefficient compared to GLI3) (27, 28). This proteasomal degradation is blocked by HH signaling, allowing full-length GLI3 (to a lesser extent GLI2) to accumulate and activate target gene transcription. GLI target genes are highly cell-type specific. However, certain general HH target genes include *PTCH1*, leading to a negative feedback loop and *GLI1*, resulting in a feed-forward loop amplifying the HH signal and altering the target gene spectrum into a more *GLI1*-directed pattern (29). In contrast to GLI2 and GLI3, GLI1 is an obligate transcriptional activator and because it is a target gene of the pathway, its mRNA levels directly correlate with pathway activity.

A ubiquitously expressed negative regulator of HH signaling is SUPPRESSOR OF FUSED (SUFU). The deletion of the *Sufu* gene in mice results in strong, ligand-independent pathway activation downstream of Ptch1 and Smo (30-32). SUFU can directly bind GLI factors and restrict their localization to the cytoplasm (33). Furthermore, due to its interaction with transcriptional corepressors (SIN3A-SAP18), it can block GLI-mediated transcription within the nucleus (34). Additionally, SUFU recruits GSK3β to full-length GLI3 favoring its truncation into the repressor form (35). The contribution of each of these inhibitory mechanisms to the full SUFU spectrum is currently not exactly clear. Interestingly, SUFU function is independent of the primary cilium (36, 37).

Recently, a so called non-canonical HH pathway induction has been described which utilizes TGFβ and is independent of Smo: TGFβ can directly activate transcription at the GLI2 promoter via SMAD3/β-Catenin in fibroblasts and pancreatic cancer cells (38-40).

4.1. Hedgehog and cancer

Hedgehog signaling was first implicated in cancer development by the discovery that inactivating mutations in the human *PTCH1* gene are the underlying genetic cause for Gorlin syndrome (also known as Nevoid Basal Cell Carcinoma Syndrome (NBCCS)), a developmental syndrome characterized by odontogenic keratocysts, skeletal anomalies and a striking predisposition to the development of basal cell carcinoma (BCC), medulloblastoma (MB) and rhabdomyosarcoma (RMS) (41, 42). It turned out that the second *PTCH1* allele was inactivated by subsequent events and that the resulting activation of HH signaling was causing the tumor development. It is now known that HH pathway activation is also associated with sporadic BCCs, MB and RMS and that in the vast majority of these cases HH pathway activation occurs through mutationally inactivated *PTCH1*, activating mutations in *SMO* or inactivating mutations in *SUFU* (43, 44). Hence, in these tumors pathway activation takes place in the tumor cells themselves.

This mode of pathway activation is in contrast to HH-mediated processes during embryonic development and is different from the situation in other cancer types, where HH signaling has been implicated (45, 46). In prostate and in pancreatic cancer it became evident that the primary signaling mode is paracrine: The tumor cells generate and secrete HH ligands whereas the surrounding stroma constitutes the responding cell population. HH-activated stroma supports tumor growth in a reciprocal manner by secreting growth-promoting factors (WNTs and IGFs in the case of pancreatic cancer; (47)).

In hematological cancers such as chronic myeloid leukemia (CML) and possibly also in multiple myeloma (MM), a reverse situation of paracrine HH signaling has been described where the stroma provides the HH ligand acting on the recipient tumor cells (48, 49). Autocrine HH pathway stimulation (the tumor cell produces and responds to HH) is currently believed to play a role in (cancer) stem cell proliferation and maintenance. Recent evidence points to HH/GLI as an inducer of the self-renewal factor NANOG, thereby promoting stemness (50, 51). The best studied cases with respect to the importance of autocrine HH signaling in tumors are neurological cancers, in particular medulloblastoma and glioblastoma (52).

5. THE RAS-HH CROSSTALK

5.1. Molecular crosstalk between RAS and HH

Data obtained from cotransfection experiments using mutant RAS or its effector molecules such as

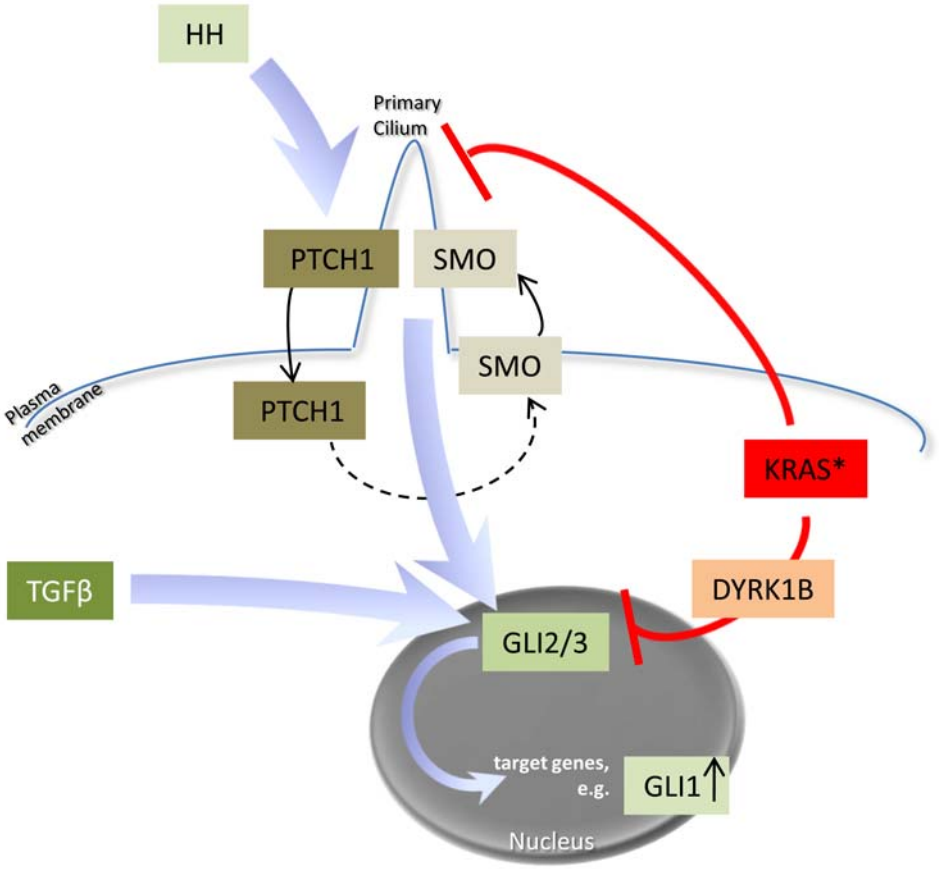

Figure 1. Schematic drawing outlining the inhibitory RAS-HH interactions. The canonical Hedgehog signaling cascade is triggered by binding of the HH ligands to PTCH1, which resides in the primary cilium. Upon HH binding, PTCH1 exits from the cilium and SMO can enter it to initiate signaling, resulting in the activation and subsequent translocation of GLI2 and GLI3 from the cilium into the nucleus. TGFβ can elicit non-canonical (SMO-independent) signaling by directly inducing GLI2 transcription. Mutant RAS (KRAS*) blocks signaling by a.) inhibiting cilium formation and b.) by interfering with GLI2 and GLI3 function.

