Table 2. Correlations between expressions of SHH, PTCH1, SMO and GLI1 mRNA and clinicopathological factors

	n	SHH	p value	PTCH1	p value	SMO	p value	GLI1	p value
Gender									
Male	30	0.026 ± 0.019		0.903 ± 0.564		0.086 ± 0.097		0.643 ± 0.632	
Female	11	0.034 ± 0.022	0.269	0.138 ± 0.751	0.361	0.167 ± 0.306	0.405	1.013 ± 0.894	0.227
Age									
<60 years	10	0.026 ± 0.014		1.241 ± 0.618		0.226 ± 0.315		1.348 ± 0.876	
≥60 years	31	0.028 ± 0.022	0.794	0.877 ± 0.601	0.106	0.070 ± 0.077	0.014	0.547 ± 0.546	0.001
Histology									
(a) Differentiated	16	0.024 ± 0.020		0.747 ± 0.603		0.068 ± 0.092		0.450 ± 0.538	
(b) Undifferentiated	23	0.032 ± 0.020	0.217	1.175 ± 0.572	0.031	0.144 ± 0.219	0.199	1.005 ± 0.753	0.016
Stroma in tumors									
(c) Scic	13	0.030 ± 0.020		1.447 ± 0.468		0.201 ± 0.279		1.301 ± 0.854	
(d) Int or (e) Med	26	0.027 ± 0.020	0.63	0.691 ± 0.533	<0.001	0.054 ± 0.054	0.012	0.477 ± 0.488	<0.001
Depth of invasion									
T1	5	0.016 ± 0.013		0.497 ± 0.336		0.016 ± 0.016		0.189 ± 0.142	
T2–T4	36	0.030 ± 0.021	0.162	1.031 ± 0.623	0.07	0.120 ± 0.186	0.22	0.819 ± 0.734	0.066
Lymph node metastasis									
Negative	11	0.019 ± 0.015		0.746 ± 0.579		0.164 ± 0.319		0.560 ± 0.807	
Positive	30	0.031 ± 0.021	0.105	1.046 ± 0.622	0.172	0.087 ± 0.082	0.223	0.809 ± 0.687	0.331
Liver metastasis									
Absent	37	0.028 ± 0.020		0.973 ± 0.593		0.115 ± 0.186		0.766 ± 0.729	
Positive	4	0.028 ± 0.026	0.959	0.898 ± 0.930	0.822	0.045 ± 0.015	0.461	0.525 ± 0.674	0.531
Peritoneal metastasis									
Absent	35	0.027 ± 0.019		0.895 ± 0.626		0.077 ± 0.088		0.656 ± 0.614	
Positive	6	0.034 ± 0.028	0.406	1.376 ± 0.394	0.079	0.286 ± 0.393	0.006	1.246 ± 1.103	0.062
Cytology of the peritoneal surface									
Negative	37	0.028 ± 0.021		0.891 ± 0.581		0.107 ± 0.185		0.680 ± 0.696	
Positive	4	0.031 ± 0.004	0.779	1.656 ± 0.098	0.017	0.116 ± 0.098	0.926	1.318 ± 0.776	0.092
Lymphatic invasion									
Negative	4	0.016 ± 0.017		0.943 ± 0.655		0.120 ± 0.151		0.385 ± 0.254	
Positive	37	0.029 ± 0.020	0.227	0.968 ± 0.624	0.94	0.106 ± 0.182	0.888	0.781 ± 0.741	0.302
Venous invasion									
Negative	5	0.021 ± 0.013		0.720 ± 0.600		0.231 ± 0.470		0.811 ± 1.193	
Positive	36	0.029 ± 0.021	0.444	1.000 ± 0.621	0.349	0.091 ± 0.091	0.096	0.733 ± 0.654	0.823
Conclusive stage									
I, II	14	0.021 ± 0.015		0.720 ± 0.579		0.060 ± 0.096		0.320 ± 0.189	
III, IV	27	0.032 ± 0.022	0.118	1.093 ± 0.609	0.066	0.133 ± 0.205	0.216	0.961 ± 0.795	0.005

Table 3. Correlations between SHH pathway molecules

		SHH	PTCH1	SMO	GLI1
SHH	Correlation coefficient		0.428	−0.108	0.183
	Significance probability		0.005	0.501	0.251
PTCH1	Correlation coefficient	0.428		0.349	0.675
	Significance probability	0.005		0.025	<0.001
SMO	Correlation coefficient	−0.108	0.349		0.695
	Significance probability	0.501	0.025		<0.001
GLI1	Correlation coefficient	0.183	0.675	0.695	
	Significance probability	0.251	<0.001	<0.001	

Most molecules correlated with each other.

