Cell Biochem Biophys (2011) 61:703–710
DOI 10.1007/s12013-011-9229-x

TRANSLATIONAL BIOMEDICAL RESEARCH

The Co-expression of USP22 and BMI-1 May Promote Cancer Progression and Predict Therapy Failure in Gastric Carcinoma

Dong-Dong Yang · Bin-Bin Cui · Ling-yu Sun · Hong-qun Zheng · Qi Huang · Jin-Xue Tong · Qi-Fan Zhang

Published online: 7 July 2011

Abstract Recent experimental evidence support the model in which the simultaneous induction of BMI-1 and USP22 is critical during cancer progression. Whether this model may affect gastric cancer (GC) progression is worthy of additional study. In this study, we examined the significance of the USP22 and BMI-1 expression in GC ($n = 219$), non-cancerous mucosa ($n = 37$), and lymph node metastasis ($n = 37$). The protein expression level of USP22 and BMI-1 were concomitantly up-regulated from non-cancerous mucosa to primary carcinoma and from carcinomas to lymph node metastasis ($P < 0.001$). A statistical correlation was observed between USP22 and BMI-1 expression in GC tissues ($n = 219$, $r = 0.634$, $P < 0.001$) and in lymph node metastasis ($n = 37$, $r = 0.689$, $P < 0.001$). The incidence of positive expression was 57.08% for USP22, 49.32% for BMI-1, and 45.21% for USP22/BMI-1 in 219 GC tissues, respectively. Co-positive of USP22/BMI-1 was significantly correlated with gross features ($x^2 = 14.256$, $P < 0.001$), differentiation ($x^2 = 5.872$, $P = 0.015$), pT classification ($x^2 = 18.486$, $P < 0.001$), pN classification ($x^2 = 9.604$, $P = 0.002$), pM classification ($x^2 = 32.766$, $P < 0.001$), and AJCC stage ($x^2 = 58.278$, $P < 0.001$). Notably, high USP22/BMI-1 expression was significantly associated with shorter disease-specific survival ($P < 0.001$). By Cox regression analysis, co-positive of USP22/BMI-1 was found to be an independent prognostic factor ($P = 0.002$). Our results indicated the simultaneous activation of USP22 and BMI-1 may associate with GC progression and therapy failure.

Keywords USP22 · BMI-1 · Cancer progression · Gastric carcinoma

Introduction

Recently, Glinsky GV and colleagues apply a mouse/human comparative translational genomics approach to identify an 11-gene Polycomb/cancer stem cell signature by the analysis of metastases and primary tumors from a transgenic mouse model of prostate cancer and cancer patients, which could powerfully predict the therapeutic outcome of individual cancer patients [1–4]. Cancer cells manifesting this signature would manifest aneuploid, anoikis-resistant, metastasis-enabling phenotype with altered cell cycle control [5]. Moreover, recent experimental and clinical observations identify the BMI-1 oncogene-driven pathway including 11-gene signature as one of the key regulatory mechanisms of "stemness" functions in both normal and cancer stem cells which are essential for tumor progression and metastases of epithelial malignancies [6–9].

Dong-Dong Yang and Bin-Bin Cui contributed equally to this work.

D.-D. Yang · L. Sun · H. Zheng · J.-X. Tong · Q.-F. Zhang (✉)
Department of Oncology, The Fourth Affiliated Hospital of Harbin Medical University, Yiyuan Street 37, Nangang District, Harbin 150001, Heilongjiang, People's Republic of China
e-mail: qifanzhang@hotmail.com

B.-B. Cui
Colorectal Surgery, The Third Affiliated Hospital of Harbin Medical University, Harbin 150001, Heilongjiang, People's Republic of China

Q. Huang
Center of Morphology, Harbin Medical University, Harbin 150001, Heilongjiang, People's Republic of China

This 11-gene signature contains Polycomb genes such as BMI-1 and RNF2/Ring1b and also a number of genes that are coregulated with BMI-1, potentially by the elevation of BMI-1 itself. In fact, increased expression of the BMI-1 oncogene is one of the key regulatory factors determining a cellular phenotype captured by the expression of death-from-cancer signature in a broad spectrum of therapy-resistant clinically lethal malignancies [1, 2, 5]. Recent observations indicate that an oncogenic role of the BMI-1 activation is a common event, which may contribute to progression of many types of the epithelial malignancies and other solid tumors [10].