activated MEK or AKT show that these molecules are able to stimulate GLI function in different cell systems (53-56). At least for GLI1, the N-terminus seems to be the integrator part of the molecule for the activating effects of MEK1 (54), suggesting that MEK or ERK kinase directly phosphorylate this region. Data obtained using melanoma cells demonstrate that active RAS, MEK or AKT increase the nuclear presence of GLI1 (53). Functional synergism between EGF receptor signaling and its downstream effector JUN and GLI1 was also reported (57). In addition, fibroblast growth factor (FGF) enhanced the transcriptional activity of transfected GLI1 in a MEK-dependent manner (54). In line with these results, our laboratory could also observe a synergism between GLI1 and cotransfected RAS in luciferase reporter assays measuring GLI1 activity. Based on these findings we anticipated that knocking down *KRAS* by means of siRNA would decrease the HH signaling activity in cancer cells harboring mutant RAS. Surprisingly however, we were unable to measure a decline in GLI1 activity in RAS-positive PDAC cells when knocking down *KRAS* (58). In contrast to our initial belief, the majority of cell lines responded with an upregulation of the HH target gene *GLI1* to the reduction of *KRAS* levels, implying a suppressive effect of RAS on endogenous HH signaling. In line with these findings, ectopically expressed mutant KRAS inhibited ligand-induced HH signaling in fibroblasts. This negative regulation is, at least partially, mediated by activation of the kinase DYRK1B (see next section for more information on DYRK kinases) and is independent of primary cilia (58). However, in addition to the DYRK1B-mediated effect there is also a cilium-dependent impact of KRAS on HH signaling, at least in pancreatic cancer cells: The mutation of *KRAS* leads to the abrogation of primary cilia, which are crucial for ligand-induced HH signaling (59). Taken together, there seem to be cilia-dependent and cilia-independent negative effects of RAS on HH signaling. A schematic diagram of these findings is shown as Figure 1. In support of these finding of a negative relationship between the RAS and HH pathways, a report by Fogarty et al. described a negative impact of FGF (which is expected to activate Ras) on HH signaling in cerebellar granule cell precursors, medulloblastoma tumor cells and fibroblasts (60).

In summary, the literature provides experimental evidence for both positive as well as negative crosstalk of RAS and the HH pathway. A potential explanation of these seemingly paradoxical results is that the positive functional interaction between RAS and GLI can only be seen in certain situations with high levels of unrestrained GLI (as in e.g. transfection experiments or experimental overexpression). In these settings, endogenous negative regulators are too low in abundance to counteract the stimulatory effects of RAS on GLI. This does not necessarily mean that no (patho)physiological situation exists, in which a positive RAS-HH crosstalk could be seen under endogenous conditions: Loss of *SUFU* or a gene amplification of *GLI1* would presumably sensitize a cell towards the stimularory effects of RAS on GLI.

However, under physiological levels of GLI and RAS and with modulators such as SUFU present, the negative regulation seems more prominent. Since in PDAC cell lines, the RAS-induced HH pathway suppression is mediated by the DYRK1B kinase, the expression levels of such mediators might also determine the final outcome of the RAS-HH crosstalk and might explain the partially contradictory results in the literature.

Most of the currently available studies investigated the influence of 'RAS on HH' signaling. Much less is known about a 'HH to RAS' crosstalk. In some settings, overexpression of *GLI2* or treatment of cells with SHH ligand induced the phosphorylation and thus activation of RAS downstream effectors such as MEK/ERK and/or AKT ((61); (62); (55); (63)). However, since these molecules are also activated by RAS itself, it is unclear what the biological significance of such a HH-induced MEK/AKT activation would be when RAS is simultaneously activated through a mutation. A recent report suggests that SMO interacts and activates TIAM1 and subsequently Rac1 in neuronal cells (64). It will be interesting to see if this finding applies to other cell types as well. Nothing is currently known about the effects of HH signaling on other RAS effector molecules (e.g. RALs, PLCε) or on RAS itself.

5.2. The DYRK family of kinases - mediators of the RAS/HH crosstalk?

The mammalian Dual-specificity tyrosine (Y) – regulated kinase (DYRK) family consists of five members: DYRK1A, DYRK1B, DYRK2, DYRK3 and DYRK4. These kinases possess a tyrosine-directed autophosphorylation specificity which is required for their activation. Upon autophosphorylation and activation, phosphorylation of substrate proteins by DYRK kinases occurs on serines and threonines (hence their name) (65).

The best studied member of the DYRK family is DYRK1A, which is strongly expressed in the brain and which maps to the Down syndrome critical region on chromosome 21q22.2. Hence, DYRK1A is overexpressed in Down syndrome patients and rodents overexpressing DYRK1A display mental defects, suggesting that DYRK1A levels are critical for the development and/or function of the nervous system (66-68).

DYRK1B kinase is strongly expressed in muscle tissue and is important for skeletal muscle cell differentiation where it destabilizes D-type cyclins (69, 70). DYRK2, which is predominantly expressed in immune cells, has been implicated in diverse functions such as calcium signaling, glucose metabolism and gene expression (65). DYRK3 and DYRK4 are not very well characterized and are expressed in erythropoietic cells and sperm, respectively. However, it should be noted that most of the different DYRK isoforms are expressed at low levels in many more tissues than the ones indicated above.

With respect to their role in tumor biology, DYRK1B (also known as Mirk) and DYRK2 are the best studied family members. *DYRK1B* is overexpressed in lung tumors and the gene can be found amplified in pancreatic cancer (71-73). Here, DYRK1B is an effector molecule of oncogenic KRAS and most likely functions as a survival-promoting kinase (72). Comparably, the *DYRK2* gene is amplified in adenocarcinomas of the oesophagus and lung (74).

Interestingly, crosstalk at several levels has been described between members of the DYRK family and the HH pathway: DYRK1A has a positive impact on HH signaling by phosphorylating GLI transcription factors. This phosphorylation leads to nuclear enrichment and increased transcriptional activity (75). DYRK1B and DYRK2 function instead as negative regulators of the HH pathway: While the exact mechanism of how DYRK1B-mediated inhibition is achieved needs further investigation (58), DYRK2 has been shown to act directly on the GLI2 protein leading to its degradation (76). Taken together, at least three out of the five DYRK family members exert an impact on HH signaling, arguing for a close functional interaction between DYRK and HH pathway proteins. At least one DYRK kinase (DYRK1B) is activated by oncogenic RAS. It will be interesting to learn if this applies to other DYRK family members as well and if these kinases contribute to the RAS-HH crosstalk on a broader scale.