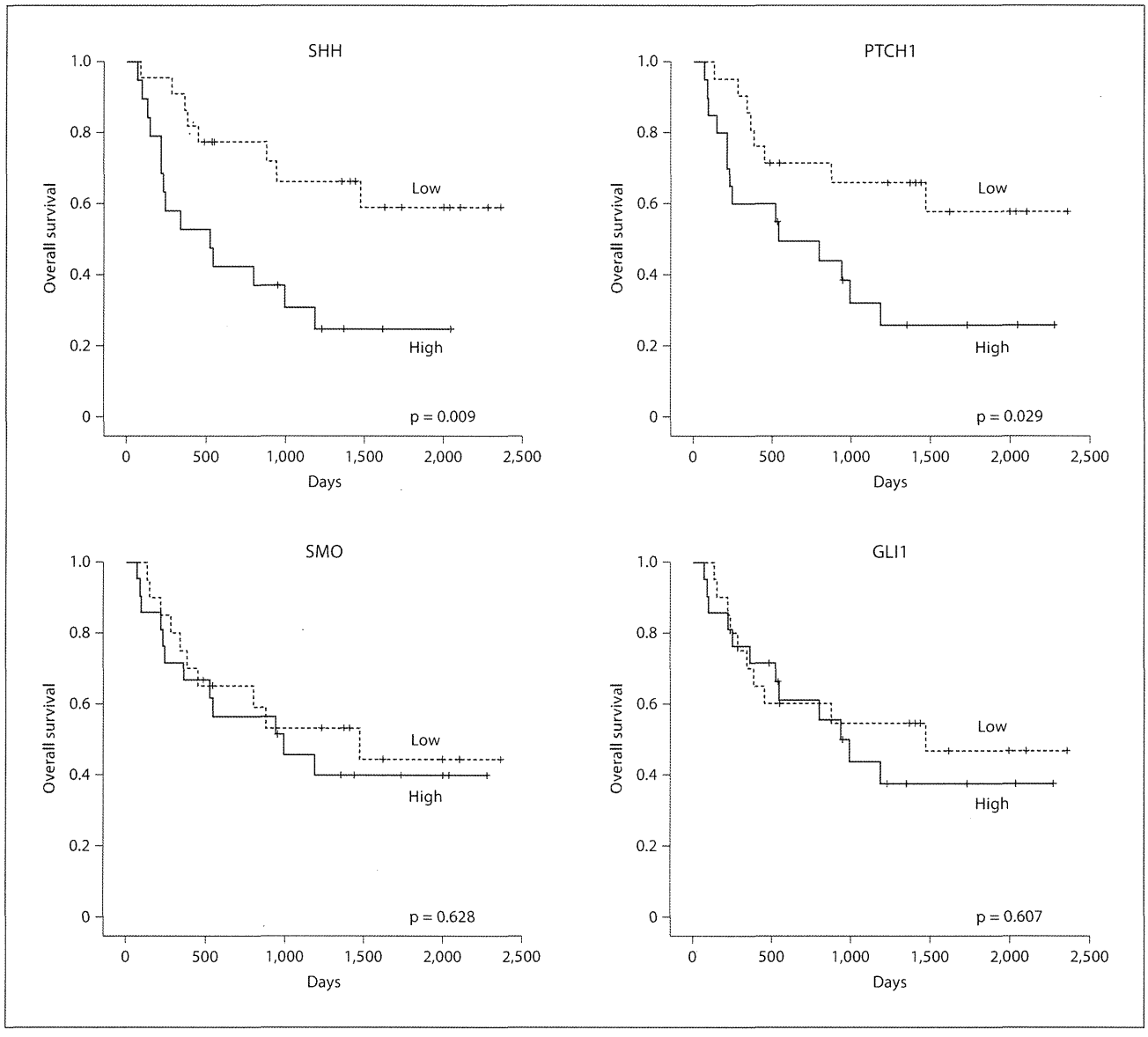

Fig. 3. Overall survival analysis with respect to the mRNA expression levels of SHH pathway-associated molecules. High *SHH* expression and high *PTCH1* expression were significantly associated with decreased overall survival.

tients were divided into high- and low-expressing groups, according to the median expression of each target. Patient survival time was then compared between the two groups. Overall survival time was significantly shorter in patients with high expression levels of *SHH* and *PTCH1* compared to patients with low expression levels (fig. 3), whereas no significant differences in survival time were seen with respect to levels of *SMO* or *GLI1* expression.