Unlike the other genes in this signature, no direct mechanistic link to human gastric cancer (GC) has been described to USP22. Sequence analysis reveals that USP22, a novel deubiquitinating enzyme gene, belongs to a large family of proteins with ubiquitin hydrolase activity [11]. Moreover, USP22 as one subunit of the human SAGA complex, can function as activator for nuclear receptor-mediated transactivation [12]. More recently, two independent studies provide compelling evidence that USP22 can deubiquitinate H2Aub1 or H2Bub1 in vitro, which is intimately linked to transcription activation, epigenetic regulation, and cancer progression [13–15].

Until now, many observations show the biological function of USP22 and BMI-1 are largely overlapping. For example, USP22 and BMI-1 are overexpressed in aggressive tumors which all target G1 phase [13, 16]. The PRC1 subunit BMI-1 and the hSAGA subunit USP22 are required for appropriate transit through the cell cycle by regulating the same genes such as CDK2NA [13, 17]. USP22 is required for MYC function [13] and BMI-1 is originally discovered as an oncogene capable of cooperating with MYC during transformation [18]. Interestingly, whereas BMI-1 and RNF2/Ring1b form the E3 ligase that directs H2A ubiquitylation to repress global transcription [19], USP22 can remove this same modification from H2A [12]. Then, some authors propose a model in which the simultaneous induction of Polycomb ubiquitin ligases, such as BMI-1 and the ubiquitin hydrolase USP22, is critical during cancer progression because USP22 can recruit to allow certain essential cell cycle genes to be transcriptionally activated in the face of the global transcriptional repression catalyzed by the PRC1 complex [13].

Based on this idea, the essential role of USP22 and BMI-1 in GC progression should be documented. Here, we tested the validity of this concept with respect to primary GC and demonstrated that the USP22 and BMI-1 were activated in a majority of clinical samples of GC and co-expression of USP22 and BMI-1 was essential for cancer progression and poor prognosis of human GC.

Materials and Methods

Tumor Samples

The 293 formalin-fixed paraffin embedded specimens used to IHC were collected from 219 GC patients undergoing surgery between January 2004 and December 2005, and were grouped as non-cancerous mucosa ($n = 37$), primary carcinoma ($n = 219$), and lymph nodal metastasis ($n = 37$). The matching normal and lymph nodal metastasis was available for 37 of the primary carcinomas. Primary cancers were evaluated in accordance with the American Joint Committee on Cancer, 7th ed., staging system. All patients were followed-up to August 2010 or until death. Median follow-up time for survivors was 29.57 months (range 1.90–78.10 months). No patient received preoperative chemotherapy or radiotherapy.

All cases representing a spectrum of GC were retrieved from the Affiliated Tumor Hospital of Harbin Medical University. The Hospital Ethics Committee granted permission for the study.

Immunohistochemistry

Immunophenotype analysis of USP22 and BMI-1 was done as previously described [20, 21]. In brief, the formalin-fixed, paraffin-embedded sections (4 μm) were deparaffinized in xylene and rehydrated in a graded series of ethanol solutions. The sections were subsequently submerged in EDTA (pH 8) and autoclave at 121°C for 5 min to retrieve the antigenicity. Endogenous peroxidase was quenched with 3% H_2O_2 for 15 min. After washing with PBS, the sections were incubated with USP22 antibody (Abcam, ab4812, diluted at 1:400) and BMI-1 antibody (LifeSpan Biosciences, LS-C98480, diluted at 1:60) overnight at 4°C. The sections were incubated with peroxidase-conjugated streptavidin for 30 min and the reaction products were visualized with diaminobenzidine as a chromogen and counterstained with commercial hematoxylin. Negative controls were obtained by omitting the primary antibodies. Positive cases exhibited cytoplasmic signal associated to nuclear staining, whereas negative cases exhibited an unspecific cytoplasmic staining. USP22 and BMI-1 was considered positive in tissue samples exhibiting nuclear staining in $\geq$20% of gastric endothelial cells. The results of staining were evaluated by two independent pathologists who were blinded to the clinical data.

Date Analysis

Statistical analyses were performed by using the SPSS software package version 13.0. Correlation between

expression levels was studied using the Pearson coefficient. The chi-square test was used for comparison between groups. The disease-specific survival (DSS) was defined as that from the date of the operation to the date of death due to cancer. Survival curves were plotted by the Kaplan–Meier method and assessed using log-rank test. To evaluate the increment statistical power of the individual covariates as predictor of unfavorable prognosis, we performed Cox regression analyses. $P < 0.05$ was considered statistically significant.