5.3. Functional implications of a RAS-HH crosstalk

Numerous reports document a positive functional interaction of the RAS and HH pathways in the process of tumor formation and/or maintenance. Both, KRAS and HH signaling components were identified in a global genomic screen detecting 12 core signaling pathways altered in pancreatic cancer (77). Furthermore, ectopic expression of SHH in the developing mouse pancreas results in precancerous lesions, some of which even harbored a *Kras* mutation (3). Enhanced *Shh* expression was also found to correlate with the *KRAS* mutational status (47, 58) and Shh-positivity of tumor cells was detected in *Kras*-induced mouse models of pancreatic cancer (78). The exact mechanism of *Shh*-induction by RAS is not fully elucidated but most likely involves the activation of NFκB (79).

In addition to the link between RAS and SHH ligand, transgenic overexpression of an activated version of *GLI2* in the pancreatic epithelium led to hyperproliferative

changes. When this transgene was combined with activated *Kras* the phenotype was more severe and the histology showed similarity to human pancreatic intraepithelial neoplasia (PanIn), which are precursor lesions of pancreatic ductal adenocarcinoma (PDAC) (61). However, it should be noted that the alterations obtained with the combined expression of *GLI2* and mutant *Kras* did not fully resemble human PDAC.

From a therapeutic point of view, interesting data were reported using Cyclopamine, a Smo inhibitor, in the treatment of pancreatic cancer (80). In xenograft experiments using human PDAC cells, Cyclopamine treatment had no impact on the growth of the primary tumor, but completely eradicated the growth of metastases. While this result was very interesting from a clinical aspect, the underlying reason why the primary tumor was unaffected became apparent later on: Nolan-Stevaux et al. (2009) genetically deleted epithelial *Smo* in a *Kras*-induced mouse model of PDAC and found that pancreatic epithelial Smo was dispensable for tumor development. In agreement with this report, a complementary approach found that expressing an activated form of Smo (SmoM2) together with mutant Kras selectively in the mouse pancreatic epithelium did not impact on the development of pancreatic neoplasia (81).

Taken together, these data describe a paradigm shift with respect to the mode of HH signaling in tumors harboring RAS mutations: While initial data implied an autocrine scenario in which the tumor cells produced HH ligand and also responded to it, it is now evident that a paracrine signaling mode is best suited to describe the *in vivo* situation. In such a paracrine model, the tumor cells generate and release HH ligands and the surrounding normal stromal cells constitute the responding cell population. In concordance with such a model, expression of the HH target gene *GLI1* can primarily be found associated with stromal cells and is only weakly expressed in the epithelial tumor cells (58). Moreover, in contrast to *Smo-/-* fibroblasts, *Smo+/+* (wildtype) fibroblasts were able to support xenograft growth in mice when being coinjected with the cancer cells. This growth support is most likely mediated by the SHH-induced expression of Wnt and Igf ligands by stromal cells which feed back on the tumor cells in a positive manner (47). Impressive, but unfortunately transient therapeutic results were obtained using a Smo inhibitor in a *Kras*-driven mouse model of PDAC: Realizing the importance of the abundant stroma, Olive et al. (2009) were able to minimize stromal proliferation and thereby normalize the vascularization of the tumor by pharmacological inhibition of stromal Smo. This was used to enhance the perfusion of the tumor tissue with the cytotoxic agent Gemcitabine, which normally is very poorly distributed in the tumor due to a pronounced hypovascularization (82). Collectively, these data strongly suggest an important role for the ligand-induced HH pathway not in the tumor epithelium, but in the surrounding stroma.

The functional implications of RAS/HH interactions are the subject of intense studies. While it would be easy to envision a possible positive interaction between RAS and GLI as a synergistic driver for malignant behavior, interpreting a negative relationship at the molecular level is not so straight forward. I would like to propose two hypotheses about how a RAS-mediated HH suppression could contribute to enhanced tumorigenesis and use PDAC as an example. A schematic diagram depicting these two scenarios is provided as figure 2.

Hypothesis 1: Protection of early stem cells (Figure 2a): It was recently shown that high *GLI1* levels are detrimental to Nestin-positive neural stem cells in the brain (83). Forced expression of *Gli1* resulted in the induction of apoptosis and in cell cycle arrest. In contrast, neural stem cells isolated from medulloblastoma from *Ptch1+/-* animals were resistant to high levels of *Gli1*. Conveying this picture to the development of a RAS-dependent cancer, such as pancreatic cancer, would imply that too high levels of *GLI1* at an early time point would harm stem cells harboring the *KRAS* mutation. In this model, KRAS would prevent the apoptosis of early stem cells and allow for the expansion of cells carrying the *KRAS* mutation. In later disease stages, an increase in GLI1 might be tolerated due to adaptive changes, such as loss of the tumor suppressors *TP53* and *INK4A*.

With respect to the role of GLIs in tumor formation it will be interesting to see how *Gli1 (or Gli2)* depletion affects tumor development in mouse models of pancreatic cancer. Another form of tumorigenic GLI effects through gene repression was recently reported by Kurita et al. (84). In cholangiocarcinoma cells GLI3 represses the TRAIL receptor DR4 and therefore protects from TRAIL-induced apoptosis. Given that KRAS enforces the formation of the GLI3 repressor (58), comparable scenarios could be envisioned for RAS-positive tumors.

Hypothesis 2: KRAS locks pancreatic epithelial cells in a Pdx1-positive precursor state and thus expands the stem cell pool (Figure 2b): Previous reports suggest that, for unknown reasons, the normal pancreatic epithelium is not responsive to HH ligands (5, 81). The responsiveness towards HH seems to change in situations of inflammation: Pancreatic epithelial cells switch on HH signaling upon induction of an acute pancreatitis (85). This correlates with a dedifferentiation process in which the former acinar cells acquire precursor cell characteristics like *Pdx1*- and *Nestin*-positivity. Importantly, blocking Smo function in this animal model *prevented* the redifferentiation of precursor cells into mature acinar cells, showing that HH signaling is required for the last step in this differentiation cycle. Chronic pancreatitis is one of the risk factors for the development of PDAC (86) and therefore a certain percentage of dedifferentiated precursor cells can be assumed to exist in these patients. If a *KRAS* mutation occurs in this situation, it would be predicted to suppress HH signaling and thus block the redifferentiation into mature acinar cells. The epithelial cells would thus be locked in a *PDX-1*-positive precursor state. Indeed, *PDX-1* expression as well as gene expression signatures indicative of precursor states can frequently be found in PDAC (86-88).

