Biochemical and Biophysical Research Communications 425 (2012) 64–69

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Hedgehog pathway regulators influence cervical cancer cell proliferation, survival and migration

Ivana Samarzija, Peter Beard *

Ecole Polytechnique Fédérale Lausanne (EPFL), Department of Life Sciences, Swiss Institute for Experimental Cancer Research (ISREC), 1015 Lausanne, Switzerland

ARTICLE INFO

Article history:
Received 10 July 2012
Available online 20 July 2012

Keywords:
Cervical cancer
Hedgehog signaling
Proliferation
Apoptosis
Papillomavirus

ABSTRACT

Human papillomavirus (HPV) infection is considered to be a primary hit that causes cervical cancer. However, infection with this agent, although needed, is not sufficient for a cancer to develop. Additional cellular changes are required to complement the action of HPV, but the precise nature of these changes is not clear. Here, we studied the function of the Hedgehog (Hh) signaling pathway in cervical cancer. The Hh pathway can have a role in a number of cancers, including those of liver, lung and digestive tract. We found that components of the Hh pathway are expressed in several cervical cancer cell lines, indicating that there could exist an autocrine Hh signaling loop in these cells. Inhibition of Hh signaling reduces proliferation and survival of the cervical cancer cells and induces their apoptosis as seen by the up-regulation of the pro-apoptotic protein cleaved caspase 3. Our results indicate that Hh signaling is not induced directly by HPV-encoded proteins but rather that Hh-activating mutations are selected in cells initially immortalized by HPV. Sonic Hedgehog (Shh) ligand induces proliferation and promotes migration of the cervical cancer cells studied. Together, these results indicate pro-survival and protective roles of an activated Hh signaling pathway in cervical cancer-derived cells, and suggest that inhibition of this pathway may be a therapeutic option in fighting cervical cancer.

1. Introduction

Cervical cancer is the second most common malignancy among women worldwide [1]. Although the incidence of this disease is slowly decreasing in developed countries due to early diagnosis, it remains an important health issue in developing countries where diagnostic programs are still not well established. The primary hit in the etiology of cervical cancer is infection by HPV, a small non-enveloped virus of the family of papillomaviruses. While the presence of HPV is detected in 99% of all cases [2], infection with this agent is not sufficient to cause cancer. Additional cellular changes are needed to bring about complete cellular transformation and carcinogenesis. In order to identify these cellular changes, we investigated the role of the Hedgehog (Hh) signaling pathway in cervical cancer.

The Hh pathway is one of the core developmental signaling pathways whose mutation during development causes congenital malformations [3]. The pathway is activated by binding of the ligand Hh to its receptor patched (Ptch). This binding releases from repression a second receptor, Smoothened (Smo), which moves to the membrane and triggers a series of reactions that result in translocation of transcription activators encoded by glioma associated oncogenes (Gli1, Gli2 and Gli3) into the nucleus and subsequent transcription of target genes.

A role for Hh signaling in certain cancers has been described. There are two different scenarios by which this pathway may be involved in carcinogenesis. One of them is by mutation of pathway components, as exemplified by the mutation of Ptch1 and Smo in basal cell carcinoma, or mutations of Ptch1 and Suppressor of Fused (SuFu) in medulloblastoma and rhabdomyosarcoma. The other scenario is autocrine requirement for Hh ligand, as seen in small-cell lung carcinoma, and cancers of the digestive tract, prostate, breast and liver [4].

It was previously reported that components of the Hh signaling pathway are gradually up-regulated as the normal epithelium progresses to squamous cell carcinoma [5], suggesting a role for Hh pathway molecules in development of this cancer. In the present work, we studied the role of the Hedgehog pathway in cell proliferation, apoptosis and migration, using a panel of cervical cancer cells that either express or not HPV transforming genes, and non-tumorigenic immortalized primary keratinocytes. By employing recombinant Shh ligand and pathway inhibitors, we found that interference with the pathway reduces survival, proliferation and migration of cervical cancer cell lines. We also found that Shh ligand has a promoting role for proliferation and migration of some of the cell-types tested.

* Corresponding author. Address: EPFL SV-DEC, SV-3516, Station 19, CH-1015 Lausanne, Switzerland. Fax: +41 21 693 0720.
E-mail address: peter.beard@epfl.ch (P. Beard).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.07.051

2. Materials and methods

2.1. Cell culture and reagents

The human cervical cancer cell lines C33, SiHa, C4-1, CasKi, and HeLa (American Type Culture Collection, Manassas, VA, USA), as well as the immortalized keratinocyte line HaCaT, were cultivated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Amimed, Allschwil, Switzerland) and penicillin and streptomycin. Recombinant Shh protein was purchased from R&D Systems, Minneapolis, MN, USA. Hedgehog pathway inhibitors KAAD-cyclopamine and GANT61 were purchased from Calbiochem, Darmstadt, Germany. Cleaved caspase 3 and Shh (H-160) antibodies were obtained from Cell Signaling, Danvers, MA, USA and Santa Cruz Biotechnology, Santa Cruz, CA, USA, respectively. Reporter Lysis Buffer (RLB) was purchased from Promega, Madison, WI, USA. Protease Inhibitor Cocktail, set III, was obtained from Calbiochem.