Results

Analysis of Correlation of USP22 and BMI-1 Expression in Primary GC

Increased expression of oncogenes is often associated with gene amplification. In agreement with the proposed oncogenic role of the USP22 and BMI-1, we evaluated the protein expression level of USP22 and BMI-1 in primary GCs ($n = 219$), the paired non-cancerous mucosa tissues ($n = 37$), and lymph node metastasis tissues ($n = 37$). We found USP22 staining and BMI-1 staining were localized within the nuclei of gastric epithelial cells as well as some scattered infiltrated lymphocytes (Fig. 1). The USP22 expression increased significantly from non-cancerous mucosa to carcinomas ($P < 0.001$) and from carcinomas to lymph node metastasis ($P < 0.001$) (Fig. 2). Meanwhile, The BMI-1 expression also increased significantly from non-cancerous mucosa to carcinomas ($P < 0.001$) and from carcinomas to lymph node metastasis ($P < 0.001$) (Fig. 2). A statistical correlation was observed between USP22 and BMI-1 expression in GC tissues ($n = 219$, $r = 0.634$, $P < 0.001$; Fig. 1) and in lymph node metastasis ($n = 37$, $r = 0.689$, $P < 0.001$; Fig. 1). Among the 219 GC samples, the positive frequency was 57.08% for USP22, 49.32.3% for BMI-1, and 45.21% for USP22/BMI-1, respectively.

Analysis of Correlation of USP22 and BMI-1 with Clinicopathologic Findings

The correlation between the protein expression of USP22 and BMI-1 and clinicopathologic variables of GC were shown in Table 1. USP22 expression was significantly correlated with gross features ($x^2 = 16.157$, $P < 0.001$), differentiation ($x^2 = 6.817$, $P = 0.009$), pT classification ($x^2 = 27.871$, $P < 0.001$), pN classification ($x^2 = 11.959$, $P = 0.001$), pM classification ($x^2 = 21.550$, $P < 0.001$), and AJCC stage ($x^2 = 58.111$, $P < 0.001$). BMI-1 expression was significantly correlated with gross features ($x^2 = 14.824$, $P < 0.001$), differentiation ($x^2 = 4.948$, $P = 0.026$), pT classification ($x^2 = 22.319$, $P < 0.001$), pN classification ($x^2 = 11.118$, $P = 0.001$), pM classification ($x^2 = 26.894$, $P < 0.001$), and AJCC stage ($x^2 = 58.397$, $P < 0.001$). Moreover, co-positive USP22/BMI-1 was significantly correlated with gross features ($x^2 = 14.256$, $P < 0.001$), differentiation ($x^2 = 5.872$, $P = 0.015$), pT classification ($x^2 = 18.486$, $P < 0.001$), pN classification ($x^2 = 9.604$, $P = 0.002$), pM classification ($x^2 = 32.766$, $P < 0.001$), and AJCC stage ($x^2 = 58.278$, $P < 0.001$).

Analysis of Correlation of USP22 and BMI-1 with Therapy Outcome

We evaluated the prognostic value of USP22 and BMI-1 on disease-specific survival (DSS) in all patients. Kaplan–Meier analysis demonstrated that GC patients with USP22, BMI-1, and USP22/BMI-1 positive expression in GC had a significantly wore DSS compared to the patients with negative expression (58.8 ± 3.2 vs. 26.2 ± 2.2 months, $P < 0.001$; 48.4 ± 3.0 vs. 26.9 ± 2.4 months, $P < 0.001$; 48.2 ± 2.9 vs. 25.7 ± 2.4, $P < 0.001$; respectively) (Fig. 3). Of note, co-positive of USP22/BMI-1 remained a statistically significant prognostic marker in the Cox regression analysis ($P = 0.002$; Table 2). These results indicated that increased USP22/BMI-1 expression was associated with high likelihood of therapy failure in GC patients.

Discussion

Recent experimental observations have identified a conserved BMI-1-driven Polycomb signature as a key regulator of "stemness" in both tissue stem cells and cancer stem cells [22]; cancer stem cells are considered essential for tumor progression and metastases of epithelial malignancies. 11-gene Polycomb/cancer stem cell signature, one of conserved BMI-1-driven pathway, has been identified that a stem cell-like expression profile of this signature in primary tumors is a consistent powerful predictor of a short interval to disease recurrence, distant metastasis, and death after therapy in cancer patients diagnosed with 11 distinct types of cancer [2]. USP22, a novel deubiquitinating enzyme gene, posses the functions such as transcription activation, epigenetic regulation, and cancer progression [13–15]. Take together with the results of this study, these data support a model in which the simultaneous induction of BMI-1 and USP22, is critical during cancer progression [13]. Here, we concluded our results of experimental analyses and attempted to shed light into the clinic significance of USP22 and BMI-1 in GC.

