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Original Article

Increased expression of αSMA is associated with poor prognosis in patients with gastric cancer after surgical resection

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Abstract: Background: Cancer associated fibroblast (CAF), whose marker is α-smooth muscle actin (αSMA), plays vital roles in the oncogenesis and progression of various types of cancer, but its role in prognosis of gastric cancer patients remains unknown. The aim of this study was to investigate its prognostic value in patients with gastric cancer after surgical resection. Methods: αSMA expression was evaluated by tissue microarrays from 387 gastric cancer patients and statistically assessed for correlations with the clinical profiles and the prognosis of the patients with gastric cancer. The prognostic nomogram was designed to predict 3-year and 5-year overall survival probability. Results: αSMA expression in gastric cancer was increased compared with that in non-tumor tissues. (P = 0.001), and was significantly associated with Lauren classification (P = 0.041). Increased expression of αSMA in tumoral tissue was associated with decreased overall survival rate (P = 0.011). Multivariate Cox regression analysis suggested that αSMA expression was an independent prognostic indicator for gastric cancer except for T and N classification (P = 0.019). Using multivariate analysis, αSMA expression, T classification, and N classification were selected to generate the nomogram to predict the 3-year and 5-year overall survival. The c-index of this model was 0.673. The calibration curve for probability of survival showed good agreement between prediction by nomogram and actual observation. Conclusion: αSMA expression might be an independent prognostic factor for gastric cancer after surgical resection and could potentially be a high-priority therapeutic target. Incorporating αSMA expression into T and N classification can provide a good prognostic model.

Keywords: Gastric cancer, αSMA, overall survival, prognostic biomarker, nomogram

Introduction

Gastric cancer is one of the most common malignancies of the gastrointestinal tract [1, 2], and China is one of the countries with the highest incidence of gastric cancer and accounts for over 40% of all new cases worldwide. Despite the advancement of surgery, chemotherapy, and molecular-targeted therapy, the prognosis of advanced gastric cancer in China tends to be dismal. Recurrence after surgical resection and resistance to current therapeutic modalities are the major obstacles to improve the outcome. Prediction and effective management of recurrence are the most significant strategies to improve overall survival after surgical resection of gastric cancer. The tumor-node-metastasis (TNM) staging system of the Union for International Cancer Control/American Joint Committee on Cancer (UICC/AJCC) is a traditional model and an important prognostic factor for predicting the survival of patients with gastric cancer. However, gastric cancer patients in the same TNM stage may have different outcomes, partly owing to the heterogeneity at the molecular level of the disease. It is insufficient to only rely on TNM stage to predict the prognosis of gastric cancer. Therefore, there is an urgent need to explore and identify specific and sensitive markers as supplementary to TNM stage for the prediction of different risk stratum of gastric cancer.

Table 1. Relation between αSMA expression and clinical characteristics of patients with gastric cancer

Factor	Patients No.	αSMA		*P*-value
		High	Low	
Age (years)				0.656
≤ 60	211	109	102	
> 60	176	86	90	
Gender				0.760
Female	119	63	56	
Male	268	148	120	
Localization				0.600
Proximal	88	46	42	
Middle	58	29	29	
Distal	241	136	105	
Tumor size (cm)[a]				0.348
< 3.5	191	99	92	
≥ 3.5	196	112	84	
Neural invasion				0.257
No	285	150	135	
Yes	102	61	41	
Differentiation				0.784
Well	23	11	12	
Moderately	158	86	72	
Poorly	206	114	92	
Lauren classification				**0.041**
Intestinal type	239	120	119	
Diffuse type	94	54	40	
Mixed type	54	37	17	
T classification				0.812
Tis	10	5	5	
T1	70	41	29	
T2	51	25	26	
T3	70	36	34	
T4	186	104	82	
N classification				0.273
N0	154	78	76	
N1	43	24	19	
N2	68	34	34	
N3	122	75	47	

Abbreviations: αSMA = α-smooth muscle actin; *P*-value < 0.05 marked in bold font shows statistical significant.
[a]Split at median.