2.2. Gene expression analysis by PCR

Cervical cancer cell lines were grown in 6-well plates in complete growth medium. RNA was isolated using the RNeasy Mini-Kit (Qiagen) following the instructions from the manufacturer. cDNA was obtained using Ready-To-Go You-Prime First-Strand Beads (Amersham Biosciences). Semi-quantitative PCR was done using primers for *SHH* [6], *PTCH1* [7], *SMO* [8], *GLI1* [6], *GLI2* [8], *GLI3* [8] and *GAPDH* [8].

2.3. Detection of Shh protein by Western blot

Cervical cancer and other cell lines were grown in 6-well plates in complete growth medium. Cells were collected in RLB supplemented with protease inhibitors and incubated on ice for 30 min. Isolated protein samples were denatured for 10 min at 95 °C and separated by SDS–polyacrylamide gel electrophoresis (SDS–PAGE). Transfer of the proteins to nitrocellulose membranes was done using iBlot Dry Blotting System (Invitrogen). Western blot analysis was done using the SNAP i.d. protein detection system (Millipore) with antibody against Shh in 0.1% dried milk in TBS-T buffer (0.2 M NaCl, 25 mM Tris, pH 7.5, 0.5 ml/L Tween-20); followed by incubation with anti-rabbit-HRP conjugated secondary antibody. The signal detection was performed with ChemiGlow (Alpha Innotech) and the membranes were then exposed using a Fluor Chem 8900 camera.

2.4. BrdU incorporation assay for cell proliferation

Cells were plated on a 96-well plate at a density of 5000 cells/well and incubated overnight in complete growth medium. The next day, medium was changed to DMEM supplemented with 0.5% FBS. Twenty-four hours later cells were treated with recombinant Shh (100 and 200 ng/ml), or KAAD-cyclopamine (5 and 10 μM), or GANT61 (5, 10, 15 and 20 μM) or an equal volume of DMSO. The treatments were done in DMEM supplemented with 0.5% FBS. BrdU was added to cells 12 h prior to the assay, which was performed 48 h after treatment with the compounds. BrdU detection was done using the Cell Proliferation Biotrak ELISA System (GE Healthcare) as has been described previously [9].

2.5. Immunofluorescence

Cervical cancer cells were plated in 12-well plates on microscope cover glasses (Ø 18 mm, Thermo Scientific) in complete growth medium. Twenty-four hours later cells were treated with GANT61 (20 μM) in DMEM supplemented with 0.5% FBS. Forty-eight hours after treatment cells were washed with PBS and fixed with 4% paraformaldehyde (PFA). Subsequently, cells were washed with PBS, permeabilized and blocked with 0.25% Triton X-100 in 5% powdered milk for 10 min. Primary antibody was added in 1% milk in PBS and incubated for 1 h. Cells were washed three times with PBS-Triton X-100 (PBS-T) for 10 min. Secondary antibody was added in 1% milk in PBS and incubated for 30 min, followed by washing three times for 10 min with PBS-T. Cells were stained with 0.1 μg/ml DAPI (4′,6′-diamidino-2-phenylindole) for 1 min. Finally, they were washed with PBS and distilled water and mounted onto diazabicyclooctane-glycerol (50%). Images were obtained using a Zeiss Axioplan microscope and were acquired with a Zeiss AxioCam MRm camera using Axiovision 4.5 software. The 20× objective was used.

2.6. Wound-closure (scratch) assay

Cells were plated in 12-well plates in complete growth medium. Twenty-four hours later cells were treated with recombinant Shh (500 ng/ml) or GANT61 (20 μM) and a scratch was made through the confluent cell monolayer using 100 μl pipette tip. CasKi cells were imaged 0 and 20 h after scratching, while SiHa cells were imaged 0 and 96 h after scratching. Images were captured with the Olympus System CELLR using a 10× objective.

3. Results

3.1. Cervical cancer cell lines express Hh pathway components

Certain components of the Hh signaling pathway have been reported to be over-expressed in cervical cancer in comparison to normal tissue [5]. To confirm this and to determine its importance, we tested the expression of Hh pathway components using semi-quantitative RT-PCR and a panel of cell-types including cervical cancer-derived cells (C33, SiHa, C4-1, CasKi, and HeLa) as well as the non-tumorigenic immortalized keratinocyte line HaCaT. We found that all components of the pathway tested (Shh, Ptch1, Smo, Gli1, Gli2 and Gli3) were expressed in the cervical cell lines (Fig. 1A). In addition, we confirmed that the Shh ligand is present in all cell lines tested also at the protein level (Fig. 1B). Production of the Hh pathway components appears to be independent of the presence of the HPV genome, because the expression of Hh signaling components was similar in HPV-negative cells (such as C33 cervical cancer cells and the HaCaT immortalized line) to that in cells containing HPV: CasKi, C-41, SiHa and HeLa.