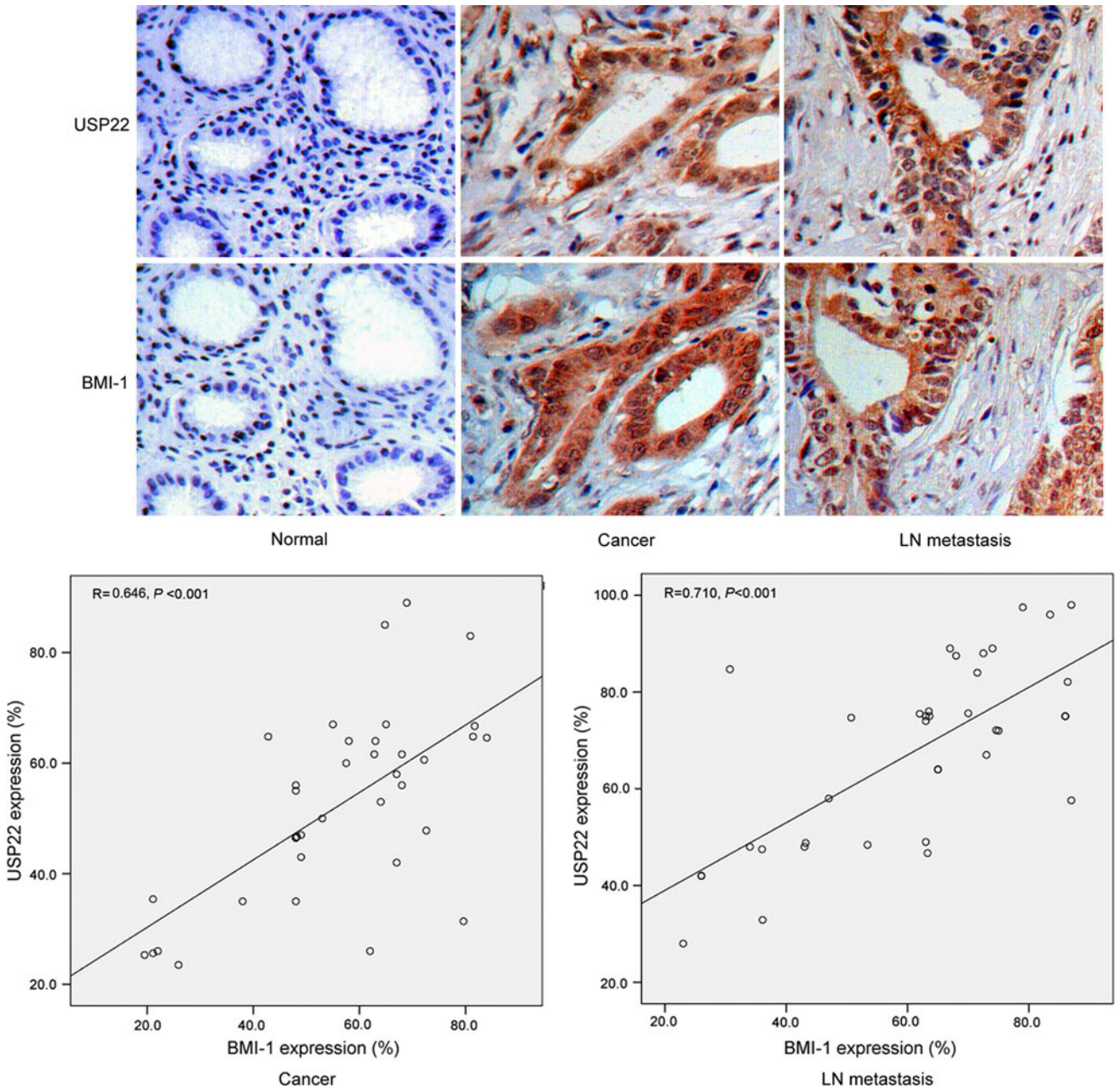

Fig. 1 Immunohistochemistry for USP22 and BMI-1 in the specimens. Strong USP22 and BMI-1 immunoreactivity was observed in the nuclear of tumor cells, as well as in some scattered infiltrated lymphocytes. A statistical correlation was observed between USP22 and BMI-1 expression in GC tissues ($n = 219$, $r = 0.634$, $P < 0.001$) and in lymph node metastasis ($n = 37$, $r = 0.689$, $P < 0.001$). Magnification: ×200

