EXPRESSION OF THE SONIC HEDGEHOG EMBRYONIC SIGNALLING PATHWAY COMPONENTS IN MATCHED PRE-TREATMENT AND RELAPSED SMALL CELL LUNG CANCER BIOPSIES

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Cancer stem cells may be responsible for tumour regrowth and acquisition of resistance in small cell lung cancer (SCLC). The Hedgehog pathway regulates survival and proliferation of tissue progenitor and stem cell populations, promoting the expression of stem cell related and proliferative genes. We evaluated the Sonic Hedgehog (Shh) embryonic signalling pathway in relapsed SCLC. Expression levels of Shh related genes GLI1, SMO, SUFU, PTCH1, HHIP, BCL2, BMI, ZEB1, ZEB2, N-MYC, Twist1 were analysed by qRT-PCR in matched pre-treatment and relapsed tumour fresh frozen biopsies of three SCLC patients. Expression of each gene was compared using the paired samples t-test, as well as comparison of mean expression levels was done. Data were statistically interpreted using the MedCalc version 10.2.0.0 software. 2.9-fold lower mean mRNA expression of the major Hedgehog activation indicator GLI1 was observed in relapsed samples ($p = 0.0529$). Mean expression of six Shh inducible genes, PTCH1, HHIP, N-MYC, ZEB2, Twist1, ZEB1, was also downregulated by 2.6-, 2.2-, 1.9-, 1.8-, 1.2-, 1.1-fold, respectively ($p = 0.4252$, $p = 0.1268$, $p = 0.2480$, $p = 0.1169$, $p = 0.1480$, $p = 0.7595$, respectively). 1.8-fold mean expression decrease was found for Gli activator Smo ($p = 0.4111$). Only the Shh pathway inhibitor SUFU and two other examined Hedgehog signalling inducible genes BCL2 and BMI in relapsed SCLC showed 0.8-, 0.9-, and 0.8-fold increase of expression, respectively ($p = 0.3074$, $p = 0.7921$, and $p = 0.3822$, respectively). To our knowledge, this is the first report of comparison of Shh signalling in matched pre-treatment and relapsed SCLC biopsies. Our data show decreased activity for majority of Shh pathway components in relapsed SCLC, although difference did not reach statistical significance.

Keywords: relapsed SCLC, cancer stem cells, Sonic Hedgehog pathway, gene expression, matched biopsies.

INTRODUCTION

Small cell lung cancer is a malignant disease, where presence of a cancer stem cell (CSC) subpopulation is suggested by clinical practice. Standard chemotherapy may reduce tumour mass very effectively and even induce complete remission in the majority of patients (Okuno and Jett, 2002). Unfortunately, recurrence almost always occurs and virtu-

ally no extensive-stage disease patients remain alive for five years (Jett *et al.*, 2013). This can be explained by the survival of a small fraction of dormant and treatment-resistant CSC during initial chemotherapy and further repopulation of the tumour. The majority of cells, like CSC in recurrent tumours, acquire resistance to chemotherapy. One major characteristic feature of CSC is its ability to exclude Hoechst dye from the cell due to high expression of drug

transporters like the ABC transporter family, including ABCB1 and ABCG2 (Goodell *et al.*, 1996). Selection and further study of CSC therefore became possible in small cell lung cancer (SCLC) cell lines and mouse models within the so-called side population fraction (with poor Hoechst dye saturation). Many stemness and drug resistance associated genes were found to be overexpressed in SCLC CSC, as well as genes involved in the embryonic signalling pathways — Notch and Hedgehog (Salcido *et al.*, 2010). The Hedgehog pathway activation was found in SCLC tumours (Watkins *et al.*, 2003) and its blockade inhibited growth of SCLC tumour xenografts in mice (Vestergaard *et al.*, 2006). Additionally, increased Hedgehog signalling was found to be associated with activation of neuroendocrine precursors within the airway epithelial compartment, which could be of importance in SCLC with its primitive neuroendocrine features (Zochbauer-Muller *et al.*, 2002).