When these markers and clinicopathological factors were tested for their predictability of patient prognosis, Cox multivariate analysis for overall survival revealed that high expression of *PTCH1*, as well as liver metastasis, were independently associated with significantly decreased survival (table 4).

When these patients were divided into diffuse- and focal expression group according to SHH and PTCH1 pos-

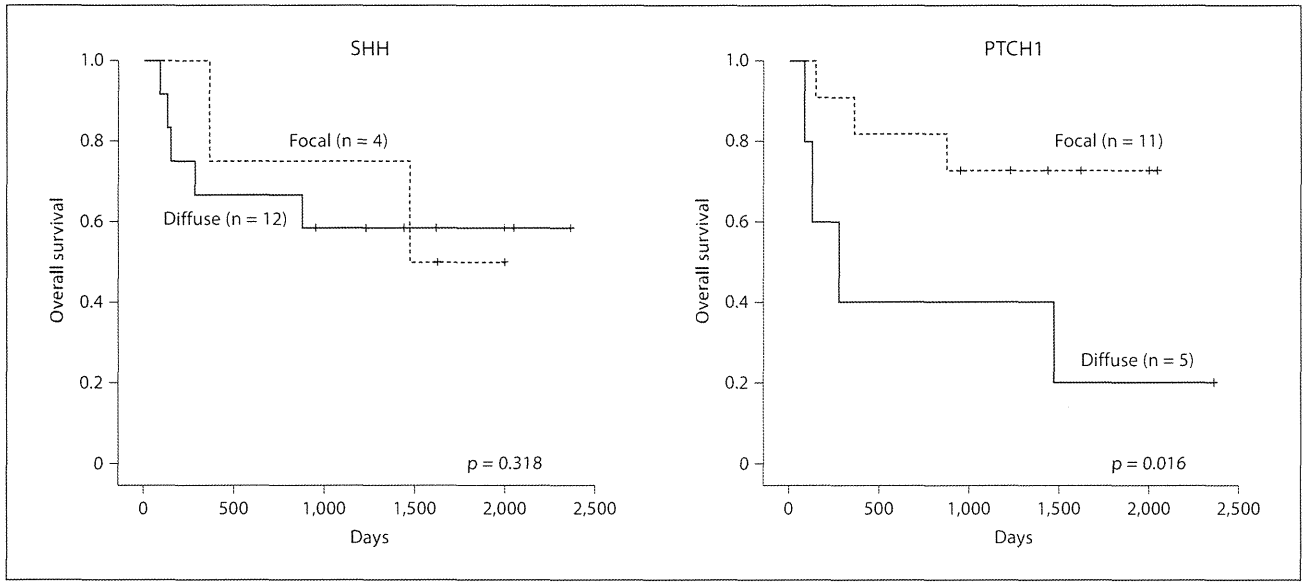

Fig. 4. Overall survival analysis with respect to the immunohistochemical expression levels of SHH and PTCH1 in differentiated type of cancer. Diffuse PTCH1 expression was significantly associated with decreased overall survival.

Table 4. Multivariate overall survival analysis (Cox proportional hazard model)

Variable	b	SE	p	HR	95% CI
Male vs. female	0.497	0.556	0.371	0.61	0.20–1.81
Age <60 vs. >60	–0.913	0.734	0.214	2.49	0.59–10.53
Differentiated vs. undifferentiated	–0.505	0.741	0.496	0.6	0.14–2.58
T1 vs. T2/3/4	–0.741	1.01	0.463	0.48	0.07–3.46
N0 vs. N1/2/3	1.405	0.733	0.055	0.24	0.06–1.03
H01 vs. H0	2.312	0.929	0.013	10.09	1.63–62.31
P1 vs. P0	0.266	0.934	0.776	1.31	0.20–8.14
SHH expression; high vs. low	–0.877	0.602	0.145	2.4	0.74–7.81
PTCH1 expression; high vs. low	–2.481	1.096	0.024	11.9	1.40–100.0
SMO expression; high vs. low	–0.13	0.995	0.896	1.12	0.16–8.00
GLI1 expression; high vs. low	2.097	1.246	0.092	0.12	0.01–1.41

SE = Standard error; HR = hazard ratio; CI = confidence interval.