Emerging evidence demonstrates that mesenchyme is essential in tissue homeostasis, and fibroblasts in the microenvironment of tumor mesenchyme, often referred to as cancer-associated fibroblasts (CAFs) [3, 4], could alter the important functions in neoplastic cells and play crucial roles in cancer initiation, development, and progression. Neoplastic cells of different origins differ in their responses upon stimulation from CAFs, illustrating a unique relationship with CAFs across tumor types. The source of CAFs mostly derives from resident tissue fibroblasts and mesenchymal stem cells (MSCs), and the abundance of CAFs varies between different types of cancers. It has been reported that there are more CAFs in breast, prostate, and pancreatic cancers, whereas in brain, renal, and ovarian cancers, there are fewer [5-7]. Previous studies have revealed that CAFs may participate in the initiation and progression of cancer by altering tumor cells function [8]. Tumor cells derived from different tissues reacted differently to CAFs, thus, making the unique relationship with CAFs across tumor types [9]. However, an extensive analysis of the clinical significance of CAF activation in correlated to prognosis of gastric cancer patients has not been performed, and further intensive investigation is substantial.

The principle aim of this study is to evaluate the relationship between the expression status of CAF and the clinicopathologic features of gastric cancer patients in an effort to identify the prognostic significance of CAF. The results of this study exhibit that overexpression of αSMA is associated with poor prognosis for patients with gastric cancer. In addition, we developed an elaborative nomogram that predicts the 3-year and 5-year OS with tumor classification, node classification and αSMA expression.

Materials and methods

Patients and specimens

Between January 2008 and December 2008, a total of 387 patients underwent surgical resection of gastric adenocarcinoma were collected in the Department of General Surgery of Zhongshan Hospital of Fudan University (Shanghai, China). Specimens were reassessed by two gastroenterology pathologists independently. A retrospective review of clinical data was performed, and the clinicopathological features (patient's age, gender, tumor localization, degree of tumor differentiation, tumor size, Lauren classification, depth of invasion, lymphatic vessel invasion, TNM stage) and the oncological results (overall survival rate) were analyzed. The follow-up was conducted until the November 31, 2015 or until death, and the median follow-up for the patients was 49 months (range, 2-79 months). No patients had

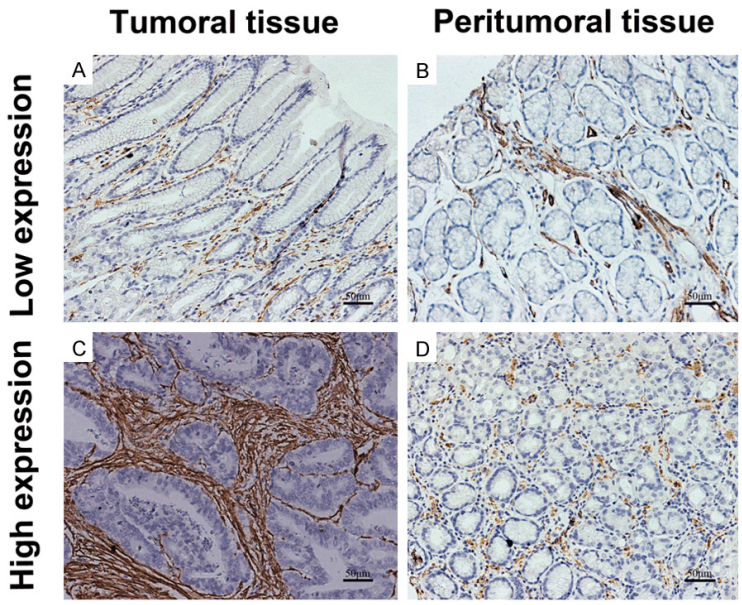

Figure 1. αSMA expression in tumoral tissue and peritumoral tissue. The micrographs showed weak staining of αSMA in tumoral tissues (A) and peritumoral tissues (B), and strong staining of tumoral tissues (C) and peritumoral tissues (D). Original magnification: × 200.

been lost to follow-up. Ethical approval was granted by the Clinical Research Ethics Committee of Zhongshan Hospital of Fudan University (Shanghai, China). Signed informed consent was obtained from all patients for the acquisition and use of anonymized clinical data.