The aim of this study was to investigate and compare Sonic Hedgehog (Shh) embryonic signalling pathway activity in small cell lung cancer biopsies before and after acquisition of resistance. In this retrospective study we evaluated mRNA expression of key Shh pathway member genes in advanced stage SCLC samples before treatment and at progression of the disease.

The following genes were evaluated:

1) the major indicator of Hedgehog activation — positive pathway regulator and pathway transcriptional target *Glioma-Associated Oncogene Homolog 1 (Gli1)* (Katoh and Katoh, 2009a), as well as *Gli* activator *Smoothed (Smo)* (Gupta *et al.*, 2010);

2) pathway inhibitor *Suppressor Of Fused Homolog (SUFU)* (Sabol *et al.*, 2018), and

3) the most important genes inducible by Hedgehog signalling: pathway canonical negative feedback regulators — *Protein Patched Homolog 1 (PTCH1)* and *Hedgehog Interacting Protein (HHIP1)* (Pietrobono *et al.*, 2019), antiapoptotic *B-Cell CLL/Lymphoma 2 (BCL2)*, CSC marker and epigenetic regulator *BM1 polycomb ring finger oncogene (BMI)*, epithelial-to-mesenchymal transition inducers *Zinc Finger E-Box Binding Homeobox 1 and 2 (ZEB1, ZEB2)* (Katoh and Katoh, 2009a), drug resistance associated *NMYC* (Salcido *et al.*, 2010) and direct transcriptional target of *Gli1* — *Twist Family BHLH Transcription Factor 1 (Twist1)* (Kong *et al.*, 2015).

MATERIALS AND METHODS

Patients and tissue samples. Pre-treatment tissue samples for the study were collected along with standard biopsies during fibrobronchoscopy (FBS) from patients who were diagnosed with advanced (stage III B and IV) SCLC at Pauls Stradiņš Clinical University Hospital between October 2010 and January 2014. Tissue specimens were submerged in RNAlater solution (Thermo Fisher Scientific, USA) and stored at -20°C . The presence of tumour cells in the study

samples was verified by cytological evaluation of a smear, which was obtained before placing the sample in RNAlater. All diagnoses were histologically confirmed as SCLC in standard biopsies by board-certified pathologists; the presence of tumour cells in study smear was done by a board-certified cytologist. Pre-treatment biopsies from 20 SCLC patients were obtained and they were followed during treatment. At the moment of disease progression, repeated FBS and study biopsies of relapsed SCLC was done in five patients. Two patients were excluded from the analysis (one due to the lack of cytological confirmation of tumour cell presence in the pre-treatment study biopsy the other due to the bad RNA quality in the relapsed tumour biopsy). A small cohort of samples that was available for testing consisted of matched pre-treatment and relapsed SCLC biopsies from the same patient ($n = 3$).

Demographics and clinical characteristics of the studied patients and comparison of three patients, who had qualitative both pre-treatment and relapsed SCLC biopsies vs patients not included in paired gene expression analysis are shown in Table 1. Statistically significant age and ECOG differences were found between groups, while patients were comparable in terms of gender, disease stage, toxicity, received treatment, best response to therapy, disease progression type, and survival.

The study was approved by the Ethics Committee of the Institute of Experimental and Clinical Medicine, University of Latvia. The tissue samples were collected after written informed consent was obtained from the patients.

Preparation of tissue mRNA. Biopsy homogenisation was done by FastPrep®-24 Instrument and Lysing Matrix D (MP Biomedicals, USA) at 0.4 m/s for 40 seconds. Total RNA was isolated using the MirVana total RNA Isolation Kit (Thermo Fisher Scientific, USA) and was treated with DNase I (Thermo Fisher Scientific, USA) according to the manufacturer's protocol. RNA concentration and purity was quantified by a Nanodrop ND-100 spectrophotometer. cDNA was synthesised by random hexamer priming from 1 µg of total RNA by using the Revert Aid First Strand cDNA Synthesis kit (Thermo Fisher Scientific, MA, USA) according to the manufacturer's instructions.