3.2. Inhibition of the Hh pathway reduces proliferation and survival of cervical cancer cells

To test the importance of this pathway for the continued proliferation or survival of cervical cancer cells, we carried out BrdU incorporation assays with and without treatment with pathway activators or inhibitors. A role for the Hh pathway in the growth of a number of cancer cell lines has been suggested. We used Shh as a pathway activator, and two different inhibitors that interfere with the pathway either at the level of the receptor Smo (KAAD-cyclopamine), or the transcription factor Gli (GANT61, Fig. 2). KAAD cyclopamine is a more soluble and more potent cyclopamine derivative, while GANT61 is a small molecule inhibitor that blocks binding of Gli to DNA [10]. We found that addition of Shh ligand increases proliferation of the CasKi cervical cancer line and of HaCaT cells, while inhibition of the pathway at both levels (Smo and Gli) reduces cell numbers and BrdU incorporation for all the cell-types tested (except HeLa where the effect was

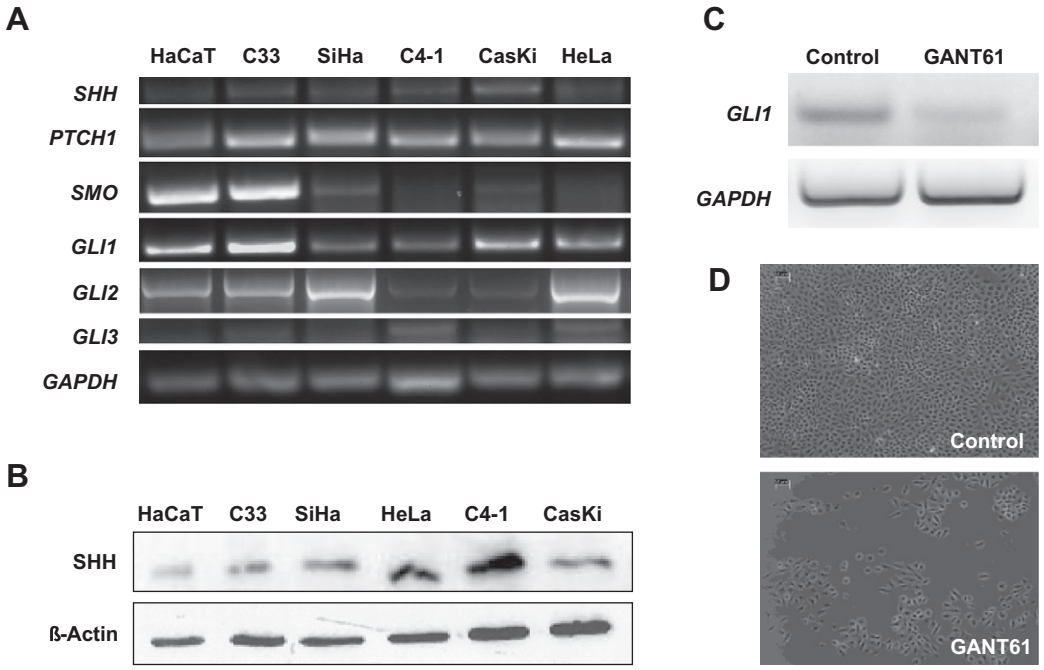

Fig. 1. Expression of components of the Hh signaling pathway by cervical cancer and other cell lines. The expression of Hh signaling pathway components in a panel of cell-types (non-tumorigenic as well as tumorigenic) was assessed by (A) semi-quantitative RT-PCR or (B) Western blot. (C) Inhibition of Hh signaling pathway by GANT61. CaSki cells were treated with GANT61 inhibitor (20 μM), and the status of GLI1 mRNA was assessed by semi-quantitative PCR. (D) Cell proliferation following treatment with an Hh pathway inhibitor. SiHa cells were treated for 72 h with GANT61 inhibitor (20 μM) and control and treated cells were photographed using phase contrast microscopy.