Tissue microarray and immunohistochemistry

Formalin-fixed and paraffin-embedded surgical specimens were used for tissue microarray construction and subsequent IHC study. The IHC were performed as described previously [10]. The primary antibody against αSMA (Abcam, Cambridge, MA, USA) was used for IHC analysis and immunoreactivity score was determined by proportion of the square of immunoreactive cells occupied compared with that of the whole area in high power field. The cutoff value for high and low expression was defined as 9.5% according to the 'minimum *P*-value method' based on its relation with overall survival. The results were confirmed by two experienced gastroenterology pathologists who were blinded to the clinicopathological data.

Statistics

Statistical analyses were performed using SPSS Software (version 19.0; SPSS Inc., Chicago, IL, USA) and R 3.2.0 software (https://www.r-project.org/). The statistical significance of categorical data was evaluated using χ^2 test or t test as appropriate. Cumulative survival time was calculated by Kaplan-Meier method and analyzed by log-rank test. Numbers at risk were calculated for the beginning of each time period. The Cox proportional hazards regression model was used to perform univariate and multivariate analyses in order to determine the independent prognostic factors, and the Cox model was the basis for the nomogram. We also performed calibration using a calibration curve, a graphic representation of the relationship between the observed outcome frequencies and the predicted probabilities. All data were analyzed using two-tail test and $P < 0.05$ was considered statistically significant.

Results

Characteristics of patients and immunohistochemical staining of αSMA

The detailed characteristics of patients enrolled in this study were listed in **Table 1**. Overall survival was defined as the interval between surgery and last visit or death. Most patents were male (69.2%) and old (> 60 years, 49.6%), and had a distal-located cancer (62.3%), poorly differentiation (53.2%), larger tumor size (≥ 3.5 cm, 50.6%), lymphatic vessel invasion (60.5%). The 3-year and 5-year overall survival rates of this study population were 72.6% and 55.2% respectively.

To ascertain the expression of αSMA in gastric cancer tissues, we examined the expression of αSMA in tumoral and non-tumoral tissues by IHC staining. The expression of αSMA was mainly localized in the stromal cell, whereas tumor cells showed negative staining. Compared with paired non-tumoral tissues, tumoral tissues had significantly up-regulated expression of αSMA (mean ± SD, 11.10 ± 6.57% vs.3.98 ± 2.34%, $P = 0.001$, **Figure 1**).

