Pathol. Oncol. Res. (2011) 17:859–866
DOI 10.1007/s12253-011-9394-7

RESEARCH

Identification of PEG10 and TSG101 as Carcinogenesis, Progression, and Poor-Prognosis Related Biomarkers for Gallbladder Adenocarcinoma

Dong-cai Liu · Zhu-lin Yang · Song Jiang

Received: 13 November 2010 / Accepted: 16 March 2011 / Published online: 1 April 2011

Abstract PEG10 is a transcriptional factor while TSG101 is involved in numerous cellular processes, including apoptotic resistance. Overexpression of PEG10 and TSG101 were observed in a variety of human cancers. However, their expression and clinical significance in gallbladder cancer (GBC) have not yet been identified. To understand the tumor biology of GBC at the molecular level, we examined PEG10 and TSG101 expression in 108 adenocarcinomas, 15 gallbladder polyps, 35 chronic cholecystitis tissues, and 46 peritumoral tissues by using immunohistochemistry. Overexpression of PEG10 and TSG101 was detected in gallbladder adenocarcinoma (48.1% and 47.2%, respectively). Conversely, there was less expression detected in the peritumoral tissues (19.6%), adenomatous polyps (13.3%), and gallbladder epithelium with chronic cholecystitis (5.1%) ($p<0.01$, $p<0.05$, and $p<0.01$, respectively). Notably, the benign lesions with positive PEG10 and/or TSG101 expression showed moderately or severely atypical hyperplasia in gallbladder epithelium. The overexpression of PEG10 and TSG101 was significantly associated with differentiation, tumor mass, lymph node metastasis and invasion of adenocarcinoma. Univariate Kaplan-Meier analysis showed that overexpression of PEG10 ($p=0.041$) and TSG101 ($p=0.025$) was closely associated with decreased overall survival. Multivariate Cox regression analysis revealed that positive expression of PEG10 ($p=0.036$) or TSG101 ($p=0.022$) is a predictor of poor prognosis in gallbladder adenocarcinoma. Our study suggested that overexpression of PEG10 and TSG101 might be closely related to the carcinogenesis, progression, clinical biological behaviors, and prognosis of gallbladder adenocarcinoma.

Keywords Gallbladder cancer · Gallbladder polyp · Chronic cholecystitis · PEG10 · TSG101

D.-c. Liu
Department of Geriatric Surgery,
Central South University, Changsha,
Hunan 410011, China

Z.-l. Yang (✉) · S. Jiang
Research Laboratory of Hepatobiliary Diseases,
Second Xiangya Hospital,
Central South University, Changsha,
Hunan 410011, China
e-mail: yangzhulin8@sina.com

Introduction

PEG10 was first identified by Ono et al. as an imprinted gene that is paternally expressed and maternally silenced [1]. The human PEG10 gene is located on chromosome band 7q21, functioning as a transcriptional factor. PEG10 expression was detected in a variety of human normal tissues, including brain, kidney, lung, placenta, testis, ovary, spleen, lymphoblast, endothelial cells and thymus [2, 3], however, its exact roles remain unclear. Recently, the overexpression of PEG10 gene was detected in human cancers, including leukemia, breast cancer, hepatocellular carcinoma (HCC), prostate cancer, and pancreatic cancer [4]. However, the exact relationship between PEG10 and tumorigenesis has not yet been determined. The accumulated evidence suggested that PEG10 may be involved in oncogenesis by increasing the apoptotic resistance of tumor cells. For instance, overexpression of PEG10 increased apoptotic resistance in B cell lineage, acute and chronic lymphocytic leukemia CD23+CD5+ B cells [3, 5]. In HCC, PEG10 decreased cell death through interaction with the SIAH1, a mediator of apoptosis [2]. Moreover, PEG10 knockdown inhibits the proliferation of pancreatic carcino-

ma and HepG2 HCC cells [6], while knockout of PEG gene caused early embryonic lethality [10]. A recent report demonstrated that PEG10 expression can be regulated by the proto-oncogene c-myc via the binding of c-myc oncoprotein to the E-box containing region of the first intron of PEG10 [6–8]. PEG10 also interacts with TGF-β type I receptor ALK1 [9]. This evidence suggests that PEG10 might play a crucial role in carcinogenesis and tumor cell growth. Therefore, PEG10 might be a new biomarker for cancer diagnosis and prognosis as well as a target for developing new treatment agents. However, PEG10 expression in the benign and malignant lesions of the gallbladder has not yet been identified.