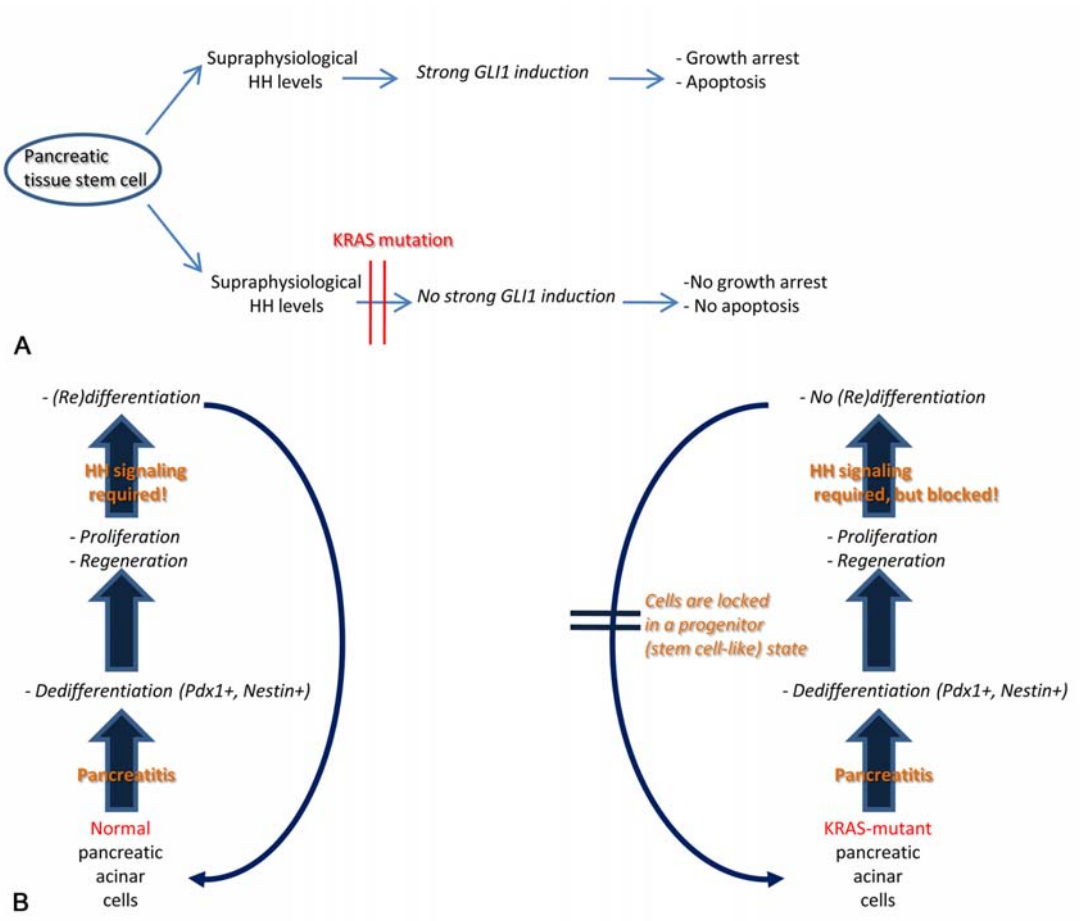

Figure 2. Hypothetical models on the functional role of HH pathway suppression in PDAC development. Model 1: Inflammatory processes and a KRAS mutation induce the generation of high HH levels around pancreatic tissue stem cells. Since high GLI1 expression has been shown to harm neural stem cells, one prediction of a KRAS-mediated HH suppression would be that tissue stem cells are protected from GLI1high-induced growth arrest and apoptosis. Model 2: During inflammation (pancreatitis), pancreatic acini dedifferentiate into Pdx1- and Nestin-positive progenitor cells. In addition, HH signaling is activated in the epithelial compartment where it is specifically required for the redifferentiation of progenitor cells into mature acini. A KRAS mutation could block this redifferentiation step and arrest cells in a progenitor (stem cell-like) state, thus expanding the stem cell pool.

Taken together, the concept underlying these hypotheses is that the *suppression* of an oncogene (i.e. *GLI1*) could in theory be resulting in pro-cancerous alterations. On the other hand it should be noted that *GLI* has been shown to drive proliferation and protect cancer cells from apoptosis *in vitro* (40, 61). Therefore, it will be interesting to learn how cancer cells adjust their *GLI* levels or if mechanisms for a variable modulation of *GLI* levels in distinct phases of carcinogenesis exist.

6. SUMMARY

There is currently very little doubt that Hedgehog (HH) pathway activation occurs in RAS-dependent tumorigenesis of e.g. the pancreas, the lung and the colon. However, only recently it became clear that the prime target cell population for the tumor cell-secreted HH ligands is not the cancer cell compartment but the stroma. Conflicting literature data exist about the molecular crosstalk of RAS and HH pathway components within the cancer cells. Data derived from endogenous *GLI* activity in pancreatic cancer cell lines and from a PDAC mouse model strongly suggest a negative regulation of HH activity by mutant RAS (89). This contrasts with the induction of SHH expression and activation of signaling in the stroma by mutant RAS. Hence, RAS diverts the HH pathway from the cancer cell to the stromal compartment. The hypothetical functional consequences of this unequal distribution of HH/GLI activity await experimental confirmation.

7. PERSPECTIVE

The *RAS* oncogene has fascinated cancer scientists for over 30 years but even today the entire spectrum of actions that this molecule can exert are not fully understood. This includes for instance the crosstalk mechanisms of RAS with other signaling entities, such as the Hedgehog pathway. Understanding these molecular

connections in greater detail will be of utmost importance for a directed pharmacological intervention of RAS-driven malignancies in the future. Only if one knows which wheels to turn will it be feasible to successfully impinge on disease development and progression. With respect to the RAS-HH crosstalk the open issues include the positive versus negative regulation of HH/GLI signaling by RAS, the characterization of the cell types in which such a regulation occurs and the possible non-canonical crosstalk levels between the two signaling cascades.

8. ACKNOWLEDGEMENTS

I would like to apologize to all researchers in the field whose work could not be addressed in this review. Work in the Lauth group is supported by grants from the DFG and by LOEWE (Tumor & Inflammation).

9. REFERENCES

1. M. Malumbres and M. Barbacid: RAS oncogenes: the first 30 years. *Nat Rev Cancer*, 3(6), 459-65 (2003)

2. D. N. Watkins, D. M. Berman, S. G. Burkholder, B. Wang, P. A. Beachy and S. B. Baylin: Hedgehog signalling within airway epithelial progenitors and in small-cell lung cancer. *Nature*, 422(6929), 313-7 (2003)

3. S. P. Thayer, M. P. di Magliano, P. W. Heiser, C. M. Nielsen, D. J. Roberts, G. Y. Lauwers, Y. P. Qi, S. Gysin, C. Fernandez-del Castillo, V. Yajnik, B. Antoniu, M. McMahon, A. L. Warshaw and M. Hebrok: Hedgehog is an early and late mediator of pancreatic cancer tumorigenesis. *Nature*, 425(6960), 851-6 (2003)

4. C. W. Mechlin, M. J. Tanner, M. Chen, R. Buttyan, R. M. Levin and B. M. Mian: Gli2 expression and human bladder transitional carcinoma cell invasiveness. *J Urol*, 184(1), 344-51 (2010)

5. J. Mao, K. L. Ligon, E. Y. Rakhlin, S. P. Thayer, R. T. Bronson, D. Rowitch and A. P. McMahon: A novel somatic mouse model to survey tumorigenic potential applied to the Hedgehog pathway. *Cancer Res*, 66(20), 10171-8 (2006)