Quantitative RT-PCR. Quantitative real-time PCR (qRT-PCR) was performed using 2 µl 1 : 10 diluted cDNA reaction mixtures, ABSolute Blue™ SYBR green Low ROX (Thermo Fisher Scientific, USA) and ViiA 7 real-time PCR system (Applied Biosystems, Life Technologies, USA). Sequences of primers used in this study are available on request. To normalise the expression data, the normalisation factor was calculated for each cDNA from the expression values of the three most stable reference genes (*ACTB*, *LRP10*, *YWHAZ*) selected among seven frequently used housekeeping genes by using geNorm software. All qRT-PCR experiments were performed in duplicate and the data were presented in graphs as means $\pm$ standard deviation.

Table 1. Characteristics of three patients with pre-treatment and relapsed SCLC biopsies vs patients not included in paired gene expression analysis. The comparison between groups was carried out by the Student's t-test or the Chi Square test, according to the type of variable

Patient characteristics	Patients included in paired gene expression analysis (n = 3)	Patients NOT included in paired gene expression analysis (n = 17)	p-value
Gender			0.348
Male	3	13	
Female	0	4	
Age			0.037
Mean (range)	60 (49–77)	56.9 (47–70)	
ECOG			0.048
0	2	1	
1	0	7	
2	1	7	
3	0	2	
Stage			0.565
III A	0	2	
III B	1	5	
IV	2	10	
Deviations of treatment dosing/timing due toxicity			0.212
none	1	12	
present	2	5	
Treatment received			0.948
Chemotherapy only	1	6	
Chemotherapy and radiation therapy	2	11	
Best response			0.093
CR	0	2	
PR	2	13	
SD	0	2	
PD	1	0	
Disease progression type			0.075
local	1	4	
distant	0	8	
combined	2	1	
NK	0	2	
not progressed	0	2	
Survival			
Mean PFS (months)	6.7	25.8	0.469
Mean OS (months)	11	28.2	0.506

SCLC, small cell lung cancer; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NK, not known; PFS, progression-free survival; OS, overall survival; PD, progressive disease

Statistical analysis. The mRNA level of each Shh related gene was compared in the same patient matched pre-treatment vs relapsed SCLC samples using the two-tailed paired samples t-test, as well as comparison of mean expression levels of each tested gene was done in all pre-treatment vs relapsed samples with calculation of 95% confidence intervals. Data were statistically interpreted using MedCalc version 10.2.0.0 software. *p* values of < 0.05 were considered significant.

RESULTS

To our knowledge, this is the first report that compares expression of the Shh signalling pathway components in matched pre-treatment and relapsed SCLC biopsies. Our results show decreased activity of majority of Shh pathway components in the relapsed SCLC tumour tissues, although the differences did not reach statistical significance.

2.9-fold lower mean mRNA expression of the major Hedgehog activation indicator *GLI1* was observed in relapsed SCLC biopsies when compared with matched pre-treatment biopsies, although the difference did not reached statistical significance ($p = 0.0529$, 95% CI: -0.0002023 to 0.000003115 , two-tailed paired samples t-test) (Fig. 1, Table 2).

Mean expression of six genes inducible by Hedgehog signalling — *PTCH1*, *HHIP*, *N-MYC*, *ZEB2*, *Twist1*, and *ZEB1* was also downregulated by 2.6-, 2.2-, 1.9-, 1.8-, 1.2-, and 1.1-fold, respectively, in relapsed SCLC samples without a statistical significant difference ($p = 0.4252$, $p = 0.1268$, $p = 0.2480$, $p = 0.1169$, $p = 0.1480$, and $p = 0.7595$, respectively) (Figs. 1 and 2, Table 2).