Liu et al. reported overexpression of tumor susceptibility gene 101 (TSG101) in human papillary thyroid carcinomas, which provided the earliest evidence linking TSG101 to carcinogenesis [11]. Subsequent studies identified overexpression of TSG101 in several other human cancers, including ovarian cancer [12], gastrointestinal tumors [13], colorectal carcinoma [14]. TSG101 was thought to be involved in tumorigenesis through close interaction with p53 within the p53/MDM2 feedback control loop [15]. TSG101 is originally identified as a potential tumor suppressor gene [16]. However, subsequent studies showed that deletion of TSG101 in cell cultures did not lead to uncontrolled cell growth while conditional knockout of TSG101 in mice did not result in neoplastic transformation. On the contrary, homozygous deletion of TSG101 in gene knockout mice resulted in embryonic lethality, whereas silencing of TSG101 expression in mammalian cells resulted in cell cycle arrest and cell death [12]. For example, gene silencing of TSG101 leads to growth arrest and cell death in breast and prostate cancer cells [17]. These results suggested that TSG101 plays a crucial role in cell survival. Recent studies indicated that TSG101 is an essential protein involved in numerous cellular processes associated with cell growth and signal transduction, such as transcriptional regulation, protein ubiquitination, cell cycle control, and vesicular transport [18]. However, the expression of TSG101 in gallbladder cancer and its clinical significance have not been reported.

Gallbladder cancer (GBC) is the most common biliary tract malignancy with overall poor prognosis. Its incidence exhibits a tremendous regional variability [19]. A progressive increase in incidence and mortality for this cancer has been reported worldwide [20]. Early diagnosis is generally impossible and most cases are diagnosed when the tumors are unresectable. Among these cases, palliative treatment remains as the only possible service that could be provided to patients, but with little benefit to the patient's survival [21]. The survival time of patients with GBC usually did not exceed 12 months. Unfortunately, there is almost no significant improvement in diagnostic methods and treatments during the past several decades. Thus, understanding tumor biology at the molecular level may help in developing a more successful strategy for early diagnosis and treatment. However, studies currently available in literature do not clearly define the molecular mechanisms involved in the pathogenesis of GBC. Although biomarkers for screening programs and predicting prognosis are under investigation, currently none of the proposed markers have reached clinical application [22]. For all these considerations, gallbladder cancers should merit much more scientific attention.

In this study, the expressions of PEG10 and TSG101 in resection specimens, including 108 adenocarcinomas, 46 peritumoral tissues, 15 adenomatous polyps, and 35 chronic cholecystitis of gallbladder, were examined using immunohistochemistry. The correlations of PEG10 and TSG101 expression with the progression and prognosis of adenocarcinoma as well as its clinical significance were evaluated.

Materials and Methods

Case Selection

A total of 204 specimens were studied ethically with pre-approval from The Ethics Committee for Human Research, Central South University. Totally, 108 adenocarcinoma samples underwent surgical resection or biopsy between 1996 and 2006. Forty-six peritumoral tissues were collected from the 108 adenocarcinoma. Thirty-five chronic cholecystitis tissues and 15 gallbladder polyp were harvested during the same period. All diagnoses were based on morphological criteria, immunohistochemical staining, and the clinical findings. Among the 108 adenocarcinoma, 31 subjects are male (28.7%) and 77 subjects are female (71.3%) with an age variation from 35 to 70 (52.6±11.2) years. Invasion of gallbladder surrounding tissues and organs was found in 59 subjects (54.6%) with adenocarcinoma, while 59 subjects had regional lymph node metastasis (54.6%); 58 subjects had gallstone (53.7%). Surgery included radical resection for 34 adenocarcinomas (31.5%), palliative surgery for 48 adenocarcinomas (44.4%) and no operation for 26 cases (24.1%) with only surgery biopsy. Survival information of 67 subjects among the 108 adenocarcinomas was obtained through letters and phone calls. Of which 20 subjects survived over 1 year and 47 subjects survived less than 1 year. The histopathologic subtypes of the 108 adenocarcinoma include 9 adenoma cancerations (8.2%, 7 well-differentiated and 2 moderately-differentiated), 29 well-differentiated adenocarcinomas (26.9%), 29 moderately-differentiated adenocarcinomas (26.9%), 30 poorly-differentiated adenocarcinomas (27.8%), and 11 mucinous adenocarcinomas (10.2%).