6. A. E. Karnoub and R. A. Weinberg: Ras oncogenes: split personalities. *Nat Rev Mol Cell Biol*, 9(7), 517-31 (2008)

7. A. R. Ramjaun and J. Downward: Ras and phosphoinositide 3-kinase: partners in development and tumorigenesis. *Cell Cycle*, 6(23), 2902-5 (2007)

8. A. Young, J. Lyons, A. L. Miller, V. T. Phan, I. R. Alarcon and F. McCormick: Ras signaling and therapies. *Adv Cancer Res*, 102, 1-17 (2009)

9. S. Schubbert, K. Shannon and G. Bollag: Hyperactive Ras in developmental disorders and cancer. *Nat Rev Cancer*, 7(4), 295-308 (2007)

10. M. P. Quinlan, S. E. Quatela, M. R. Philips and J. Settleman: Activated Kras, but not Hras or Nras, may initiate tumors of endodermal origin via stem cell expansion. *Mol Cell Biol*, 28(8), 2659-74 (2008)

11. K. M. Haigis, K. R. Kendall, Y. Wang, A. Cheung, M. C. Haigis, J. N. Glickman, M. Niwa-Kawakita, A. Sweet-Cordero, J. Sebolt-Leopold, K. M. Shannon, J. Settleman, M. Giovannini and T. Jacks: Differential effects of oncogenic K-Ras and N-Ras on proliferation, differentiation and tumor progression in the colon. *Nat Genet*, 40(5), 600-8 (2008)

12. M. Drosten, A. Dhawahir, E. Y. Sum, J. Urosevic, C. G. Lechuga, L. M. Esteban, E. Castellano, C. Guerra, E. Santos and M. Barbacid: Genetic analysis of Ras signalling pathways in cell proliferation, migration and survival. *Embo J*, 29(6), 1091-104 (2010)

13. C. J. Sarkisian, B. A. Keister, D. B. Stairs, R. B. Boxer, S. E. Moody and L. A. Chodosh: Dose-dependent oncogene-induced senescence in vivo and its evasion during mammary tumorigenesis. *Nat Cell Biol*, 9(5), 493-505 (2007)

14. B. Ji, L. Tsou, H. Wang, S. Gaiser, D. Z. Chang, J. Daniluk, Y. Bi, T. Grote, D. S. Longnecker and C. D. Logsdon: Ras activity levels control the development of pancreatic diseases. *Gastroenterology*, 137(3), 1072-82, 1082 e1-6 (2009)

15. C. Guerra, A. J. Schuhmacher, M. Canamero, P. J. Grippo, L. Verdaguer, L. Perez-Gallego, P. Dubus, E. P. Sandgren and M. Barbacid: Chronic pancreatitis is essential for induction of pancreatic ductal adenocarcinoma by K-Ras oncogenes in adult mice. *Cancer Cell*, 11(3), 291-302 (2007)

16. K. H. Lim, B. B. Ancrile, D. F. Kashatus and C. M. Counter: Tumour maintenance is mediated by eNOS. *Nature*, 452(7187), 646-9 (2008)

17. Y. Mebratu and Y. Tesfaigzi: How ERK1/2 activation controls cell proliferation and cell death: Is subcellular localization the answer? *Cell Cycle*, 8(8), 1168-75 (2009)

18. K. H. Lim, A. T. Baines, J. J. Fiordalisi, M. Shipitsin, L. A. Feig, A. D. Cox, C. J. Der and C. M. Counter: Activation of RalA is critical for Ras-induced tumorigenesis of human cells. *Cancer Cell*, 7(6), 533-45 (2005)

19. G. Feldmann, A. Mishra, S. M. Hong, S. Bisht, C. J. Strock, D. W. Ball, M. Goggins, A. Maitra and B. D. Nelkin: Inhibiting the cyclin-dependent kinase CDK5 blocks pancreatic cancer formation and progression through the suppression of Ras-Ral signaling. *Cancer Res*, 70(11), 4460-9 (2010)

20. P. A. Konstantinopoulos, M. V. Karamouzis and A. G. Papavassiliou: Post-translational modifications and regulation of the RAS superfamily of GTPases as anticancer targets. *Nat Rev Drug Discov*, 6(7), 541-55 (2007)

21. E. Dessaud, A. P. McMahon and J. Briscoe: Pattern formation in the vertebrate neural tube: a sonic hedgehog morphogen-regulated transcriptional network. *Development*, 135(15), 2489-503 (2008)

22. J. A. Porter, K. E. Young and P. A. Beachy: Cholesterol modification of hedgehog signaling proteins in animal development. *Science*, 274(5285), 255-9 (1996)

23. M. F. Bijlsma, C. A. Spek, D. Zivkovic, S. van de Water, F. Rezaee and M. P. Peppelenbosch: Repression of smoothened by patched-dependent (pro-)vitamin D3 secretion. *PLoS Biol*, 4(8), e232 (2006)

24. Y. Wang, Z. Zhou, C. T. Walsh and A. P. McMahon: Selective translocation of intracellular Smoothened to the primary cilium in response to Hedgehog pathway modulation. *Proc Natl Acad Sci U S A*, 106(8), 2623-8 (2009)

25. W. Chen, X. R. Ren, C. D. Nelson, L. S. Barak, J. K. Chen, P. A. Beachy, F. de Sauvage and R. J. Lefkowitz: Activity-dependent internalization of smoothened mediated by beta-arrestin 2 and GRK2. *Science*, 306(5705), 2257-60 (2004)

26. A. R. Meloni, G. B. Fralish, P. Kelly, A. Salahpour, J. K. Chen, R. J. Wechsler-Reya, R. J. Lefkowitz and M. G. Caron: Smoothened signal transduction is promoted by G protein-coupled receptor kinase 2. *Mol Cell Biol*, 26(20), 7550-60 (2006)

27. B. Wang, J. F. Fallon and P. A. Beachy: Hedgehog-regulated processing of Gli3 produces an anterior/posterior repressor gradient in the developing vertebrate limb. *Cell*, 100(4), 423-34 (2000)

28. Y. Pan, C. B. Bai, A. L. Joyner and B. Wang: Sonic hedgehog signaling regulates Gli2 transcriptional activity by suppressing its processing and degradation. *Mol Cell Biol*, 26(9), 3365-77 (2006)

29. T. Eichberger, V. Sander, H. Schnidar, G. Regl, M. Kasper, C. Schmid, S. Plamberger, A. Kaser, F. Aberger and A. M. Frischauf: Overlapping and distinct transcriptional regulator properties of the GLI1 and GLI2 oncogenes. *Genomics*, 87(5), 616-32 (2006)

30. J. Svard, K. Heby-Henricson, M. Persson-Lek, B. Rozell, M. Lauth, A. Bergstrom, J. Ericson, R. Toftgard and S. Teglund: Genetic elimination of Suppressor of fused reveals an essential repressor function in the mammalian Hedgehog signaling pathway. *Dev Cell*, 10(2), 187-97 (2006)