Cancer cell with an activated 11-gene signature would be expected to exhibit a concomitantly high expression of USP22 and BMI-1. In this study, we experimentally tested the relevance of this concept for GC. We applied immunohistochemistry analysis to measure the expression of both genes. We demonstrated that the protein expression level of USP22 and BMI-1 was concomitantly upregulated from non-cancerous mucosa to primary carcinoma and from carcinomas to lymph node metastasis ($P < 0.001$). A statistical correlation was observed between USP22 and BMI-1 expression in GC tissues and in lymph node metastasis. The incidence of positive expression was 57.08% for USP22, 49.32% for BMI-1, and 45.21% for USP22/BMI-1 in 219 GC tissues, respectively. Co-positive expression of both genes was associated with certain clinical-pathologic variables such as gross features, differentiation, pT classification, pN classification, pM classification, and AJCC stage. Those results confirmed that an oncogenic role of USP22 and BMI-1 activation may contribute to progression of GC.

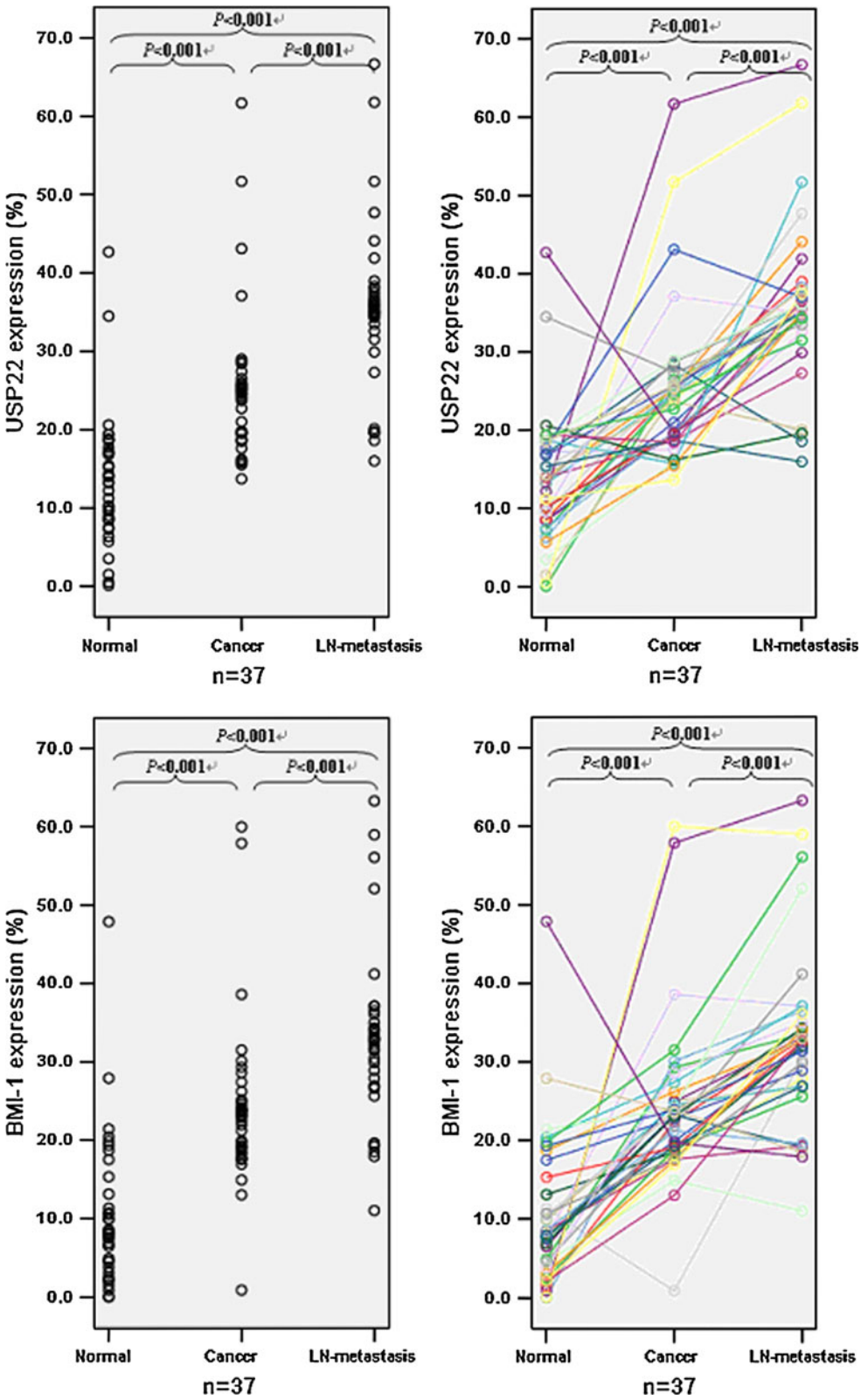

Fig. 2 Increase of USP22 and BMI-1 staining in GC progression. The protein expression level of USP22 and BMI-1 were concomitantly up-regulated from non-cancerous mucosa to primary carcinoma and from carcinomas to lymph node metastasis ($P < 0.001$)