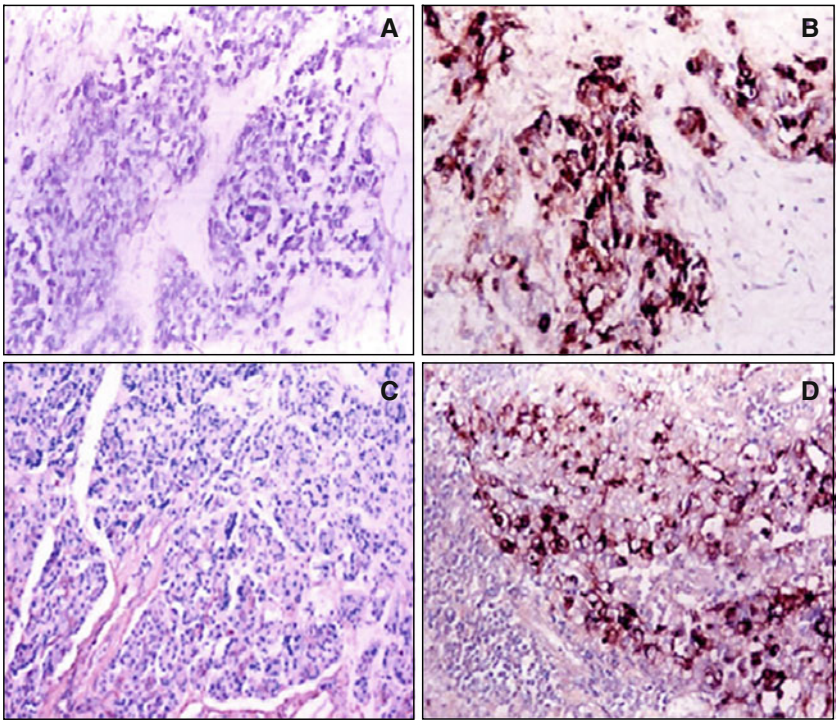

Fig. 1 PEG10 and TSG101 expression in the benign and malignant lesions of gallbladder. EnVision immunohistochemistry, original magnification x200. PEG10 positive reaction was mainly localized in the nucleus and cytoplasm and TSG101 positive expression was mainly localized in the cytoplasm and membrane **a**, Poorly-differentiated adenocarcinoma, PEG10 (−). **b**, Moderately-differentiated adenocarcinoma, PEG10 (+). **c**, Poorly-differentiated adenocarcinoma, TSG101 (−). **d**, Moderately-differentiated adenocarcinoma, TSG101 (+)

Among the 46 peritumoral tissues (distance from cancer≥ 3mm), 10 tissues are normal, 10 tissues are mild dysplastic, 12 tissues are moderate dysplastic, and 14 tissues are severe dysplastic gallbladder epithelium. Among the 15 subjects with gallbladder polyp, 5 subjects are males (33.3%) and 10 are females (66.7%). The polyp diameter is within 8–15 mm. The pathological examination confirmed that 10 polyps had normal gallbladder epithelium to mild dysplastic epithelium, and 5 had moderate to severe dysplastic epithelium. Among the 35 chronic cholecystitis (15 have chronic cholecystitis only and 20 had chronic cholecystitis accompanied by gallbladder stone), 11 had normal, 12 had mild, 7 had moderate, and 5 had severe dysplastic gallbladder epithelium.

EnVision Immunohistochemistry

Four-micrometer-thick sections were cut from routinely paraffin-embedded tissues. Immunostaining was conducted by use of the ready-to-use, peroxidase-based EnVision™ Detection kit (Dako Laboratories, CA, USA) following the user manual. The rabbit anti-PEG10, mouse anti-TSG101 and HRP-conjugated anti-mouse and anti-rabbit second antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The positive control is the PEG10- and TSG101-positve hepatocellular carcinoma biopsy provided by EnVision kit. The negative control was included in every staining by replace of the primary antibody with PBS. PEG10 positive cells exhibited brown nuclear and cytoplasm staining. TSG101 positive cells exhibited brown membrane and cytoplasmic staining. The positive case was determined mainly by the percentage of positive cells with a cutoff value of 25% and secondarily by strength of PEG10 or TSG101 staining. The positive and negative cells were counted from 10 random fields and the value of positivity was calculated in the whole 10 fields. The staining strength was graded from 1 to 3: 1 score for no positive staining or a uncertainly weak staining; 2 scores for weak to moderate staining; 3 scores for moderate to strong staining. The case with both positive cells≥25% and scores≥2 was considered positive whereas was considered negative [23–27]. Few cases with 10% to 25% positive cells and 3 scores in staining were also regarded as positive.

Table 1 PEG10 and TSG101 expression in benign and malignant lesions of gallbladder

Tissue Types	Total No:	PEG10			TSG101		
		Pos No:(%)	χ^2	[a]P	Pos No:(%)	χ^2	[a]P
GBC	108	52(48.1)			51(47.2)		
PT. tissue	46	9(19.6)	11.02	<0.01	9(19.6)	10.38	<0.01
A. polyp	15	2(13.3)	6.48	<0.05	2(13.3)	6.16	<0.05
C. cholecystitis	35	2(5.7)	20.25	<0.01	2(5.7)	19.52	<0.01

[a] compared with gallbladder adenocarcinoma (GBC). PT tissue: peritumoral tissue; A. Polyp: adenomatous polyp; C. Cholecystitis: chronic cholecystitis; Pos No: positive numbers.