Cox multivariate analysis revealed that liver metastasis and high expression of PTCH1 mRNA were independently associated with significantly decreased overall survival (p = 0.013 and p = 0.024, respectively).

itive area ratio in cancer tissue, overall survival time was significantly short in patients with diffuse expression of PTCH1 compared to patients with focal expression in differentiated type of cancer (fig. 4). However, it was not the case with undifferentiated type of cancer or if all cases were included for analysis.

Discussion

Recent studies have reported that alteration of the SHH signaling pathway results in distinct human cancer and that the SHH signaling pathway is required for the survival of several tumors, suggesting that SHH signaling may play a critical role in tumor proliferation or mainte-

nance [1]. Deregulation of SHH signaling has been reported to occur in gastric cancer, mainly using immunohistochemical analysis [15, 16, 18, 19]. However, the clinical impact, especially with respect to patient survival, of the SHH pathway is not yet determined. In this report, we have clearly shown that the expression of SHH pathway-related molecules significantly correlated with certain clinicopathological factors and with patients' survival.

Previous studies, using immunohistochemistry, have shown that the expression of SHH correlated with advanced stage tumors or with tumor differentiation [15, 16, 19]. In our study, we failed to show significant correlations between the expressions of SHH related molecules and clinicopathological factors of tumor. Meanwhile we found was significant correlation in RQ-PCR assay. Fukaya et al. [17] have demonstrated that SHH was not expressed in actual cancer cells, but was expressed in myofibroblastic cells in the interstitial tissue of the tumor. There were some same cases in our study. Accordingly, our result of RQ-PCR might reflect not only tumor cells but also interstitial cells surrounding the tumor. Meanwhile, immunohistochemistry showed that SHH expression in undifferentiated tumor was low [18]. In contrast, we found that *SHH* mRNA levels were high in undifferentiated tumor, as previously shown by Fukaya et al. [15]. This suggests an interaction between the interstitial tissue and the tumor [20], but this needs further elucidation.

We reveal here, for the first time, that *PTCH1* mRNA level was significantly higher in peritoneal cytology positive cases compared to cytology negative cases, and that expression of *SMO* was significantly higher in cases with peritoneal metastasis compared to cases without peritoneal metastasis. It can be reasonably assumed that the alterations of *PTCH1* and possibly *GLI1* might be involved in tumor progression, including positive peritoneal cytology, since *GLI1* has been reported to modify several important molecules for tumor progression, such as cell adhesion molecules [21]. However, their involvement requires further elucidation.

We have also found that SHH signaling correlated with the prognosis of gastric cancer patients. High *PTCH1* mRNA level was recognized as an independent prognostic factor for survival using multivariate Cox proportional hazard analysis. Since *SHH* expression alone did not correlate with any clinicopathological factors, we determined the strength of correlation between the molecules in the pathway. *SHH* expression correlated with that of *PTCH1*, and *PTCH1* expression correlated with that of all of the molecules examined. Expression of *SMO* correlated with that of *GLI1*. This suggests that the pathway was activated in gastric cancer.

We tried to evaluate a role of SHH pathway activation on patient survival from another viewpoint. We divided the patients into three categories: all molecules were upregulated (group A), none of the molecules were activated (group B) and others (group C). We found that the worst prognosis was for group A, then group C and then group B. The same group order was also found for the depth of invasion and positive cytology of the peritoneal surface (data not shown). Together, our data could provide an important model to select patients who need adjuvant therapy.

Recent studies have shown that the SHH pathway is probably involved in carcinogenesis and/or progression of human cancer. Our study demonstrates an important role of SHH signaling as a prognostic indicator for patients with gastric cancer. For patients with differentiated type of tumor immunohistological evaluation of PTCH1 could be a simple prognostic indicator. Further studies will be needed to confirm the clinical impact of our results and to investigate the mechanisms of this pathway in gastric cancer.

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Disclosure Statement

There is no conflict of interests to disclose.

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