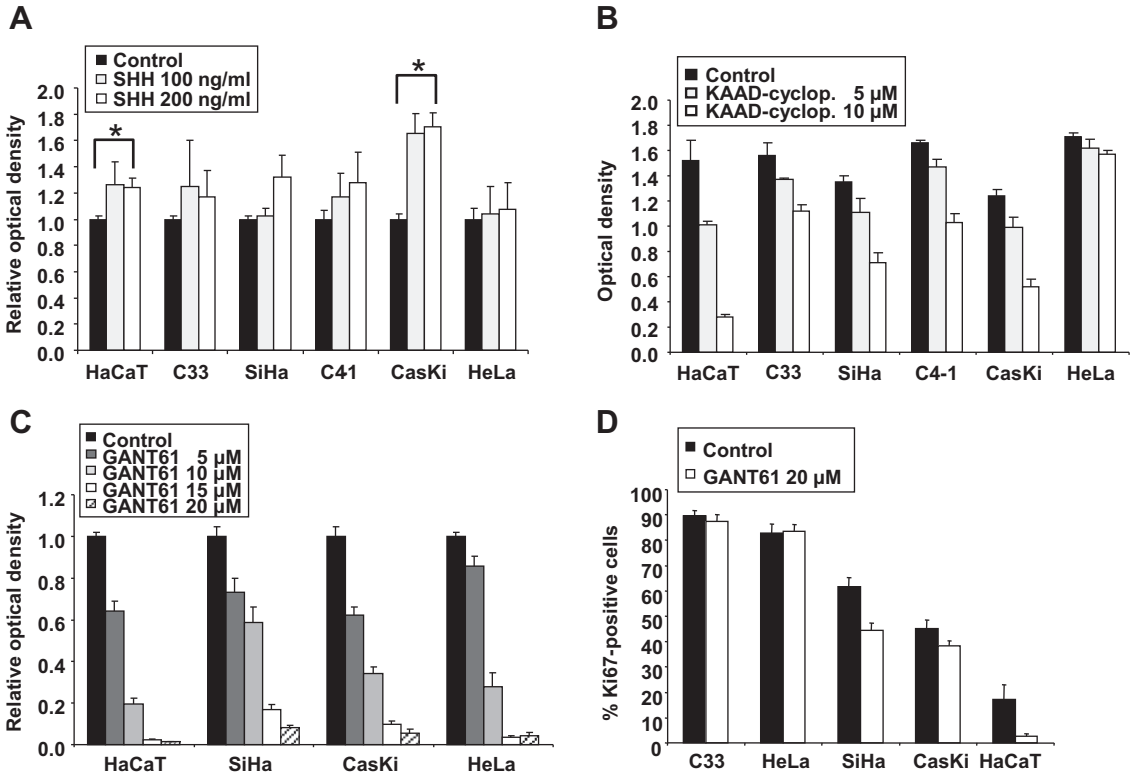

Fig. 2. Proliferation assays following treatment of cells with Shh ligand or Hh pathway inhibitors. Cells were incubated with the indicated concentrations of (A) Shh ligand, (B) KAAD-cyclopamine or (C) GANT61 for 48 h at 37 °C. Proliferation was assessed using an in vitro cell proliferation BrdU ELISA kit, $^{*}P < 0.05$. (D) Percentage of Ki67-positive cells in controls or after treatment with GANT61 (20 μM).

minimal), and this in a dose dependent manner (see representative microscopy Fig. 1D and quantification Fig. 2). Similar experiments were performed using siRNA directed against Gli1 to inhibit Hh pathway function, in the place of inhibitors, with similar results. Gli1 siRNA, but not control siRNA, clearly reduced the growth of SiHa cells, and the Gli1 siRNA was, as expected, able to reduce

expression of Gli1 as assessed by semi-quantitative RT-PCR (data not shown). To check that the Hh pathway is suppressed after treatment with GANT61, we measured the levels of mRNA for Gli1, a Hh pathway target gene, by semi-quantitative PCR in CasKi cells. As seen in Fig. 1C, GANT61 does indeed inhibit Hh pathway activity.

Since the reduced signal in the BrdU incorporation assay could be due to decreased proliferation or reduced survival, or both, we performed Ki67 staining to specifically label proliferating cells after treatment with GANT61. As seen in Fig. 2D, GANT61 reduced the percentage of Ki67-positive cells with SiHa and HaCaT; there was a similar trend with CasKi cells, but it did not reach statistical significance. Finally, consistent with our observation (Fig. 1) that the expression of Hh signaling components was comparable in HPV-negative and HPV-positive cells, the sensitivity of cell proliferation to Hh pathway inhibitors appeared also to be independent of HPV.

3.3. The Hh signaling inhibitor GANT61 induces caspase 3 cleavage in SiHa and CasKi cervical cancer cells

The Hh signaling pathway can have a pro-survival role in cancer cells [11–15]. Because the reduction in cervical cancer cell number that we detected using BrdU incorporation assays could be due to decreased cell survival, as well as a reduction in proliferation, we directly assayed for apoptotic cells to test the ability of these cells to survive upon treatment with the GANT61 inhibitor. As a readout of apoptotic pathway induction, we checked for the levels of cleaved caspase 3. We found that treatment with GANT61 increased the appearance of cleaved caspase 3 immuno-staining, pointing to an increase in apoptosis (Fig. 3A and B). From this result we conclude that the reduction of cell number seen in the proliferation assays is at least partly due to an increase in apoptosis upon treatment with GANT61.

3.4. The Hh pathway regulates migration of cervical cancer cells

The ability of cancer cells to migrate is closely associated with their ability to colonize distant organs. It was shown that the Hh signaling pathway can be involved in migration of cancer cell lines. To test the migratory ability of cervical cancer cells in response to recombinant Shh protein and to the pathway inhibitor GANT61, we performed wound closure (scratch) assays. The basic steps of this assay involve creating a "scratch" in a cell monolayer and capturing images at the beginning and at regular intervals during cell migration as the cells close the scratch. Finally, these images are quantified to express the migration rate of the cells [16]. We treated migrating SiHa and CasKi cells with recombinant Shh and GANT61. We observed that SiHa cells treated with recombinant Shh protein were able to fill and close the scratched area significantly faster than non-treated cells (Fig. 4). Conversely, CasKi cells treated with GANT61 were slower in closing the scratched area than the control cells (Fig. 4). In conclusion, the recombinant Shh ligand increases the ability of cervical cancer cells to migrate, while the inhibitor GANT61 reduces their ability to migrate and survive.