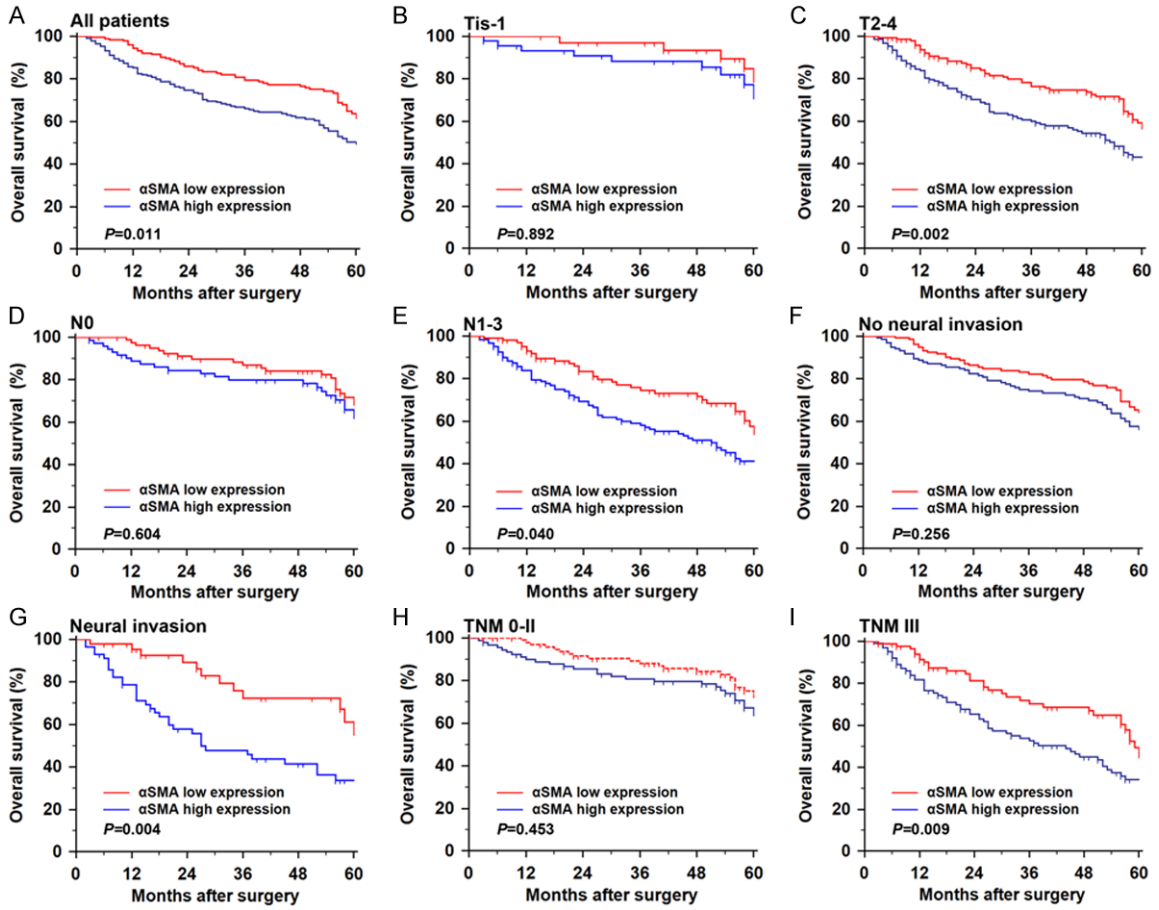

Figure 2. Kaplan-Meier analysis for OS of patients with gastric cancer according to the αSMA expression. Kaplan-Meier analysis for OS of patients with gastric cancer according to αSMA expression in all patients (A), Tis-1 (B), T2-4 (C), N0 (D), N1-3 (E), No neural invasion (F), Neural invasion (G), TNM 0-II (H), TNM III (I).

Relation between αSMA expression and clinicopathological features

To evaluate the association of αSMA expression with tumor biology, comparisons of the clinicopathological features with αSMA expression were made. Patients were divided into high and low αSMA expression group according to the 'minimum *P*-value method' based on its relation with overall survival (cut-off ratio = 9.5%). As shown in **Table 1**, patients with mixed-type gastric cancer were more likely to exhibit high αSMA expression, compared with intestinal-type and diffuse-type ($P = 0.041$).

Prognostic significance of αSMA for gastric cancer

In order to estimate the clinical prognostic significance of αSMA expression that might influence the overall survival of patients enrolled in this study, Kaplan-Meier survival analysis was performed. As shown in **Figure 2**, patients with higher expression of αSMA in tumor tissues were prone to lower OS. Low expression of αSMA has a survival benefit compared with high expression ($P = 0.011$). To further explore the prognostic significance of αSMA expression according to different clinicopathological factors, we performed Kaplan-Meier analysis in patients with early disease (Tis-1, **Figure 2B**) or advanced disease (T2-4, **Figure 2C**), in patients without lymph node metastasis (N0, **Figure 2D**) or with lymph metastasis (N1-3, **Figure 2E**), in patients without neural invasion (**Figure 2F**) or with neural invasion (**Figure 2G**). In those with advanced disease (**Figure 2C**), or with lymph metastasis (**Figure 2E**), or with neural invasion subgroups (**Figure 2G**), patients with αSMA low expression gained a survival benefit compared with those with αSMA high expression ($P = 0.002$, $P = 0.040$ and $P = 0.004$, respectively).

Table 2. Univariate and multivariate analyses of factors associated with survival

	Univariate	*P* value	Multivariate	
			HR (95% CI)	*P* value
Age (years): > 60 vs ≤ 60	0.758		NA	NA
Gender: Female vs Male	0.902		NA	NA
Localization: Middle + Distal vs Proximal	0.171		NA	NA
Tumor size (cm)[a]: ≥ 3.5 vs < 3.5		**0.006**	1.054 (0.954-1.169)	0.298
Differentiation: Moderately + Poorly vs Well	0.246		NA	NA
Lauren classification: Diffuse + Mixed vs Intestinal		0.393	NA	NA
T classification: T2-4 vs Tis-1	**< 0.001**		**1.191** (1.024-1.385)	**0.024**
N classification: N1-3 vs N0	**< 0.001**		**1.608** (1.144-2.259)	**0.007**
αSMA expression: High vs Low	**0.011**		**1.434** (1.064-1.943)	**0.019**

Abbreviations: αSMA = α-smooth muscle actin; *P*-value < 0.05 marked in bold font shows statistical significant. [a]Split at median.