Table 2 The association of PEG10 and TSG101 expression with the clinicopathological characteristics of gallbladder adenocarcinoma

Clinicopathological characteristics	Total No:	PEG10			TSG101		
		Pos No:(%)	χ^2	*P*	Pos No:(%)	χ^2	*P*
Sex							
Male	31	15(48.4)	0.01	>0.05	11(35.5)		
Female	77	53(49.4)			40(51.9)	2.40	>0.05
Age(years)							
≤45	24	10(41.7)	0.62	>0.05	9(37.5)	1.17	>0.05
>45	84	42(50.0)			42(50.0)		
Differentiation*							
Well	36	11(30.5)	10.24	<0.05	10(27.8)	10.26	<0.05
Moderately	31	15(48.4)			16(51.6)		
Poorly	30	21(70.0)			20(66.7)		
Mucinous A.	11	5(45.5)			5(45.5)		
Tumor size (cm)							
<2	31	10(32.2)	4.40	<0.05	10 (32.2)	3.91	<0.05
≥2	77	42(54.5)			41 (53.3)		
Lymph node metastasis							
no	49	17(34.7)	6.50	<0.05	17 (34.7)	5.65	<0.05
yes	59	35(59.3)			34 (57.6)		
Invasion of peritumoral tissue							
no	49	17(34.7)	6.50	<0.05	16 (34.7)	7.64	<0.05
yes	59	35(59.3)			35 (59.3)		
Gallstones							
no	50	21(42.0)	1.41	>0.05	22 (44.0)	0.39	>0.05
yes	58	31(53.4)			29 (50.0)		

*Comparation between well differentiated and moderately differentiated adenocarcinoma: $\chi_{PEG10}^2 = 2.23$, $p > 0.05$; $\chi_{TSG101}^2 = 3.99$, $p < 0.05$.

*Comparation between well-differentiated and poorly-differentiated adenocarcinoma: $\chi^2_{PEG10} = 10.19$, $p < 0.01$; $\chi^2_{TSG101} = 9.98$, $p < 0.01$.

*Comparation between moderately differentiated and poorly differentiated adenocarcinoma: $\chi^2_{PEG10} = 2.94$, $p > 0.05$; $\chi^2_{TSG101} = 1.43$, $p > 0.05$.

Mucinous A.: mucinous adenocarcinoma.

Statistical Analysis

Data was analyzed using the statistical package for the Social Sciences Version 13.0 (SPSS 13.0). The inter-relationship of PEG10 or TSG101 expression with histology or clinical factors was analyzed using χ^2 or Fisher's exact test. Kaplan-Meier and log-rank test were used for univariate survival analysis. Cox proportional hazards model was used for multivariate analysis and to determine the 95% confidence interval.

Results

PEG10 and TSG101 Expression in Gallbladder Adenocarcinoma, Peritumoral Tissue, Adenomatous Polyp, and Gallbladder Epithelium with Chronic Cholecystitis

Immunohistochemistry revealed that PEG10 positive reaction was mainly localized in the nucleus and cytoplasm while TSG101 positive expression was localized in the cytoplasm and membrane (Fig. 1). The rate of PEG10 and TSG101 overexpression was significantly higher in adenocarcinoma (48.1% and 47.2%, respectively) than that in the peritumoral tissues (19.6%), adenomatous polyps (13.3%), or gallbladder epithelium with chronic cholecystitis (5.1%) ($p < 0.01$, $p < 0.05$, and $p < 0.01$, respectively). The epithelium of benign gallbladder with higher PEG10 and/or TSG101 expression showed moderate to severe dysplasia (Table 1). These results suggested that both PEG10 and TSG101 are high expressed in gallbladder adenocarcinoma and they could be regarded as molecular marker to evaluate the malignancy of metaplasia. Among the 52 PEG10 positive cases, TSG101 was positively expressed in 38 cases. In contrast, among the 56 PEG10 negative cases, TSG101 was negatively expressed in 43 cases ($\chi^2 = 26.90$, $p < 0.01$), suggesting a high consistency between these two markers.