4. Discussion

The incidence of cervical cancer is still one of the important health care issues. There are 500,000 new cases of cervical cancer

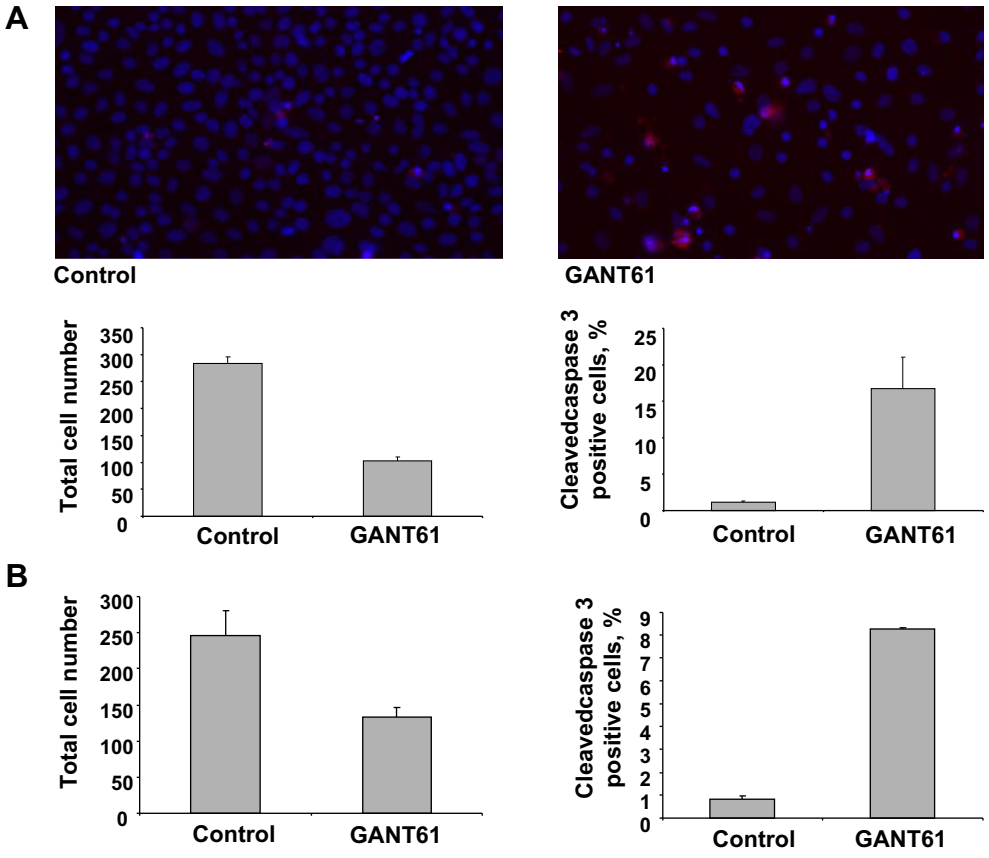

Fig. 3. Cleaved caspase 3 staining as readout of apoptotic pathway activation. (A) SiHa and (B) CasKi cells were treated with GANT61 (20 μM) for 48 h at 37 °C. Cleaved caspase 3 immuno-staining was performed, and total cell number (blue staining) as well as cleaved caspase 3-positive cells (red staining) were counted.