To further investigate the prognostic significance of αSMA expression in different TNM stratum, we performed Kaplan-Meier analysis in patients with TNM staging according to αSMA expression. We found that in TNM stage III patients, those with αSMA low expression gained a survival benefit compared with those with αSMA high expression (**Figure 2I**, *P* = 0.009), while no such difference found in patients with TNM 0-II disease (**Figure 2H**, *P* = 0.453). All these results here indicated a vital impact of αSMA expression on clinical outcome in gastric cancer patients, especially for the advanced stage disease. In addition, univariate analyses for overall survival in this study exhibited that high αSMA expression is a significant negative prognostic predictor for patients with gastric cancer (*P* = 0.011, **Table 2**). Besides, tumor size (*P* = 0.006), T classification (*P* < 0.001), and N classification (*P* < 0.001) all also significantly affected the survival of gastric cancer (**Table 2**). Furthermore, Cox multivariate regression analyses were performed to derive independent risk estimates related to overall survival. As shown in the **Table 2**, αSMA expression (hazard ratio (HR), 1.434; 95% CI, 1.064-1.943; *P* = 0.019), T classification (HR, 1.191; 95% CI, 1.024-1.385; *P* = 0.024), N classification (HR, 1.608; 95% CI, 1.144-2.259; *P* = 0.007) were all recognized as independent prognostic factors.

Construction of the nomogram

To predict the 3-year and 5-year OS rates of gastric cancer, the following three independent variables, including αSMA expression, T classification and N classification, were selected in the nomogram. The sum of the each variable point was plotted on the total point axis, and the estimated median 3-year and 5-year survival rates were obtained by drawing a vertical line from the plotted total point axis straight down to the outcome axis. The c-index of this model was 0.673. **Figure 3** showed the calibration graph for the nomogram, in which the probability of 3-year and 5-year survival as predicted by the nomogram is plotted against the corresponding observed survival rates obtained by the Kaplan-Meier method.

Discussion

Although the incidence of gastric cancer has decreased during recent decades in many industrialized nations, China is still one of the countries with the highest incidence and advanced stage disease already present in the vast majority of patients. Nowadays, TNM staging system of UICC/AJCC is the most important prognostic factor for gastric cancer. However, it is insufficient to only rely on TNM stage to predict the prognosis of gastric cancer because of the heterogeneity of gastric cancer. Therefore, the exploration and identification of molecular markers that is predictive of gastric cancer prognosis of patient as supplementary to TNM stage has the potential to provide important clinically relevant insights into gastric cancer treatment. Recently, research concerning the relationship between tumor microenvironment and gastric cancer has gradually gained attention from researchers in the general surgery field.