1.8-fold mean expression decrease was found in paired biopsies at relapse for Gli activator *Smo* with no statistically significant difference ($p = 0.4111$) (Fig. 2, Table 2).

For relapsed SCLC, only Shh pathway inhibitor *SUFU* and 2 examined Hedgehog signalling inducible genes – *BCL2* and *BMI* showed increase by 0.8-, 0.9- and 0.8-fold, respectively, in their mean expression level, without statistically significant difference ($p = 0.3074$, $p = 0.7921$ and $p = 0.3822$, respectively) (Fig. 3, Table 2).

DISCUSSION

To our knowledge, this is the first report that directly compares expression of the Shh signalling pathway components in matched pre-treatment and relapsed SCLC biopsies.

2.9 fold lower mean mRNA expression of the major Hedgehog activation indicator *GLI1* was observed in relapsed SCLC biopsies compared to matched pre-treatment biopsies, although the difference did not reach statistical significance ($p = 0.0529$).

Mean expression of six genes inducible by Hedgehog signalling — *PTCH1*, *HHIP*, *N-MYC*, *ZEB2*, *Twist1*, and *ZEB1* was also downregulated by 2.6-, 2.2-, 1.9-, 1.8-, 1.2- and 1.1-fold, respectively, in relapsed SCLC, without statistically a significant difference ($p = 0.4252$, $p = 0.1268$, $p = 0.2480$, $p = 0.1169$, $p = 0.1480$, and $p = 0.7595$, respectively), as well as 1.8-fold mean expression decrease in relapsed samples for Gli activator *Smo*, with no statistically significant difference ($p = 0.4111$).

For relapsed biopsies, only Shh pathway inhibitor *SUFU* and the other two examined Hedgehog signalling inducible genes — *BCL2* and *BMI* showed increase by 0.8-, 0.9-, and

Fig. 1. Dot and line diagram with normalised gene expression levels of each Shh-related gene plotted as individual dots connected by a line for the same SCLC patient pre-treatment (Pre) and relapsed (Rel) samples. Bars for means with 95% CI for the mean. Data presented in graphs are means obtained from experiments in duplicates ± standard deviation. SCLC, small cell lung cancer; CI, confidence interval; *Gli1*, Glioma-Associated Oncogene Homolog 1; *PTCH1*, Protein Patched Homolog 1; *HHIP1*, Hedgehog Interacting Protein; *N-MYC*, a member of the MYC family of transcription factors.

0.8-fold, respectively, in their mean expression level, without statistically significant difference ($p = 0.3074$, $p = 0.7921$, and $p = 0.3822$, respectively).

Cancer stem cells may be responsible for tumour initiation, regrowth and acquisition of resistance. Many studies have linked the aberrant hedgehog signalling to renewal and sur-

vival of cancer stem cells in SCLC, thus making it a promising therapeutic target.

Preclinical research in SCLC is relying mainly on cell lines and xenografts, due to the lack of widely available tumour samples (Hamilton *et al.*, 2012). However, it has been observed that establishment of SCLC cell line/xenograft leads

Table 2. Comparison of mean expression level of Shh related genes in pre-treatment vs relapsed samples