31. M. Varjosalo, S. P. Li and J. Taipale: Divergence of hedgehog signal transduction mechanism between Drosophila and mammals. *Dev Cell*, 10(2), 177-86 (2006)

32. A. F. Cooper, K. P. Yu, M. Brueckner, L. L. Brailey, L. Johnson, J. M. McGrath and A. E. Bale: Cardiac and CNS defects in a mouse with targeted disruption of suppressor of fused. *Development*, 132(19), 4407-17 (2005)

33. P. Kogerman, T. Grimm, L. Kogerman, D. Krause, A. B. Unden, B. Sandstedt, R. Toftgard and P. G. Zaphiropoulos: Mammalian suppressor-of-fused modulates nuclear-cytoplasmic shuttling of Gli-1. *Nat Cell Biol*, 1(5), 312-9 (1999)

34. S. Y. Cheng and J. M. Bishop: Suppressor of Fused represses Gli-mediated transcription by recruiting the SAP18-mSin3 corepressor complex. *Proc Natl Acad Sci U S A*, 99(8), 5442-7 (2002)

35. Y. Kise, A. Morinaka, S. Teglund and H. Miki: Sufu recruits GSK3beta for efficient processing of Gli3. *Biochem Biophys Res Commun*, 387(3), 569-74 (2009)

36. J. Jia, A. Kolterud, H. Zeng, A. Hoover, S. Teglund, R. Toftgard and A. Liu: Suppressor of Fused inhibits mammalian Hedgehog signaling in the absence of cilia. *Dev Biol*, 330(2), 452-60 (2009)

37. M. H. Chen, C. W. Wilson, Y. J. Li, K. K. Law, C. S. Lu, R. Gacayan, X. Zhang, C. C. Hui and P. T. Chuang: Cilium-independent regulation of Gli protein function by Sufu in Hedgehog signaling is evolutionarily conserved. *Genes Dev*, 23(16), 1910-28 (2009)

38. S. Dennler, J. Andre, I. Alexaki, A. Li, T. Magnaldo, P. ten Dijke, X. J. Wang, F. Verrecchia and A. Mauviel: Induction of sonic hedgehog mediators by transforming growth factor-beta: Smad3-dependent activation of Gli2 and Gli1 expression in vitro and in vivo. *Cancer Res*, 67(14), 6981-6 (2007)

39. S. Dennler, J. Andre, F. Verrecchia and A. Mauviel: Cloning of the human GLI2 Promoter: transcriptional activation by transforming growth factor-beta via SMAD3/beta-catenin cooperation. *J Biol Chem*, 284(46), 31523-31 (2009)

40. O. Nolan-Stevaux, J. Lau, M. L. Truitt, G. C. Chu, M. Hebrok, M. E. Fernandez-Zapico and D. Hanahan: GLI1 is regulated through Smoothened-independent mechanisms in neoplastic pancreatic ducts and mediates PDAC cell survival and transformation. *Genes Dev*, 23(1), 24-36 (2009)

41. H. Hahn, C. Wicking, P. G. Zaphiropoulous, M. R. Gailani, S. Shanley, A. Chidambaram, I. Vorechovsky, E. Holmberg, A. B. Unden, S. Gillies, K. Negus, I. Smyth, C. Pressman, D. J. Leffell, B. Gerrard, A. M. Goldstein, M. Dean, R. Toftgard, G. Chenevix-Trench, B. Wainwright and A. E. Bale: Mutations of the human homolog of Drosophila patched in the nevoid basal cell carcinoma syndrome. *Cell*, 85(6), 841-51 (1996)

42. R. L. Johnson, A. L. Rothman, J. Xie, L. V. Goodrich, J. W. Bare, J. M. Bonifas, A. G. Quinn, R. M. Myers, D. R. Cox, E. H. Epstein, Jr. and M. P. Scott: Human homolog of

patched, a candidate gene for the basal cell nevus syndrome. *Science*, 272(5268), 1668-71 (1996)

43. M. D. Taylor, L. Liu, C. Raffel, C. C. Hui, T. G. Mainprize, X. Zhang, R. Agatep, S. Chiappa, L. Gao, A. Lowrance, A. Hao, A. M. Goldstein, T. Stavrou, S. W. Scherer, W. T. Dura, B. Wainwright, J. A. Squire, J. T. Rutka and D. Hogg: Mutations in SUFU predispose to medulloblastoma. *Nat Genet*, 31(3), 306-10 (2002)

44. J. Xie, M. Murone, S. M. Luoh, A. Ryan, Q. Gu, C. Zhang, J. M. Bonifas, C. W. Lam, M. Hynes, A. Goddard, A. Rosenthal, E. H. Epstein, Jr. and F. J. de Sauvage: Activating Smoothened mutations in sporadic basal-cell carcinoma. *Nature*, 391(6662), 90-2 (1998)

45. S. Teglund and R. Toftgard: Hedgehog beyond medulloblastoma and basal cell carcinoma. *Biochim Biophys Acta*, 1805(2), 181-208 (2010)

46. S. J. Scales and F. J. de Sauvage: Mechanisms of Hedgehog pathway activation in cancer and implications for therapy. *Trends Pharmacol Sci*, 30(6), 303-12 (2009)

47. R. L. Yauch, S. E. Gould, S. J. Scales, T. Tang, H. Tian, C. P. Ahn, D. Marshall, L. Fu, T. Januario, D. Kallop, M. Nannini-Pepe, K. Kotkow, J. C. Marsters, L. L. Rubin and F. J. de Sauvage: A paracrine requirement for hedgehog signalling in cancer. *Nature*, 455(7211), 406-10 (2008)

48. C. Zhao, A. Chen, C. H. Jamieson, M. Fereshteh, A. Abrahamsson, J. Blum, H. Y. Kwon, J. Kim, J. P. Chute, D. Rizzieri, M. Munchhof, T. VanArsdale, P. A. Beachy and T. Reya: Hedgehog signalling is essential for maintenance of cancer stem cells in myeloid leukaemia. *Nature*, 458(7239), 776-9 (2009)

49. C. D. Peacock, Q. Wang, G. S. Gesell, I. M. Corcoran-Schwartz, E. Jones, J. Kim, W. L. Devereux, J. T. Rhodes, C. A. Huff, P. A. Beachy, D. N. Watkins and W. Matsui: Hedgehog signaling maintains a tumor stem cell compartment in multiple myeloma. *Proc Natl Acad Sci U S A*, 104(10), 4048-53 (2007)

50. A. Po, E. Ferretti, E. Miele, E. De Smaele, A. Paganelli, G. Canettieri, S. Coni, L. Di Marcotullio, M. Biffoni, L. Massimi, C. Di Rocco, I. Screpanti and A. Gulino: Hedgehog controls neural stem cells through p53-independent regulation of Nanog. *Embo J*, 29(15), 2646-58 (2010)