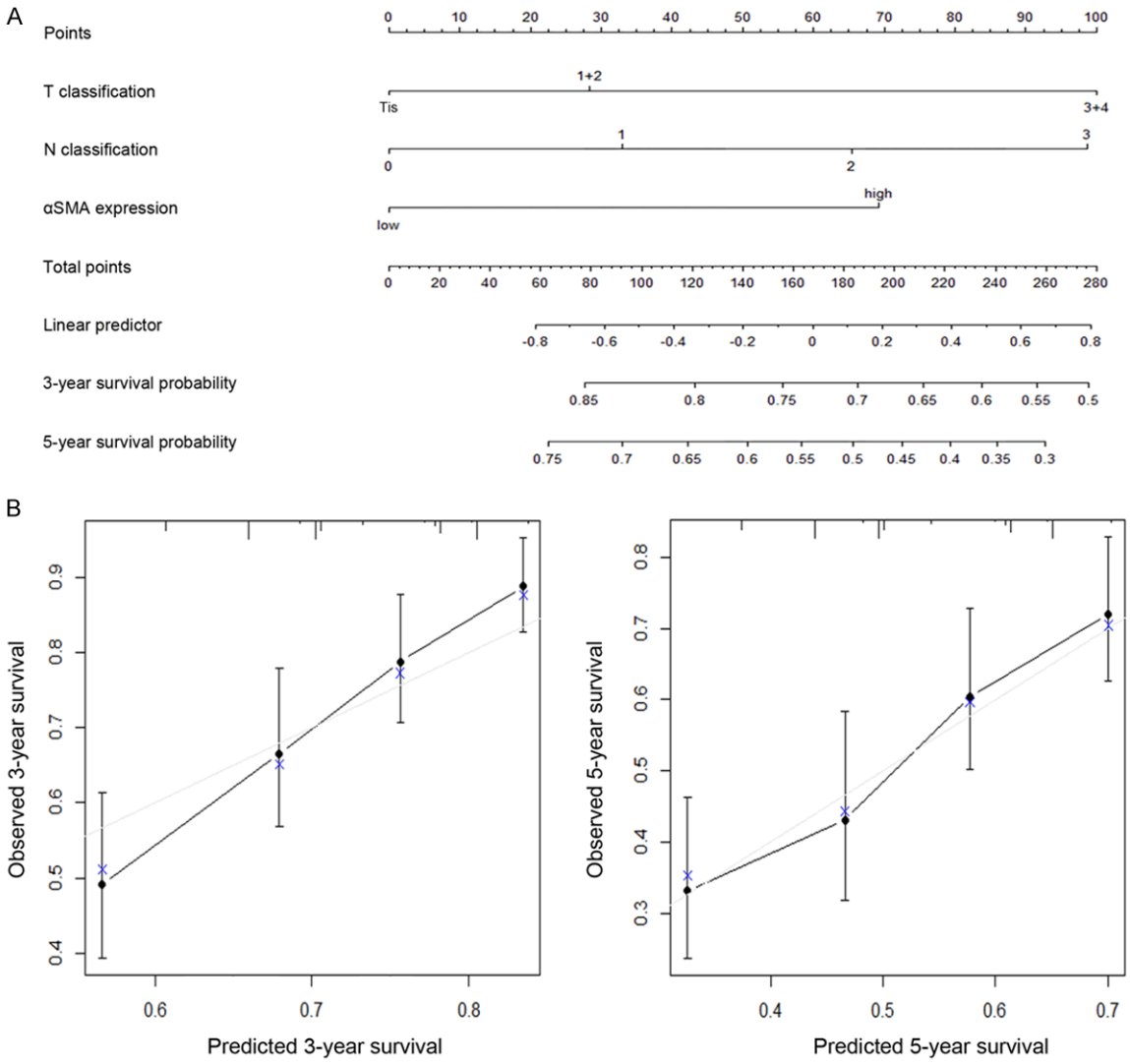

Figure 3. Prognostic nomogram generation for predicting overall survival in patients with gastric cancer. A: Nomogram for predicting postoperative 3- year and 5- year survival probabilities after surgery, summing the score of the 3 variables, that is, T classification, N classification and αSMA expression. B: Calibration of the nomogram for 3-year and 5-year overall survival. Bars indicate 95% confidence intervals.

In this study, we demonstrated that αSMA expression (a marker of CAFs) in the microenvironment of gastric cancer was a promising, independent predictor for survival of patients with gastric cancer. The patients with higher αSMA expression showed shorter overall survival time after surgical resection. This is concordant with most streams of previous observations from other cancers [11, 12]. Furthermore, αSMA expression exhibited prognostic role in gastric cancer patients with TNM stage III. These data suggest that the αSMA expression might have good discriminatory power as a supplementary risk factor in patients with late-stage gastric cancer and lead to a more accurate classification under the TNM stage system.

In addition, a prognostic nomogram for patients with gastric cancer after curative surgery was constructed. The nomogram performed well in predicting survival and its prediction was supported by the C-index (0.673) and the calibration curve. However, the profound molecular roles of CAFs in gastric cancer progression remain far from being fully elucidated and await further investigation.