Tested gene	Mean expression level in 3 SCLC pre-treatment vs relapsed samples				Estimated difference
	pre-treatment	95% CI for the mean	relapsed	95% CI for the mean	
<i>Gli1</i>	0.0001526	0.000088 to 0.0002173	0.00005305	-0.000004 to 0.0001101	2.9
<i>PTCH1</i>	0.09971	-0.1515 to 0.3509	0.03881	0.01099 to 0.06664	2.6
<i>HHIP1</i>	0.0002836	0.000109 to 0.0004582	0.0001305	-0.0001989 to 0.0004598	2.2
<i>N-MYC</i>	0.02085	-0.006934 to 0.04863	0.01094	-0.001096 to 0.02297	1.9
<i>Smo</i>	0.01722	-0.0130 to 0.04744	0.009398	-0.01974 to 0.03854	1.8
<i>ZEB2</i>	0.02419	0.01042 to 0.03795	0.01354	0.004276 to 0.0228	1.8
<i>Twist1</i>	0.004609	-0.0004286 to 0.009646	0.003798	-0.002644 to 0.01024	1.2
<i>ZEB1</i>	0.007191	-0.001361 to 0.01574	0.006662	0.004601 to 0.008722	1.1
<i>BCL2</i>	0.02301	0.0006755 to 0.04534	0.02509	-0.01966 to 0.06984	0.9
<i>SUFU</i>	0.007723	0.004172 to 0.01127	0.009916	0.002686 to 0.01715	0.8
<i>BMI</i>	0.08249	0.0108 to 0.1542	0.09763	-0.02615 to 0.2214	0.8

Shh, Sonic Hedgehog; SCLC, small cell lung cancer; CI, confidence interval; *Gli1*, Glioma-Associated Oncogene Homolog 1; *PTCH1*, Protein Patched Homolog 1; *HHIP1*, Hedgehog Interacting Protein; *N-MYC*, a member of the MYC family of transcription factors; *Smo*, Smoothened; *ZEB1*, *ZEB2*, Zinc Finger E-Box Binding Homeobox 1 and 2; *Twist1*, Twist Family BHLH Transcription Factor 1; *BCL2*, B-Cell CLL/Lymphoma 2; *SUFU*, Suppressor Of Fused Homolog; *BMI*, BMI1 polycomb ring finger oncogene

Fig. 2. Dot and line diagram with normalised gene expression levels of each Shh-related gene plotted as individual dots connected by a line for the same SCLC patient pre-treatment (Pre) and relapsed (Rel) samples. Bars for means with 95% CI for the mean. Data presented in graphs are means obtained from experiments in duplicates ± standard deviation. SCLC, small cell lung cancer; CI, confidence interval; *Smo*, Smoothed; *ZEB1*, *ZEB2*, Zinc Finger E-Box Binding Homeobox 1 and 2; *Twist1*, Twist Family BHLH Transcription Factor 1.

Fig. 3. Dot and line diagram with normalised gene expression levels of each Shh-related gene plotted as individual dots connected by a line for the same SCLC patient pre-treatment (Pre) and relapsed (Rel) samples. Bars for means with 95% CI for the mean. Data presented in graphs are means obtained from experiments in duplicates ± standard deviation. SCLC, small cell lung cancer; CI, confidence interval; *BCL2*, B-Cell CLL/Lymphoma 2; *SUFU*, Suppressor of Fused Homolog; *BMI*, *BMI1* polycomb ring finger oncogene

to irreversible changes in gene expression (Daniel *et al.*, 2009). Regarding Hedgehog signalling, it was shown previously that significantly higher mean *GLII* expression is present in SCLC tumour biopsies tissues compared to that in SCLC cell lines (Pedersen *et al.*, 2003). Thus, caution should be taken when working with tumour-derived cell lines, as the expression and signalling may not reflect the *in vivo* situation. The advantage of this study is that mRNA expression of key Shh pathway members was evaluated in SCLC fresh biopsies (*in vivo*). To our knowledge, this is the first report of such analysis of Shh signalling pathway components in matched pre-treatment and relapsed SCLC samples.