The Association of PEG10 and TSG101 Expression with Clinicopathological Characteristics of Gallbladder Adenocarcinoma

As shown in Table 2, the rate of PEG10 and TSG101 overexpression in tumor tissues was significantly lower in cases with small tumor size (< 2 cm), no lymph node metastasis, and no invasion compared to cases with larger tumor size (> 2 cm), having lymph node metastasis and invasion to the gallbladder surrounding tissues and organs ($ps<0.05$). However, PEG10 and TSG101 expression in poorly-differentiated adenocarcinoma was significantly higher than that in the well-differentiated adenocarcinoma ($\chi^2_{PEG10}=10.19$, $p<0.01$; $\chi^2_{TSG101}=9.98$, $p<0.01$); PEG10 expression in well-differentiated adenocarcinoma was not different from moderately-differentiated adenocarcinoma ($\chi_{PEG10}{}^2=2.23$, $p>0.05$), whereas TSG101 expression in well-differentiated adenocarcinoma was significantly lower than that in moderately-differentiated adenocarcinoma

Table 3 Relationship between PEG10 and TSP101 expression, clinicopathological characteristics and average survival of gallbladder adenocarcinoma patients

Clinicopathological Characteristics	Samples (n)	Average survival(Month)	*P value*
Sex			
Male	19	10.0 (4–16)	0.910
Female	48	10.0 (4–18)	
Age (years)			
≤ 45	11	8.0 (4–14)	0.121
>45	56	10.0 (4–18)	
Pathological Types			
Adenoma	8	12.0 (8–18)	
Well	20	10.0 (4–18)	
Moderately	20	10.0 (4–18)	0.031
Poorly	12	8.0 (4–10)	
Mucinous A.	7	10.0 (6–16)	
Tumor size (cm)			
<2	20	14.0 (4–18)	0.003
≥2	47	8.0 (4–18)	
Lymph node metastasis			
no	36	12.0 (4–18)	0.005
yes	31	8.0 (4–18)	
Invasion of peritumoral tissue			
no	39	10.0 (4–18)	0.002
yes	28	8.0 (4–16)	
PEG10			
+	30	9.3(4–18)	0.041
−	37	11.3(4–18)	
TSG101			
+	32	9.3(4–18)	0.025
−	35	11.4(4–18)	

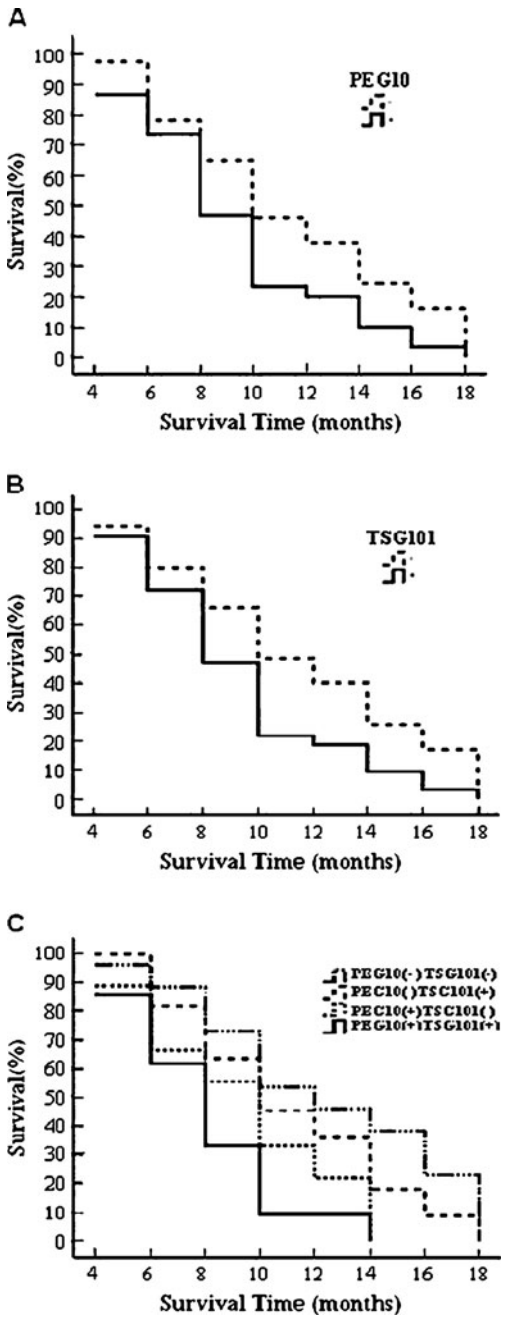

Fig. 2 PEG10 and/or TSG101 expression and survival in patients with adenocarcinoma of gallbladder. **a**, Kaplan-Meier plots of overall survival in patients with gallbladder adenocarcinoma and with PEG10 positive and negative expression. **b**, Kaplan-Meier plots of overall survival in patients with gallbladder adenocarcinoma and with TSG101 positive and negative expression. **c**, Kaplan-Meier plots of overall survival in patients with gallbladder adenocarcinoma and with PEG10(+) (−) and TSG101 (+) (−) expression

Table. 4 Multivariate Cox regression analysis of survival rate in 67 gallbladder adenocarcinoma patients