51. M. Zbinden, A. Duquet, A. Lorente-Trigos, S. N. Ngwabyt, I. Borges and A. Ruiz i Altaba: NANOG regulates glioma stem cells and is essential in vivo acting in a cross-functional network with GLI1 and p53. *Embo J*, 29(15), 2659-74 (2010)

52. V. Clement, P. Sanchez, N. de Tribolet, I. Radovanovic and A. Ruiz i Altaba: HEDGEHOG-GLI1 signaling regulates human glioma growth, cancer stem cell self-renewal, and tumorigenicity. *Curr Biol*, 17(2), 165-72 (2007)

53. B. Stecca, C. Mas, V. Clement, M. Zbinden, R. Correa, V. Piguet, F. Beermann and I. A. A. Ruiz: Melanomas require HEDGEHOG-GLI signaling regulated by interactions between GLI1 and the RAS-MEK/AKT pathways. *Proc Natl Acad Sci U S A*, 104(14), 5895-900 (2007)

54. N. A. Riobo, G. M. Haines and C. P. Emerson, Jr.: Protein kinase C-delta and mitogen-activated protein/extracellular signal-regulated kinase-1 control GLI activation in hedgehog signaling. *Cancer Res*, 66(2), 839-45 (2006)

55. N. A. Riobo, K. Lu, X. Ai, G. M. Haines and C. P. Emerson, Jr.: Phosphoinositide 3-kinase and Akt are essential for Sonic Hedgehog signaling. *Proc Natl Acad Sci U S A*, 103(12), 4505-10 (2006)

56. M. Seto, M. Ohta, Y. Asaoka, T. Ikenoue, M. Tada, K. Miyabayashi, D. Mohri, Y. Tanaka, H. Ijichi, K. Tateishi, F. Kanai, T. Kawabe and M. Omata: Regulation of the hedgehog signaling by the mitogen-activated protein kinase cascade in gastric cancer. *Mol Carcinog*, 48(8), 703-12 (2009)

57. H. Schnidar, M. Eberl, S. Klingler, D. Mangelberger, M. Kasper, C. Hauser-Kronberger, G. Regl, R. Kroismayr, R. Moriggl, M. Sibilia and F. Aberger: Epidermal growth factor receptor signaling synergizes with Hedgehog/GLI in oncogenic transformation via activation of the MEK/ERK/JUN pathway. *Cancer Res*, 69(4), 1284-92 (2009)

58. M. Lauth, A. Bergstrom, T. Shimokawa, U. Tostar, Q. Jin, V. Fendrich, C. Guerra, M. Barbacid and R. Toftgard: DYRK1B-dependent autocrine-to-paracrine shift of Hedgehog signaling by mutant RAS. *Nat Struct Mol Biol* 17(6), 718-25 (2010)

59. E. S. Seeley, C. Carriere, T. Goetze, D. S. Longnecker and M. Korc: Pancreatic cancer and precursor pancreatic intraepithelial neoplasia lesions are devoid of primary cilia. *Cancer Res*, 69(2), 422-30 (2009)

60. M. P. Fogarty, B. A. Emmenegger, L. L. Grasfeder, T. G. Oliver and R. J. Wechsler-Reya: Fibroblast growth factor blocks Sonic hedgehog signaling in neuronal precursors and tumor cells. *Proc Natl Acad Sci U S A*, 104(8), 2973-8 (2007)

61. M. Pasca di Magliano, S. Sekine, A. Ermilov, J. Ferris, A. A. Dlugosz and M. Hebrok: Hedgehog/Ras interactions regulate early stages of pancreatic cancer. *Genes Dev*, 20(22), 3161-73 (2006)

62. J. P. Morton, M. E. Mongeau, D. S. Klimstra, J. P. Morris, Y. C. Lee, Y. Kawaguchi, C. V. Wright, M. Hebrok and B. C. Lewis: Sonic hedgehog acts at multiple stages

during pancreatic tumorigenesis. *Proc Natl Acad Sci U S A*, 104(12), 5103-8 (2007)

63. H. Chang, Q. Li, R. C. Moraes, M. T. Lewis and P. A. Hamel: Activation of Erk by sonic hedgehog independent of canonical hedgehog signalling. *Int J Biochem Cell Biol* (2010)

64. N. Sasaki, J. Kurisu and M. Kengaku: Sonic hedgehog signaling regulates actin cytoskeleton via Tiam1-Rac1 cascade during spine formation. *Mol Cell Neurosci* (2010)

65. K. Yoshida: Role for DYRK family kinases on regulation of apoptosis. *Biochem Pharmacol*, 76(11), 1389-94 (2008)

66. J. Park, W. J. Song and K. C. Chung: Function and regulation of Dyrk1A: towards understanding Down syndrome. *Cell Mol Life Sci*, 66(20), 3235-40 (2009)

67. V. Fotaki, M. Dierssen, S. Alcantara, S. Martinez, E. Marti, C. Casas, J. Visa, E. Soriano, X. Estivill and M. L. Arbones: Dyrk1A haploinsufficiency affects viability and causes developmental delay and abnormal brain morphology in mice. *Mol Cell Biol*, 22(18), 6636-47 (2002)

68. X. Altafaj, M. Dierssen, C. Baamonde, E. Marti, J. Visa, J. Guimera, M. Oset, J. R. Gonzalez, J. Florez, C. Fillat and X. Estivill: Neurodevelopmental delay, motor abnormalities and cognitive deficits in transgenic mice overexpressing Dyrk1A (minibrain), a murine model of Down's syndrome. *Hum Mol Genet*, 10(18), 1915-23 (2001)

69. X. Deng, D. Z. Ewton, B. Pawlikowski, M. Maimone and E. Friedman: Mirk/dyrk1B is a Rho-induced kinase active in skeletal muscle differentiation. *J Biol Chem*, 278(42), 41347-54 (2003)

70. S. E. Mercer, D. Z. Ewton, X. Deng, S. Lim, T. R. Mazur and E. Friedman: Mirk/Dyrk1B mediates survival during the differentiation of C2C12 myoblasts. *J Biol Chem*, 280(27), 25788-801 (2005)

71. E. Friedman: Mirk/Dyrk1B in cancer. *J Cell Biochem*, 102(2), 274-9 (2007)

72. K. Jin, S. Park, D. Z. Ewton and E. Friedman: The survival kinase Mirk/Dyrk1B is a downstream effector of oncogenic K-ras in pancreatic cancer. *Cancer Res*, 67(15), 7247-55 (2007)

73. X. Deng, D. Z. Ewton, S. Li, A. Naqvi, S. E. Mercer, S. Landas and E. Friedman: The kinase Mirk/Dyrk1B mediates cell survival in pancreatic ductal adenocarcinoma. *Cancer Res*, 66(8), 4149-58 (2006)