Contrary to promising preclinical data that suggested a synergistic role between the canonical Shh pathway inhibitors and chemotherapy for targeting the chemoresistant subset of SCLC cells, no positive results from clinical trials evaluating activity of SMO inhibitors (Vismodegib, LDE-225, BMS-833923, and LY2940680) have been reported. One possible explanation of this failure in SCLC might be the non-canonical activation of Shh pathway key players. An example of ligand-independent *GLII* activation is *GLII* overexpression observed in the case of TP53 deficiency. Study of this mechanism showed that exposure of human medulloblastoma cell lines and *Ptc*^{-/-} mouse embryonic fibroblasts to DNA damaging cytotoxic drugs induced concomitant expression of p53 and sequential downregulation of GLII through its p53-dependent ubiquitination and degradation (Mazza *et al.*, 2013). This dependency may be crucial in this particular lung cancer type because bi-allelic inactivation of *TP53* and *RB1* genes is observed nearly in all SCLC (George *et al.*, 2015).

Other possible cause of increased ligand-independent Shh signalling is presence of activating mutations in RAS-RAF-MEK, PI3K-AKT oncogenic signalling pathways or downregulation of the Notch pathway. Thus, oncogenic H- or N-RAS and AKT1 potentiate GLII function, by enhancing its transcriptional activity and nuclear localisation, and counteracting its cytoplasmic retention by SUFU in melanoma, prostate cancer and human glioma cells (Stecca *et al.*, 2007). Similarly, loss of function of tumour suppressor PTEN (phosphatase and tensin homolog deleted on chromosome 10), an endogenous inhibitor of AKT, will lead to increased transcriptional activity of *GLII*. PTEN alterations can be found in 10–18% of SCLC tumours (Yokomizo *et al.*, 1998).

Another oncogene that was found to activate *GLII* independently from Shh ligand-mediated signalling is *C-MYC*. It is frequently overexpressed in both SCLC and non-small cell lung cancer (NSCLC) (Wistuba *et al.*, 2001). *C-MYC* directly binds to the *GLII* promoter and activates its transcription. Inhibition of *C-MYC* by small molecule inhibitors downregulates *GLII* mRNA and induces cell death in Burkitt lymphoma cells (Yoon *et al.*, 2013).

While KRAS/AKT aberrant activation is a rare event in SCLC (Maitra *et al.*, 2001; Wakuda *et al.*, 2014; Yamamoto

et al., 2008; Kodaz *et al.*, 2016), Notch pathway signalling was found to be downregulated in 77% of 110 SCLC clinical tumour specimens, among which Notch family genomic alterations were found in 25% of cases (George *et al.*, 2015). As Notch signals downregulate GLII expression via HES/HEY family members (Katoh and Katoh, 2009b), increased Shh signalling is possible in this case. Additional studies found reduced tumour formation, metastatic capability, cell cycle inhibition, and reduced neuroendocrine markers with Notch activation, thus demonstrating Notch as a tumour suppressor in SCLC (Hassan *et al.*, 2014). One discovered mechanism of Notch pathway downregulation is overexpression of its inhibitor DLL3, which enhances endosomal/lysosomal degradation of both activatory Notch single-pass transmembrane receptors and membrane-bound ligand DLL1 (Chapman *et al.*, 2011; Serth *et al.*, 2015).

Considering multiple possible non-canonical Shh signalling interactions, our finding with lower (although statistically non-significant) *GLII* expression in relapsed SCLC samples when compared to matched pre-treatment biopsies might reflect the further increasing dysregulation of different signalling pathways and/or epigenetic mechanisms. In this case, accumulation of additional genetic/epigenetic changes over time decreases initially high GLII levels, which is characteristic for SCLC tumour biopsies and may be explained by a few critical genetic alterations like the frequently observed TP53 deficiency or downregulated Notch pathway signalling.

A possible limitation of the study might be the inclusion of primary resistant patients in the analysis. One patient did not respond to specific therapy, thus suggesting that resistance-associated changes were already present in the pre-treatment sample.

This study was the first attempt to solve the problem of notable lack of paired samples of newly diagnosed and recurrent SCLC. Unfortunately, only five patients from 20 had undergone FBS re-biopsy of their progressed tumour and only three qualitative matched paired samples were obtained. The main causes of such a low re-biopsy rate were distant disease progression only and refusal of patients to undergo a repeated invasive procedure. An issue was also the small size of biopsy samples and exclusion of a significant part of study patients from analysis due to lack of cancer cells in a biopsy.