Groups	Factors	RC*	SE*	RR*	*P value*	95% confidence interval	
						Lower	Upper
Pathological Types:	adenoma/well/moderately/ poorly/mucinous adenocacinoma	0.716	0.394	2.046	0.097	0.95	4.43
Tumor size (cm)	< 2.0/≥ 2.0	1.002	0.466	2.724	0.041	1.09	6.79
Lymph node metastasis	no/yes	1.193	0.489	3.297	0.019	1.26	8.60
Invasion of peritumoral tissue	no/yes	0.891	0.448	2.438	0.047	1.01	5.87
PEG10	−/+	10.997	0.421	2.710	0.036	1.19	6.19
TSG101	−/+	1.116	0.508	3.053	0.022	1.13	8.26

*RR: Relative risk; *SE: standard error; *RC: Regression Coefficients

($\chi_{TSG101}{}^2$=3.99, p< 0.05). Both PEG10 and TSG101 expression had no difference between moderately-differentiated and poorly-differentiated adenocarcinoma (χ^2_{PEG10}=2.94, p> 0.05; χ^2_{TSG101}=1.43, p>0.05). PEG10 and TSG101 exhibited no significant association with mucinous adenocarcinoma and other clinicopathological characteristics, such as the sex, age, and history of gallstones.

The correlation Between PEG10 and/or TSG101 Expression with Survival in Patients with Gallbladder Adenocarcinoma

Survival information of 67 subjects among the 108 subjects of gallbladder adenocarcinoma was obtained through letters and phone calls. Of which 20 subjects survived over 1 year and 47 subjects survived less than 1 year with an average survival time of 9.6±5.2 months. Among the 67 subjects, 30 (44.8%) and 32 (47.8%) of subjects had PEG10 and TSG101 positive expression, respectively. The Kaplan-Meier survival analysis revealed that the histological type (p=0.031), tumor size (p=0.003), lymph node metastasis (p=0.005) and invasion (p=0.002) were significantly associated with the average survival time in subjects with adenocarcinoma. The average survival time in PEG10 positive subjects was significantly lower than that having negative PEG10 expression (p=0.041), but the average survival time in TSG101 positive subjects was significantly lower than that with negative TSG101 expression (p=0.025). In addition, the average survival time in patients having low/no PEG10 and TSP101 expression was significantly higher than that in other three subtypes (Table 3, Fig. 2a & c). Cox multivariate analysis showed that the tumor size (≥ 2cm), lymph node metastasis, invasion as well as PEG10-positive expression or TSG101-positive expression was negatively correlated with postoperative survival, and positively correlated with mortality, suggesting that both PEG10 and TSG101 are risk factor (Table 4).

Discussion

Although the overexpression of PEG10 and TSG101 in cancer cells was previously reported, their expression in the benign and malignant lesions of the gallbladder has not yet been identified. In this study, we identified PEG10 and TSG101 expression in an extensive collection of human gallbladder cancer samples. We found that 48.1% of adenocarcinomas exhibited overexpression of PEG10 and 47.2% exhibited a significantly elevated TSG101 level. Moreover, both the PEG10 and TSG101 overexpression were closely correlated with the differentiation of adenocarcinoma, lymph node metastasis and invasion. These correlations indicated that PEG10 and TSG101 are associated with the progression of GBC. We found that the survival time of patients with overexpression of PEG10 and TSG101 was significantly shorter than that in patients with lower expression; Cox multivariate analysis indicated that overexpression of PEG10 and TSG101 was positively correlated with mortality. These results suggested that PEG10 and TSG101 are important prognostic factors independent of other clinicopathological factors. Therefore, PEG10 and TSG101 could be biological markers to predict the progression and prognosis of GBC. In the epithelium of benign gallbladder, PEG10 and TSG101 were highly expressed in moderate to severe dysplastic epithelium. In contrast, in the mild dysplastic or normal tissues, PEG10 and TSG101 had low/no expression. Thus, PEG10 and TSG101 could be used as a diagnosis marker to screen for neoplasia within benign lesions and to identify subgroups of patients who require more aggressive treatment.

The surgical removal of the gallbladder with a part of the liver and lymph dissection is still the main treatment for resectable GBC. However, most of the patients were diagnosed at the late stage. Thus, palliative chemotherapy and radiation therapy were the only choices left [21]. Few significant improvements were made during the past three

decades regarding the treatment of GBC. Although several genetic abnormalities in GBC had been described that might be etiologically important, these studies did not clearly define the molecular mechanisms involved in the pathogenesis of GBC [28]. These included mutations in the proteins K-RAS and p53, and altered expression of P-glycoprotein, COX-2 and epidermal growth factor receptor [28]. The lack of patients with bile duct neoplasms might be the limiting factor for any improvements [29]. In this study, an extensive collection of human gallbladder cancer samples (108 adenocarcinomas) was utilized to identify PEG10 and TSG101 protein levels and compared with 96 benign lesions. A significant correlation of these two markers with the differentiation, tumor mass, lymph node metastasis, invasion, and survival of adenocarcinoma was determined. Thus, PEG10 and TSG101 might be more important markers for development of new diagnostic and treatment agents.