74. C. T. Miller, S. Aggarwal, T. K. Lin, S. L. Dagenais, J. I. Contreras, M. B. Orringer, T. W. Glover, D. G. Beer and L. Lin: Amplification and overexpression of the dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2 (DYRK2) gene in esophageal and lung adenocarcinomas. *Cancer Res*, 63(14), 4136-43 (2003)

75. J. Mao, P. Maye, P. Kogerman, F. J. Tejedor, R. Toftgard, W. Xie, G. Wu and D. Wu: Regulation of Gli1 transcriptional activity in the nucleus by Dyrk1. *J Biol Chem*, 277(38), 35156-61 (2002)

76. M. Varjosalo, M. Bjorklund, F. Cheng, H. Syvanen, T. Kivioja, S. Kilpinen, Z. Sun, O. Kallioniemi, H. G. Stunnenberg, W. W. He, P. Ojala and J. Taipale: Application of active and kinase-deficient kinome collection for identification of kinases regulating hedgehog signaling. *Cell*, 133(3), 537-48 (2008)

77. S. Jones, X. Zhang, D. W. Parsons, J. C. Lin, R. J. Leary, P. Angenendt, P. Mankoo, H. Carter, H. Kamiyama, A. Jimeno, S. M. Hong, B. Fu, M. T. Lin, E. S. Calhoun, M. Kamiyama, K. Walter, T. Nikolskaya, Y. Nikolsky, J. Hartigan, D. R. Smith, M. Hidalgo, S. D. Leach, A. P. Klein, E. M. Jaffee, M. Goggins, A. Maitra, C. Iacobuzio-Donahue, J. R. Eshleman, S. E. Kern, R. H. Hruban, R. Karchin, N. Papadopoulos, G. Parmigiani, B. Vogelstein, V. E. Velculescu and K. W. Kinzler: Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. *Science*, 321(5897), 1801-6 (2008)

78. S. R. Hingorani, L. Wang, A. S. Multani, C. Combs, T. B. Deramaudt, R. H. Hruban, A. K. Rustgi, S. Chang and D. A. Tuveson: Trp53R172H and KrasG12D cooperate to promote chromosomal instability and widely metastatic pancreatic ductal adenocarcinoma in mice. *Cancer Cell*, 7(5), 469-83 (2005)

79. H. Nakashima, M. Nakamura, H. Yamaguchi, N. Yamanaka, T. Akiyoshi, K. Koga, K. Yamaguchi, M. Tsuneyoshi, M. Tanaka and M. Katano: Nuclear factor-kappaB contributes to hedgehog signaling pathway activation through sonic hedgehog induction in pancreatic cancer. *Cancer Res*, 66(14), 7041-9 (2006)

80. G. Feldmann, S. Dhara, V. Fendrich, D. Bedja, R. Beaty, M. Mullendore, C. Karikari, H. Alvarez, C. Iacobuzio-Donahue, A. Jimeno, K. L. Gabrielson, W. Matsui and A. Maitra: Blockade of hedgehog signaling inhibits pancreatic cancer invasion and metastases: a new paradigm for combination therapy in solid cancers. *Cancer Res*, 67(5), 2187-96 (2007)

81. H. Tian, C. A. Callahan, K. J. DuPree, W. C. Darbonne, C. P. Ahn, S. J. Scales and F. J. de Sauvage: Hedgehog signaling is restricted to the stromal compartment during pancreatic carcinogenesis. *Proc Natl Acad Sci U S A*, 106(11), 4254-9 (2009)

82. K. P. Olive, M. A. Jacobetz, C. J. Davidson, A. Gopinathan, D. McIntyre, D. Honess, B. Madhu, M. A. Goldgraben, M. E. Caldwell, D. Allard, K. K. Frese, G. Denicola, C. Feig, C. Combs, S. P. Winter, H. Ireland-Zecchini, S. Reichelt, W. J. Howat, A. Chang, M. Dhara, L. Wang, F. Ruckert, R. Grutzmann, C. Pilarsky, K. Izeradjene, S. R. Hingorani, P. Huang, S. E. Davies, W.

Plunkett, M. Egorin, R. H. Hruban, N. Whitebread, K. McGovern, J. Adams, C. Iacobuzio-Donahue, J. Griffiths and D. A. Tuveson: Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. *Science*, 324(5933), 1457-61 (2009)

83. K. E. Galvin, H. Ye, D. J. Erstad, R. Feddersen and C. Wetmore: Gli1 Induces G2/M Arrest and Apoptosis in Hippocampal but not Tumor-Derived Neural Stem Cells. *Stem Cells*, 14, 14 (2008)

84. S. Kurita, J. L. Mott, L. L. Almada, S. F. Bronk, N. W. Werneburg, S. Y. Sun, L. R. Roberts, M. E. Fernandez-Zapico and G. J. Gores: GLI3-dependent repression of DR4 mediates hedgehog antagonism of TRAIL-induced apoptosis. *Oncogene* (2010)

85. V. Fendrich, F. Esni, M. V. Garay, G. Feldmann, N. Habbe, J. N. Jensen, Y. Dor, D. Stoffers, J. Jensen, S. D. Leach and A. Maitra: Hedgehog signaling is required for effective regeneration of exocrine pancreas. *Gastroenterology*, 135(2), 621-31 (2008)

86. A. F. Hezel, A. C. Kimmelman, B. Z. Stanger, N. Bardeesy and R. A. Depinho: Genetics and biology of pancreatic ductal adenocarcinoma. *Genes Dev*, 20(10), 1218-49 (2006)

87. K. Quint, S. Stintzing, B. Alinger, C. Hauser-Kronberger, O. Dietze, S. Gahr, E. G. Hahn, M. Ocker and D. Neureiter: The Expression Pattern of PDX-1, SHH, Patched and Gli-1 Is Associated with Pathological and Clinical Features in Human Pancreatic Cancer. *Pancreatology*, 9(1-2), 116-126 (2008)

88. N. B. Prasad, A. V. Biankin, N. Fukushima, A. Maitra, S. Dhara, A. G. Elkahloun, R. H. Hruban, M. Goggins and S. D. Leach: Gene expression profiles in pancreatic intraepithelial neoplasia reflect the effects of Hedgehog signaling on pancreatic ductal epithelial cells. *Cancer Res*, 65(5), 1619-26 (2005)

89. M. Lauth, A. Bergstrom, T. Shimokawa, U. Tostar, Q. Jin, V. Fendrich, C. Guerra, M. Barbacid and R. Toftgard: DYRK1B-dependent autocrine-to-paracrine shift of Hedgehog signaling by mutant RAS. *Nat Struct Mol Biol*, 17(6), 718-25 (2010)

Key Words: Molecular Biology, Cancer Biology, Hedgehog signaling, GLI, RAS, KRAS, signaling crosstalk, Review

Send correspondence to: Matthias Lauth, Institute of Molecular Biology and Tumor Research (IMT), Philipps University, 35037 Marburg, Germany, Tel: 49-0-6421-2866727, Fax: 49-0-6421-2865932, E- mail: lauth@imt.uni-marburg.de