Our data show decreased activity of the majority of Shh pathway components in the relapsed SCLC tumour tissues, although the differences did not reach statistical significance.

Thus, the activation of Shh signalling might not be a universal mechanism for SCLC relapse and resistance development.

Due to the small number of patients in this study, generalisation of our results is questionable. A longer period of time

for tissue sample collection or inclusion of additional centres in the tissue collection programme may solve this problem.

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SONIC HEDGEHOG EMBRIOĢENĒZI REGULĒJOŠĀ SIGNĀLCEĻA KOMPONENTU EKSPRESIJA SĪKŠŪNU PLAUŠU VĒŽA PACIENTU PIRMSTERAPIJAS UN RECIDĪVA PĀRA BIOPSIJĀS

Vēža cilmšūnas var būt par pamatu sīkšūnu plaušu vēža (SŠPV) recidīvam un rezistences attīstībai. *Hedgehog* signālceļš regulē vairākus ar cilmšūnām un proliferāciju saistītus gēnus, tādējādi veicinot cilmšūnu un audu progenitoro šūnu izdzīvošanu un proliferāciju. Mēs izvērtējām *Sonic Hedgehog* (Shh) embrioģenēzi regulējošo signālceļu recidivējošā SŠPV. Izmantojot reālā laika kvantitatīvo *qRT-PCR*, tika analizēti ekspresijas līmeņi Shh saistītiem gēniem *GLI1*, *SMO*, *SUFU*, *PTCH1*, *HHIP*, *BCL2*, *BMI*, *ZEB1*, *ZEB2*, *N-MYC*, *Twist1* trīs SŠPV pacientu audzēju svaigi saldēto pāra biopsiju paraugos, kas ņemti pirms terapijas un recidīva laikā. Katra gēna ekspresijas līmeņi saistītajām paraugkopām tika salīdzināti, izmantojot Stjūdenta t-testu, kā arī tika salīdzināti vidējie ekspresijas līmeņi. Datu statistiskā analīze veikta, izmantojot *MedCalc* 10.2.0.0 versijas programmu. Recidīva paraugos bija 2,9 reizes zemāka ($p = 0,0529$) vidējā mRNS ekspresija galvenajam *Hedgehog* signālceļa aktivitātes indikatoram *GLI1*. Arī sešu *Hedgehog* signālceļa inducējamo gēnu — *PTCH1*, *HHIP*, *N-MYC*, *ZEB2*, *Twist1*, *ZEB1* — vidējā ekspresija bija attiecīgi zemāka 2,6, 2,2, 1,9, 1,8, 1,2, 1,1 reizes (ar attiecīgu $p = 0,4252$, $p = 0,1268$, $p = 0,2480$, $p = 0,1169$, $p = 0,1480$, $p = 0,7595$). 1,8 reizes zemāka vidējā ekspresija bija konstatēta Gli aktivatoram *Smo* ($p = 0,4111$). Attiecīgi 0,8, 0,9 un 0,8 reizes augstāka vidējā ekspresija recidivējošā SŠPV (ar attiecīgu $p = 0,3074$, $p = 0,7921$ un $p = 0,3822$) bija vienīgi signālceļa inhibitoram *SUFU* un 2 citiem izmeklētiem *Hedgehog* signālceļa inducējamiem gēniem *BCL2* un *BMI*. Pēc mums pieejamās informācijas, šis ir pirmais ziņojums, kas salīdzina Shh signālceļa ekspresiju SŠPV pirmsterapijas un recidīva pāra biopsijās. Pētījumā iegūtie rezultāti norāda uz Shh signālceļa komponentu samazinātu aktivitāti recidivējošā SŠPV, kaut starpība nav sasniegusi statistisku ticamību.