Zhao et al. found that TSG101 is overexpressed in vincristine-resistant human gastric adenocarcinoma cell line SGC7901/VCR [30]. The same group later found that silencing of TSG101 expression significantly increased SGC7901/VCR sensitization to chemotherapeutic drugs through reducing ADR [31]. This suggests that TSG101 plays a critical role in chemoresistance. This might be an explanation for why chemotherapy and radiation therapy exhibited less effect in GBC [21]. Although no studies revealed that PEG10 is directly involved in chemoresistance, overexpressed PEG10 is involved in apoptotic resistance [5]. Previous studies demonstrated that knockdown of PEG10 inhibited the proliferation of cancer cells [6] and expression of human telomerase reverse transcriptase was downregulated when PEG10 was down knocked by siRNA [8]. These studies suggested that inhibition of PEG10 expression could be a useful strategy to treat the tumors having PEG10 overexpression. Similarly, silencing of TSG101 leads to growth arrest and cell death in breast and prostate cancer cells [17] and knockout of PEG10 gene can cause early embryonic lethality [10]. By considering that silencing of TSG101 expression can reverse chemoresistance and inhibit tumor cell proliferation while silencing of PEG10 could induce tumor cell apoptosis, the development of gene therapy to target these genes will sensitize the chemotherapy and maybe radiotherapy of GBC. There are several possible avenues to develop novel molecular targeting of TSG101 and/or PEG10. For example, a strategy that combines conditionally replicating adenovirus using the cancer-specific telomerase promoter to drive E1 expression and viral replication with E1-deleted replication defective adenovirus containing a siRNA expression cassette could selectively knockdown TSG101 and/or PEG10 expression in tumor tissue, but not in normal tissues [32–34].

In conclusion, the elevated expression of PEG10 and TSG101 in gallbladder adenocarcinoma suggested that they are important markers for the progression, clinical biological behavior and prognosis. Measurement of PEG10 and TSG101 expression could be used as a tool for early detection of gallbladder cancer in benign lesions as well as population screening. The involvement of TSG101 in chemoresistance and the role of PEG10 in apoptotic resistance suggested that these two markers have a strong potential to be selected as wonderful targets for development of agents to sensitize chemotherapy and radiotherapy. Also, patients with high PEG10 and TSG101 expression in their tumors are more likely to suffer from invasion and metastatic recurrence. These patients may need to be monitored closely for clinical signs of relapse, so that therapeutic inventions can be applied early enough for optimal outcomes.

Conflict of Interest Statement We declare that we have no conflict of interesting.

References

1. Ono R, Kobayashi S, Wagatsuma H et al (2001) A retrotransposon-derived gene, PEG10, is a novel imprinted gene located on human chromosome 7q21. Genomics 73:232–237
2. Okabe H, Satoh S, Furukawa Y et al (2003) Involvement of PEG10 in human hepatocellular carcinogenesis through interaction with SIAH1. Cancer Res 63:3043–3048
3. Kainz B, Shehata M, Bilban M et al (2007) Overexpression of the paternally expressed gene 10 (PEG10) from the imprinted locus on chromosome 7q21 in high-risk B-cell chronic lymphocytic leukemia. Int J Cancer 121:1984–1993
4. Tsuji K, Yasui K, Gen Y et al (2010) PEG10 is a probable target for the amplification at 7q21 detected in hepatocellular carcinoma. Cancer Genet Cytogenet 198:118–125
5. Chunsong H, Yuling H, Li W et al (2006) CXC chemokine ligand 13 and CC chemokine ligand 19 cooperatively render resistance to apoptosis in B cell lineage acute and chronic lymphocytic leukemia CD23 + CD5+ B cells. J Immunol 177:6713–6722
6. Li CM, Margolin AA, Salas M et al (2006) PEG10 is a c-MYC target gene in cancer cells. Cancer Res 66:665–672
7. Ip WK, Lai PB, Wong NL et al (2007) Identification of PEG10 as a progression related biomarker for hepatocellular carcinoma. Cancer Lett 250:284–91
8. Jie X, Lang C, Jian Q et al (2007) Androgen activates PEG10 to promote carcinogenesis in hepatic cancer cells. Oncogene 26:5741–5751
9. Lux A, Beil C, Majety M et al (2005) Human retroviral gag- and gag-pol-like proteins interact with the transforming growth factor-beta receptor activin receptor-like kinase 1. J Biol Chem 280:8482–8493
10. Ono R, Nakamura K, Inoue K et al (2006) Deletion of Peg10, an imprinted gene acquired from a retrotransposon, causes early embryonic lethality. Nat Genet 38:101–106
11. Liu RT, Huang CC, You HL et al (2002) Overexpression of tumor susceptibility gene TSG101 in human papillary thyroid carcinomas. Oncogene 21:4830–4837