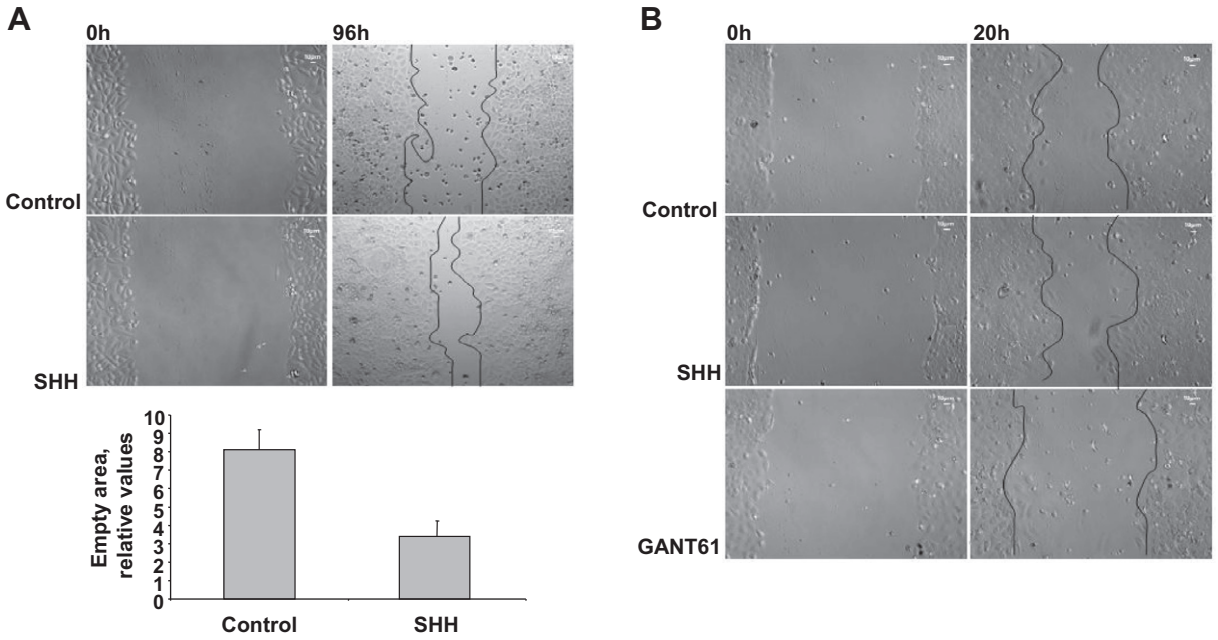

Fig. 4. Wound-closure (scratch) assay following treatment of cells with Shh ligand (500 ng/ml) or Hh pathway inhibitors (20 μM). Scratch assays were performed using (A) SiHa and (B) CasKi cells. Cells were photographed at the beginning of the assay and at the indicated time points. The surface area free of cells was calculated using Fiji software.

diagnosed per year worldwide, with mortality of approximately one third of these cases [17]. Although the occurrence rate in developed countries is decreasing, mainly due to effects of early diagnosis, cervical cancer continues to be a leading cause of mortality in developing countries where screening programs are not well established [17]. The specificity of cervical cancer in comparison to other cancers is that it is caused by a viral agent, HPV. Over 100 different genotypes of HPV have been identified, among which about 40 infect the genital mucosa. High-risk HPVs, such as HPV16, 18 and 31 are associated with more than 90% of cervical cancers [17].

HPV infection is needed for a cancer to develop, but it is not sufficient. Consequently, the genesis of cervical cancer is a multistep process. In a search for a possible 'second hit' that leads to cervical cancer we reported previously that moderate levels of Notch1 together with active PI3K can exhibit oncogenic properties when transfected into transformed primary keratinocytes [18]. It was also shown that activation of the canonical Wnt pathway can complement HPV in genital keratinocyte transformation [19]. Recapitulating an in vitro multistep carcinogenesis model for human cervical cancer, Narisawa-Saito et al. [20] have shown that oncogenic Hras provides keratinocytes with marked tumor-forming ability in nude mice. In the same work, the authors show that ErbB2 or c-Myc endow weaker but significant tumor-forming ability. Moreover, combined transduction of Myc and Hras to HPV-infected keratinocytes results in creation of highly potent tumor-initiating cells [20]. In a search for other possible complements of HPV action, here we have examined the role of the Hh signaling pathway in cervical cancer. The clue that the Hh signaling pathway might be involved in cervical carcinogenesis came from the work of Xuan et al. who showed that expression of Hh signaling molecules is enhanced in squamous cell carcinoma of the uterine cervix and its precursor lesions [5], [21].

To check the expression of Hh signaling molecules in a panel of cervical cancer cell lines we performed semi-quantitative RT-PCR. We found that all of the cell lines tested express most of the components of the Hh signaling pathway. To test whether addition of exogenous ligand can activate the Hh signaling pathway, we treated growing cervical cancer cells with Shh ligand and checked BrdU incorporation as readout of pathway activation. We found that Shh ligand increases proliferation of CasKi cells as well as of the HaCaT line. The inability of Shh ligand to increase proliferation in the other cell lines tested may indicate that the Hh signaling pathway is activated already maximally in these cells and that exogenous stimulation of this pathway is not possible.

The results from expression analysis and proliferation assays indicate the possible existence of an active autocrine Hh signaling loop within cervical cancer cells. To interfere with this signaling loop, we used Hh pathway inhibitors KAAD-cyclopamine to inhibit the pathway at the level of Smo, and GANT61 to inhibit Gli binding to DNA. Using BrdU incorporation assays we found that both inhibitors reduce proliferation or survival of the cervical cells in a dose-dependent manner. It is important to emphasize that the concentrations of GANT61 that we used in this and other experiments are within the range of, or lower than, those widely used by others ([22–24]).

Since the reduction in signal in BrdU incorporation assays could indicate either reduced proliferation or decreased cell survival, or a combination of the two, we wanted to distinguish the roles of the Hh signaling pathway in proliferation and in survival of the cervical cancer cells. Using proliferation-specific Ki67 staining, we found that GANT61 reduces the growth of SiHa and HaCaT cells. The Hh pathway has been reported to be involved in survival of cancer cell lines [11–15]. In order to test directly the involvement of the Hh pathway in the survival of cervical cancer cells, we performed immune-fluorescence staining for cleaved caspase 3 as an indicator of apoptotic cells. We found that, with SiHa and CasKi cells, GANT61 increases the frequency of cleaved caspase 3-positive cells, indicating increased apoptosis. From this result we conclude that Hh signaling promotes cervical cancer cell survival. Therefore, taken together our findings indicate that the Hh signaling pathway affects both survival and proliferation of cervical cancer cells; the relative contribution of each to the overall increase in cell number, however, varies and depends on the specific cell-type.