Current evidence has clearly demonstrated that CAF plays an important role in tumor progression, which needs a positive and reciprocal feedback between CAF and cancer cells. Secreted factors from cancer cells and CAF are believed to be responsible for this loop. Cancer

cells induce and maintain the fibroblasts activated phenotype, which, in turn, produce a series of growth factors and cytokines that sustain tumor progression. It has been demonstrated that cancer cells could recruit and activate CAFs by secreting fibroblast-activating factors, such as TGF-β and IL-6, which are significant and well-studied cancer cell-derived factors affecting CAF activation [13, 14]. Conversely, many factors secreted by CAFs, such as IL-17A, PGE2, CXCL7 and HGF, were shown to trigger the Wnt-catenin pathway in neoplastic cells and augment the cancer stem cells (CSC) population. Recently, in invasive human breast cancers, Orimo and colleagues reported that stromal fibroblasts could promote tumor angiogenesis and development through elevating CXCL12 secretion [15]. In addition, so many growth factors, including basic fibroblast growth factor (bFGF) [16], hepatocyte growth factor (HGF) [17], and connective tissue growth factor (CTGF) [18], have all been reported to be involved in this paracrine cross talk. In the present study, the results exhibited that the expression level of αSMA significantly correlates with the prognosis of patients with gastric cancer, especially for the late-stage disease. Based on this condition, we assumed that CAFs in the tumor microenvironment might induce gastric cancer cells more malignant when its number accumulated. Therefore, CAF might be used as a potential molecular therapeutic target of gastric cancer in the future.

Therapeutic resistance is the major cause for a poor prognosis of gastric cancer patients, and previous researches revealed that CAF could promote tumorigenesis and drug resistance in many cellular pathways. CAF could secrete a variety of mitogens, chemokines, and matricellular proteins that promote drug resistance. Sequestering and/or inhibiting CAF-secreted factors that stimulate neoplastic cell behavior or directly targeting the CAF themselves could disable their downstream effects and provide multiple avenues to pursue therapeutic development in the management of gastric cancer. CXCL12 is highly overexpressed in CAF [19], and its interaction with CXCR4 has been reported in many malignant tumors [20, 21]. In prostate cancer, the CXCR4 antagonist AMD3100 was shown to chemosensitize prostate cancer cells to docetaxel in a synergistic manner [22, 23]. Therefore, targeting CAF-secreted factors to block the downstream signaling transduction may be a potential therapeutic strategy. NK4, a four-kringle fragment of HGF, functions as a HGF antagonist, can be used to block the HGF/MET signaling pathway. *Wen* and his colleagues have reported that overexpression of the NK4 gene would inhibit invasive growth of colon carcinoma cells and prolong survival in mice [24]. Furthermore, normalization of the metabolic phenotype and inhibition of metabolic pathways have been suggested as a plausible way to target tumors [25, 26]. In lung cancer, recent studies have shown that Dasatinib could reverse CAF from primary carcinomas to a more "non-myofibroblastic" phenotype comparable to that of normal fibroblasts through inhibiting PDGF signaling [27]. Therefore, inhibiting CAF-secreted factors and normalization of CAF may provide novel therapeutic strategies for gastric cancer.

There are several limitations of this study. First, this study is limited by the retrospective nature of the analysis and the selection biases cannot be totally eliminated. Second, there is not including the data of disease free survival in this study. There are many factors, such as the follow-up examinations and the postoperative treatment, might influence the disease free survival. And the disease free survival data should be collected in the future researches. Thirdly, the exact mechanism to explain the tight relationship between CAF and the progression of gastric cancer remains to be elucidated in the future. Finally, the number of patients included in this study is relatively small. Large prospective randomized controlled clinical studies are needed to identify the prognostic value of αSMA expression in the patients with gastric cancer.

In conclusion, we demonstrated that the expression level of αSMA was of prognostic value in human gastric cancer. Patients with high levels of αSMA presence are characterized by worse prognoses, and might need more aggressive postoperative treatment and closer follow-up. Incorporating αSMA expression into T and N classification can provide a good prognostic model for patients with gastric cancer. Targeting CAFs may open a new avenue for treatment of late-stage gastric cancer.

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Disclosure of conflict of interest

None.

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References

[1] Siegel RL, Miller KD and Jemal A. Cancer statistics, 2015. CA Cancer J Clin 2015; 65: 5-29.

[2] Hartgrink HH, Jansen EP, van Grieken NC and van de Velde CJ. Gastric cancer. Lancet 2009; 374: 477-490.