12. Young TW, Rosen DG, Mei FC et al (2007) Up-regulation of tumor susceptibility gene 101 conveys poor prognosis through suppression of p21 expression in ovarian cancer. Clin Cancer Res 13:3848–3854
13. Koon N, Schneider-Stock R, Sarlomo-Rikala M et al (2004) Molecular targets for tumour progression in gastrointestinal stromal tumours. Gut 53:235–240
14. Ma XR, Edmund Sim UH, Pauline B et al (2008) Overexpression of WNT2 and TSG101 genes in colorectal carcinoma. Trop Biomed 25:46–57
15. Li L, Liao J, Ruland J et al (2001) A TSG101/MDM2 regulatory loop modulates MDM2 degradation and MDM2/p53 feedback control. Proc Natl Acad Sci USA 98:1619–1624
16. Li L, Cohen SN (1996) Tsg101: a novel tumor susceptibility gene isolated by controlled homozygous functional knockout of allelic loci in mammalian cells. Cell 85:319–329
17. Zhu G, Gilchrist R, Borley N et al (2004) Reduction of TSG101 protein has a negative impact on tumor cell growth. Int J Cancer 109:541–547
18. Bashirova AA, Bleiber G, Qi Y et al (2006) Consistent effects of TSG101 genetic variability on multiple outcomes of exposure to human immunodeficiency virus type 1. J Virol 80:6757–6763
19. Goldin RD, Roa JC (2009) Gallbladder cancer: a morphological and molecular update. Histopathology 55:218–229
20. Gatto M, Bragazzi MC, Semeraro R et al (2010) Cholangiocarcinoma: update and future perspectives. Dig Liver Dis 42:253–260
21. Jayaraman S, Jarnagin WR (2010) Management of gallbladder cancer. Gastroenterol Clin North Am 39:331–342
22. Maurya SK, Tewari M, Mishra RR (2010) Genetic abberations in gallbladder cancer. Surg Oncol Sep 28. [Epub ahead of print]
23. Sanada Y, Yoshida K, Ohara M, Tsutani Y (2007) Expression of orotate phosphoribosyltransferase (OPRT) in hepatobiliary and pancreatic carcinoma. Pathol Oncol Res 13:105–113
24. Chang HJ, Yoo BC, Kim SW, Lee BL, Kim WH (2007) Significance of PML and p53 protein as molecular prognostic markers of gallbladder carcinomas. Pathol Oncol Res 13:326–335
25. Németh Z, Szász AM, Somorácz A, Tátrai P, Németh J et al (2009) Zonula Occludens-1, Occludin, and E-cadherin Protein Expression in Biliary Tract cancers. Pathol Oncol Res 15:533–539
26. Li Q, Yang Z (2009) Expression of phospho-ERK1/2 and PI3-K in benign and malignant gallbladder lesions and its clinical and pathological correlations. J Exp& Clin Cancer Res 28:65–72
27. Li Q, Yang Z, Liu J, et al (2010). Expression of CDx2 and Hepatocyte antigen in benign and malignant lesions of gallbladder and its correlation with histopathologic typr and clinical outcome. Pathol Oncol Res Dec 28. 2010 [Epub ahead of print]
28. Malik IA (2004) Gallbladder cancer: current status. Expert Opin Pharmacother 5:1271–1277
29. Tucek S, Tomasek J, Halámkova J (2010) Bile duct malignancies. Klin Onkol 23:231–241
30. Zhao Y, You H, Liu F et al (2002) Differentially expressed gene profiles between multidrug resistant gastric adenocarcinoma cells and their parental cells. Cancer Lett 185:211–218
31. Shen H, Pan Y, Han Z et al (2004) Reversal of multidrug resistance of gastric cancer cells by downregulation of TSG101 with TSG101siRNA. Cancer Biol Ther 3:561–565
32. Lee CT, Park KH, Yanagisawa K et al (2004) Combination therapy with conditionally replicating adenovirus and replication defective adenovirus. Cancer Res 64:6660–6665
33. Lee CT, Lee YJ, Kwon SY et al (2006) In vivo imaging of adenovirus transduction and enhanced therapeutic efficacy of combination therapy with conditionally replicating adenovirus and adenovirus-p27. Cancer Res 66:372–377
34. Huang Q, Zhang X, Wang H et al (2004) A novel conditionally replicative adenovirus vector targeting telomerase-positive tumor cells. Clin Cancer Res 10:1439–1445