It was previously shown that introduction of components of the Hh signaling pathway into transformed but non-tumorigenic keratinocytes (HaCaT) increases their ability to infiltrate into a

collagen matrix [25]. In addition, Hh signaling can have pro-migratory effects in cancer cell lines [6,26–29]. To determine the importance of the Hh signaling pathway for the migration of cervical cancer cells, we used SiHa and CasKi cells in scratch assays. We found that Shh increases migration of SiHa cells, while conversely GANT61 decreases the ability of CasKi cells to migrate. Thus, we conclude that Hh signaling regulates the migration of cervical cancer cells.

Is the Hh pathway directly activated by HPV-encoded proteins? Our results would argue against this. Firstly, the activation of Hh signaling is comparable in cervical cancer cells that do or do not contain HPV DNA. Secondly, both HPV-containing and HPV-free cervical cancer cells are similarly slowed in their growth by Hh signaling inhibitors. Thirdly, primary human keratinocytes immortalized by the HPV16 E6 and E7 proteins (cells which are not tumorigenic) are barely affected in their proliferation by Hh pathway inhibitors (Ref. [18] and data not shown), implying that this pathway is not controlling their growth. Therefore it is more likely that the HPV E6 and E7 proteins do not directly induce Hh signaling, rather that mutations that activate the Hh pathway are selected during tumorigenesis in cells that have initially been immortalized by HPV. Taken together, our studies establish the importance of the Hh signaling pathway for the proliferation, survival and migratory ability of cervical cancer cells. They suggest that interference with this pathway could be a therapeutic option in cervical cancer.

Acknowledgments

The authors would like to thank the members of the Beard group for discussions. This work was supported by the Swiss National Science Foundation.