To further validate the potential clinical utility of USP22 and BMI-1, we evaluated the therapy outcome-predictive power. Kaplan–Meier analysis demonstrated that GC patients with USP22, BMI-1, and USP22/BMI-1 positive expression in GC had a significantly wore DSS compared to the patients with negative expression. Of note, co-positive of USP22/BMI-1 remained a statistically significant prognostic marker in the Cox regression analysis. These results

Table 1 Correlation between BMI-1 and USP22 protein expression level and clinicopathological variables

Variables	No.	USP22		x^2, P	BMI-1		x^2, P	USP22 + BMI-1		x^2, P
		Negative	Positive		Negative	Positive		Negative	Positive	
Gender				1.931, 0.165			0.339, 0.560			1.001, 0.317
Male	162	74	88		84	78		92	70	
Female	57	20	37		27	30		28	29	
Age (years)				0.010, 0.920			0.002, 0.964			0.054, 0.816
<60	97	42	55		49	48		54	43	
≥60	122	52	70		62	60		66	56	
Tumor size (cm)				1.154, 0.283			0.566, 0.452			0.449, 0.503
<5	105	49	56		56	49		60	45	
≥5	114	45	69		55	59		60	54	
Gross features				16.157, <0.001			14.824, <0.001			14.256, <0.001
Noninfiltrating	30	23	7		25	5		26	4	
Infiltrating	189	71	118		86	103		94	95	
Differentiation				6.817, 0.009			4.948,0.026			5.872, 0.015
Well/moderate	81	44	37		49	32		53	28	
Poor/mucinous	138	50	88		62	76		67	71	
pT classification				27.871, <0.001			22.319, <0.001			18.486, <0.001
T 1/2	60	43	17		46	14		47	13	
T 3/4	159	51	108		65	94		73	86	
pN classification				11.959,0.001			11.118,0.001			9.604, 0.002
N0	42	28	14		31	11		32	10	
N1–N3	177	66	111		80	97		88	89	
pM classification				21.550, <0.001			26.894, <0.001			32.766, <0.001
M0	186	92	94		108	78		117	69	
M1	33	2	31		3	30		3	30	
AJCC stage				58.111, <0.001			58.397, <0.001			58.278, <0.001
I	34	30	4		32	2		33	1	
II	48	29	19		33	15		35	13	
III	104	33	71		43	61		48	56	
IV	33	2	31		3	30		4	29	

P value is considered significant if <0.05

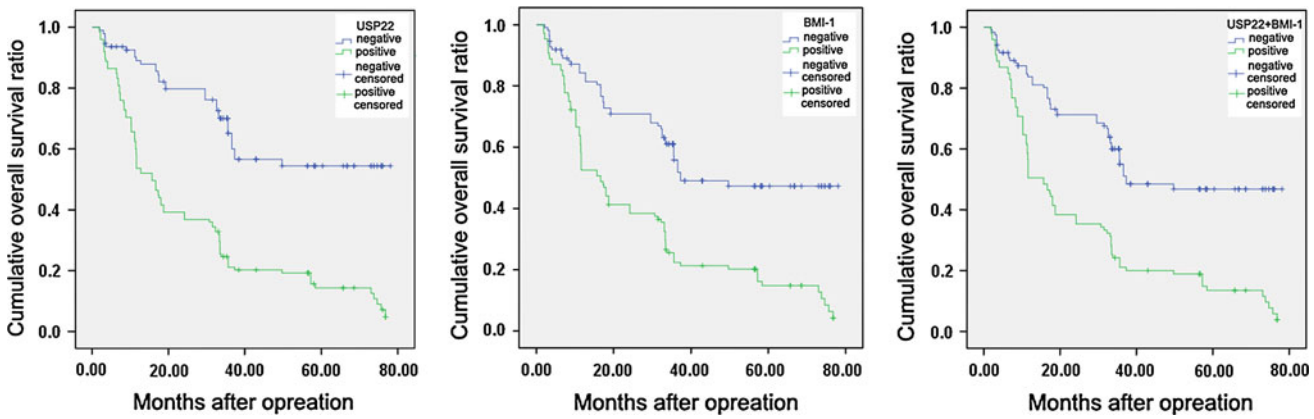

Fig. 3 Classification of GC patients into subgroups with distinct therapy outcome based on the level of proteins expression. Kaplan–Meier analysis revealed the patients with positive of USP22, BMI-1, and USP22 + BMI-1 had a significantly wore DSS after surgery than those with negative expression of corresponding proteins