References

[1] S. Movva, L. Rodriguez, H. Arias-Pulido, C. Verschraegen, Novel chemotherapy approaches for cervical cancer, Cancer 115 (2009) 3166–3180.
[2] N. Ganguly, S.P. Parihar, Human papillomavirus E6 and E7 oncoproteins as risk factors for tumorigenesis, J. Biosci. 34 (2009) 113–123.
[3] J. Jiang, C.C. Hui, Hedgehog signaling in development and cancer, Dev. Cell 15 (2008) 801–812.
[4] L.L. Rubin, F.J. de Sauvage, Targeting the Hedgehog pathway in cancer, Nat. Rev. Drug Discov. 5 (2006) 1026–1033.
[5] Y.H. Xuan, H.S. Jung, Y.L. Choi, Y.K. Shin, H.J. Kim, K.H. Kim, W.J. Kim, Y.J. Lee, S.H. Kim, Enhanced expression of hedgehog signaling molecules in squamous cell carcinoma of uterine cervix and its precursor lesions, Mod. Pathol. 19 (2006) 1139–1147.
[6] W.T. Cheng, K. Xu, D.Y. Tian, Z.G. Zhang, L.J. Liu, Y. Chen, Role of Hedgehog signaling pathway in proliferation and invasiveness of hepatocellular carcinoma cells, Int. J. Oncol. 34 (2009) 829–836.
[7] J. Vestergaard, M.W. Pedersen, N. Pedersen, C. Ensinger, Z. Tumer, N. Tommerup, H.S. Poulsen, L.A. Larsen, Hedgehog signaling in small-cell lung cancer: frequent in vivo but a rare event in vitro, Lung Cancer 52 (2006) 281–290.
[8] Y. Kim, J.W. Yoon, X. Xiao, N.M. Dean, B.P. Monia, E.G. Marcusson, Selective down-regulation of glioma-associated oncogene 2 inhibits the proliferation of hepatocellular carcinoma cells, Cancer Res. 67 (2007) 3583–3593.
[9] I. Samarzija, P. Sini, T. Schlange, G. Macdonald, N.E. Hynes, Wnt3a regulates proliferation and migration of HUVEC via canonical and non-canonical Wnt signaling pathways, Biochem. Biophys. Res. Commun. 386 (2009) 449–454.
[10] M. Lauth, A. Bergstrom, T. Shimokawa, R. Toftgard, Inhibition of GLI-mediated transcription and tumor cell growth by small-molecule antagonists, Proc. Natl. Acad. Sci. USA 104 (2007) 8455–8460.
[11] P. Desch, D. Asslaber, D. Kern, H. Schnidar, D. Mangelberger, B. Alinger, M. Stoecher, S.W. Hofbauer, D. Neureiter, I. Tinhofer, F. Aberger, T.N. Hartmann, R. Greil, Inhibition of GLI, but not Smoothened, induces apoptosis in chronic lymphocytic leukemia cells, Oncogene 29 (2010) 4885–4895.
[12] T. Mazumdar, J. DeVecchio, T. Shi, J. Jones, A. Agyeman, J.A. Houghton, Hedgehog signaling drives cellular survival in human colon carcinoma cells, Cancer Res. 71 (2011) 1092–1102.
[13] M.E. Han, Y.S. Lee, S.Y. Baek, B.S. Kim, J.B. Kim, S.O. Oh, Hedgehog signaling regulates the survival of gastric cancer cells by regulating the expression of Bcl-2, Int. J. Mol. Sci. 10 (2009) 3033–3043.
[14] X. Chen, A. Horiuchi, N. Kikuchi, R. Osada, J. Yoshida, T. Shiozawa, I. Konishi, Hedgehog signal pathway is activated in ovarian carcinomas, correlating with cell proliferation: it's inhibition leads to growth suppression and apoptosis, Cancer Sci. 98 (2007) 68–76.
[15] K. Wang, L. Pan, X. Che, D. Cui, C. Li, Gli1 inhibition induces cell-cycle arrest and enhanced apoptosis in brain glioma cell lines, J. Neurooncol. 98 (2010) 319–327.
[16] C.C. Liang, A.Y. Park, J.L. Guan, In vitro scratch assay: a convenient and inexpensive method for analysis of cell migration in vitro, Nat. Protoc. 2 (2007) 329–333.
[17] T. Yugawa, T. Kiyono, Molecular mechanisms of cervical carcinogenesis by high-risk human papillomaviruses: novel functions of E6 and E7 oncoproteins, Rev. Med. Virol. 19 (2009) 97–113.
[18] S. Lathion, J. Schaper, P. Beard, K. Raj, Notch1 can contribute to viral-induced transformation of primary human keratinocytes, Cancer Res. 63 (2003) 8687–8694.
[19] A. Uren, S. Fallen, H. Yuan, A. Usubutun, T. Kucukali, R. Schlegel, J.A. Toretsky, Activation of the canonical Wnt pathway during genital keratinocyte transformation: a model for cervical cancer progression, Cancer Res. 65 (2005) 6199–6206.
[20] M. Narisawa-Saito, Y. Yoshimatsu, S. Ohno, T. Yugawa, N. Egawa, M. Fujita, S. Hirohashi, T. Kiyono, An in vitro multistep carcinogenesis model for human cervical cancer, Cancer Res. 68 (2008) 5699–5705.
[21] N. Chaudary, M. Pintilie, D. Hedley, A.W. Fyles, M. Milosevic, B. Clarke, R.P. Hill, H. Mackay, Hedgehog pathway signaling in cervical carcinoma and outcome after chemoradiation, Cancer 118 (2012) 3105–3115.
[22] T. Mazumdar, J. Devecchio, A. Agyeman, T. Shi, J.A. Houghton, Blocking Hedgehog survival signaling at the level of the GLI genes induces DNA damage and extensive cell death in human colon carcinoma cells, Cancer Res. 71 (2011) 5904–5914.
[23] T. Mazumdar, J. DeVecchio, T. Shi, J. Jones, A. Agyeman, J.A. Houghton, Hedgehog signaling drives cellular survival in human colon carcinoma cells, Cancer Res. 71 (2011) 1092–1102.
[24] D. Pan, Y. Li, Z. Li, Y. Wang, P. Wang, Y. Liang, Gli inhibitor GANT61 causes apoptosis in myeloid leukemia cells and acts in synergy with rapamycin, Leuk. Res. 36 (2012) 742–748.
[25] R.L. Bigelow, E.Y. Jen, M. Delehedde, N.S. Chari, T.J. McDonnell, Sonic hedgehog induces epidermal growth factor dependent matrix infiltration in HaCaT keratinocytes, J. Invest. Dermatol. 124 (2005) 457–465.
[26] S. Inaguma, K. Kasai, H. Ikeda, GLI1 facilitates the migration and invasion of pancreatic cancer cells through MUC5AC-mediated attenuation of E-cadherin, Oncogene 30 (2011) 714–723.
[27] 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, A. Maitra, Blockade of hedgehog signaling inhibits pancreatic cancer invasion and metastases: a new paradigm for combination therapy in solid cancers, Cancer Res. 67 (2007) 2187–2196.
[28] V.I. Alexaki, D. Javelaud, L.C. Van Kempen, K.S. Mohammad, S. Dennler, F. Luciani, K.S. Hoek, P. Juarez, J.S. Goydos, P.J. Fournier, C. Sibon, C. Bertolotto, F. Verrecchia, S. Saule, V. Delmas, R. Ballotti, L. Larue, P. Saiag, T.A. Guise, A. Mauviel, GLI2-mediated melanoma invasion and metastasis, J. Natl. Cancer Inst. 102 (2010) 1148–1159.
[29] X. Liao, M.K. Siu, C.W. Au, E.S. Wong, H.Y. Chan, P.P. Ip, H.Y. Ngan, A.N. Cheung, Aberrant activation of hedgehog signaling pathway in ovarian cancers: effect on prognosis, cell invasion and differentiation, Carcinogenesis 30 (2009) 131–140.