Table 2 Univariate and multivariate Cox regression analyses for disease-specific survival in 219 GC patients

Variable	Univariate	Multivariate		
	P value	Risk ratio	95% CI	*P* value
Gender	0.729			
Age	0.111			
Tumor size	<0.001	2.069	1.402–3.053	<0.001
Gross features	<0.001			
Differentiation	0.002			
pT classification	<0.001	1.475	1.042–2.088	0.028
pN classification	<0.001	1.556	1.194–2.028	0.001
pM classification	<0.001			
AJCC stage	<0.001	1.494	1.063–2.152	0.014
USP22	<0.001			
BMI-1	<0.001			
USP22/BMI-1	<0.001	1.857	1.260–2.738	0.002

P value is significant if <0.05

indicated that increased USP22/BMI-1 expression was associated with high likelihood of therapy failure in GC patients, which was consisted with the results provided by 11-gene signature [5].

Since increased expression of the BMI-1 oncogene is one of the key regulatory factors determining a cellular phenotype captured by the expression of a death-from-cancer signature [4], our results support this hypothesis that cancer cells expressing USP22 may activate BMI1 oncogene-driven pathway signature which manifest a stem cell-like characteristics such as therapy-resistant and metastasis-enabling phenotypes. In fact, USP22 is required for Myc function and BMI-1 was originally discovered as an oncogene capable of cooperating with Myc during transformation [6, 13]. A possible mechanism for this collaboration is suggested by studies demonstrating that the ubiquitination/deubiquitination cycle of histones H2A and H2B is important in regulating chromatin dynamics and transcription mediated, in part, via "cross-talk" between histone ubiquitination and methylation [23], and this crosstalk could depend on the interplay between the Polycomb group, SAGA, and MLL3 (KMT2C)/MLL4(KMT2D) histone-modifying complexes [15]. The BMI-1 PcG protein is a component human Polycomb repressive complex 1-like complex, which is recently identified as the E3 ubiquitin ligase complex that is specific for histone H2A and plays a key role in Polycomb silencing [24]. USP22 as a subunit of the hSAGA coactivator complex can hydrolyze ubiquitin that has been conjugated to either histone H2A or H2B in vivo [12–14]. Taken together our results, we are tempting to speculate that the coordinate expression of USP22 and BMI-1 may target deubiquitylation of histones which is intimately linked to transcription activation, epigenetic regulation, and cancer progression. Future studies will be focused on determining how the H2Aub1 and H2Bub1 marks are linked with the global regulatory networks that are established by USP22 and BMI-1?

Acknowledgments This work was supported by Natural Science Foundation of Heilongjiang Province (ZD200920). This work was also supported by grant of Harbin Science and Technology Bureau (2007AA3CS083).

References

1. Glinsky, G. V. (2005). Death-from-cancer signatures and contribution of stem cells to metastatic cancer. *Cell Cycle, 4*, 1171–1175.
2. Glinsky, G. V., Berezovska, O., & Glinskii, A. B. (2005). Microarray analysis identifies a death from cancer signature predicting therapy failure in patients with multiple types of cancer. *Journal of Clinical Investigation, 115*, 1503–1521.
3. Glinsky, G. V. (2006). Integration of HapMap-based SNP pattern analysis and gene expression profiling reveals common SNP profiles for cancer therapy outcome predictor genes. *Cell Cycle, 5*, 2613–2625.
4. Glinsky, G. V. (2008). "Stemness" genomics law governs clinical behavior of human cancer: implications for decision making in disease management. *Journal of Clinical Oncology, 26*, 2846–2853.
5. Glinsky, G. V. (2006). Genomic models of metastatic cancer: functional analysis of death-from-cancer signature genes reveals aneuploid, anoikis-resistant, metastasis-enabling phenotype with altered cell cycle control and activated Polycomb Group (PcG) protein chromatin silencing pathway. *Cell Cycle, 5*, 1208–1216.
6. Liu, S., Dontu, G., Mantle, I. D., Patel, S., Ahn, N. S., Jackson, K. W., et al. (2006). Hedgehog signaling and bmi-1 regulate self-renewal of normal and malignant human mammary stem cell. *Cancer Research, 66*, 6063–6071.
7. Park, I. K., Qian, D., Kiel, M., Becker, M. W., Pihalja, M., Weissman, I. L., et al. (2003). Bmi-1 is required for maintenance of adult self-renewing haematopoietic stem cells. *Nature, 423*, 302–305.
8. Molofsky, A. V., Pardal, R., Iwashita, T., Park, I. K., Clarke, M. F., & Morrison, S. J. (2003). Bmi-1 dependence distinguishes neural stem cell self-renewal from progenitor proliferation. *Nature, 425*, 962–967.
9. Lessard, J., & Sauvageau, G. (2003). Bmi-1 determines the proliferative capacity of normal and leukaemic stem cells. *Nature, 423*, 255–260.
10. Berezovska, O. P. (2006). Essential role for activation of the Polycomb group (PcG) protein chromatin silencing pathway in metastatic prostate cancer. *Cell Cycle, 5*, 1886–1901.
11. Lee, H. J., Kim, M. S., Shin, J. M., Park, T. J., Chung, H. M., & Baek, K. H. (2006). The expression patterns of deubiquitinating enzymes, USP22 and Usp22. *Gene Expression Patterns, 6*, 277–284.
12. Zhang, X. Y., Pfeiffer, H. K., Thorne, A. W., & McMahon, S. B. (2008). USP22, an hSAGA subunit and potential cancer stem cell marker, reverses the polycomb-catalyzed ubiquitylation of histone H2A. *Cell Cycle, 7*, 1522–1524.
13. Zhang, X. Y., Varthi, M., Sykes, S. M., Phillips, C., Warzecha, C., Zhu, W., et al. (2008). The putative cancer stem cell marker USP22 is a subunit of the human SAGA complex required for activated transcription and cell-cycle progression. *Molecular Cell, 29*, 102–111.

14. Zhao, Y., Lang, G., Ito, S., Bonnet, J., Metzger, E., Sawatsubashi, S., et al. (2008). A TFTC/STAGA module mediates histone H2A and H2B deubiquitination, coactivates nuclear receptors, and counteracts heterochromatin silencing. *Molecular Cell, 29*, 92–101.
15. Pijnappel, W. W., & Timmers, H. T. (2008). Dubbing SAGA unveils new epigenetic crosstalk. *Molecular Cell, 29*, 152–154.
16. Yu, Q., Su, B., Liu, D., Liu, B., Fan, Y., Wang, Y., et al. (2007). Antisense RNA-mediated suppression of Bmi-1 gene expression inhibits the proliferation of lung cancer cell line A549. *Oligonucleotides, 17*, 327–335.
17. Jacobs, J. J., Kieboom, K., Marino, S., DePinho, R. A., & van Lohuizen, M. (1999). The oncogene and Polycomb-group gene bmi-1 regulates cell proliferation and senescence through the INK4A locus. *Nature, 397*, 164–168.
18. Haupt, Y., Alexander, W. S., Barri, G., Klinken, S. P., & Adams, J. M. (1991). Novel zinc finger gene implicated as myc collaborator by retrovirally accelerated lymphomagenesis in E mu-myc transgenic mice. *Cell, 65*, 753–763.
19. Schwartz, Y. B., & Pirrotta, V. (2007). Polycomb silencing mechanisms and the management of genomic programmes. *Nature Reviews. Genetics, 8*, 9–22.
20. Silva, J., García, J. M., Peña, C., García, V., Domínguez, G., Suárez, D., et al. (2006). Implication of polycomb members Bmi-1, Mel-18, and Hpc-2 in the Regulation of p16INK4a, p14ARF, h-TERT, and c-Myc expression in primary breast carcinomas. *Clinical Cancer Research, 12*, 6929–6936.
21. Liu, Y. L., Yang, Y. M., Xu, H., & Dong, X. S. (2010). Increased expression of ubiquitin-specific protease 22 can promote cancer progression and predict therapy failure in human colorectal cancer. *Journal of Gastroenterology and Hepatology, 25*, 1800–1805.
22. Glinsky, G. V. (2007). Stem cell origin of death-from-cancer phenotypes of human prostate and breast cancers. *Stem Cell Reviews, 3*, 79–93.
23. Henry, K. W., Wyce, A., Lo, W. S., Duggan, L. J., Emre, N. C., Kao, C. F., et al. (2003). Transcriptional activation via sequential histone H2B ubiquitylation and deubiquitylation, mediated by SAGA-associated Ubp8. *Genes and Development, 17*, 2648–2663.
24. Wang, H., Wang, L., Erdjument-Bromage, H., Vidal, M., Tempst, P., Jones, R. S., et al. (2004). Role of histone H2A ubiquitination in Polycomb silencing. *Nature, 431*, 873–878.