[3] Paunescu V, Bojin FM, Tatu CA, Gavriliuc OI, Rosca A, Gruia AT, Tanasie G, Bunu C, Crisnic D, Gherghiceanu M, Tatu FR, Tatu CS and Vermesan S. Tumour-associated fibroblasts and mesenchymal stem cells: more similarities than differences. J Cell Mol Med 2011; 15: 635-646.

[4] Kojima Y, Acar A, Eaton EN, Mellody KT, Scheel C, Ben-Porath I, Onder TT, Wang ZC, Richardson AL, Weinberg RA and Orimo A. Autocrine TGF-beta and stromal cell-derived factor-1 (SDF-1) signaling drives the evolution of tumor-promoting mammary stromal myofibroblasts. Proc Natl Acad Sci U S A 2010; 107: 20009-20014.

[5] Doldi V, Callari M, Giannoni E, D'Aiuto F, Maffezzini M, Valdagni R, Chiarugi P, Gandellini P and Zaffaroni N. Integrated gene and miRNA expression analysis of prostate cancer associated fibroblasts supports a prominent role for interleukin-6 in fibroblast activation. Oncotarget 2015; 6: 31441-31460.

[6] Bedussi F, Bottini A, Memo M, Fox SB, Sigala S and Generali D. Targeting fibroblast growth factor receptor in breast cancer: a promise or a pitfall? Expert Opin Ther Targets 2014; 18: 665-678.

[7] Neesse A, Michl P, Frese KK, Feig C, Cook N, Jacobetz MA, Lolkema MP, Buchholz M, Olive KP, Gress TM and Tuveson DA. Stromal biology and therapy in pancreatic cancer. Gut 2011; 60: 861-868.

[8] Shimoda M, Mellody KT and Orimo A. Carcinoma-associated fibroblasts are a rate-limiting determinant for tumour progression. Semin Cell Dev Biol 2010; 21: 19-25.

[9] Kadaba R, Birke H, Wang J, Hooper S, Andl CD, Di Maggio F, Soylu E, Ghallab M, Bor D, Froeling FE, Bhattacharya S, Rustgi AK, Sahai E, Chelala C, Sasieni P and Kocher HM. Imbalance of desmoplastic stromal cell numbers drives aggressive cancer processes. J Pathol 2013; 230: 107-117.

[10] Liu H, Xu J, Zhou L, Yun X, Chen L, Wang S, Sun L, Wen Y and Gu J. Hepatitis B virus large surface antigen promotes liver carcinogenesis by activating the Src/PI3K/Akt pathway. Cancer Res 2011; 71: 7547-7557.

[11] Dhanda J, Triantafyllou A, Liloglou T, Kalirai H, Lloyd B, Hanlon R, Shaw RJ, Sibson DR and Risk JM. SERPINE1 and SMA expression at the invasive front predict extracapsular spread and survival in oral squamous cell carcinoma. Br J Cancer 2014; 111: 2114-2121.

[12] Berdiel-Acer M, Sanz-Pamplona R, Calon A, Cuadras D, Berenguer A, Sanjuan X, Paules MJ, Salazar R, Moreno V, Batlle E, Villanueva A and Mollevi DG. Differences between CAFs and their paired NCF from adjacent colonic mucosa reveal functional heterogeneity of CAFs, providing prognostic information. Mol Oncol 2014; 8: 1290-1305.

[13] Giannoni E, Bianchini F, Masieri L, Serni S, Torre E, Calorini L and Chiarugi P. Reciprocal activation of prostate cancer cells and cancer-associated fibroblasts stimulates epithelial-mesenchymal transition and cancer stemness. Cancer Res 2010; 70: 6945-6956.

[14] Yang G, Rosen DG, Zhang Z, Bast RC Jr, Mills GB, Colacino JA, Mercado-Uribe I and Liu J. The chemokine growth-regulated oncogene 1 (Gro-1) links RAS signaling to the senescence of stromal fibroblasts and ovarian tumorigenesis. Proc Natl Acad Sci U S A 2006; 103: 16472-16477.

[15] Sugihara H, Ishimoto T, Yasuda T, Izumi D, Eto K, Sawayama H, Miyake K, Kurashige J, Imamura Y, Hiyoshi Y, Iwatsuki M, Iwagami S, Baba Y, Sakamoto Y, Miyamoto Y, Yoshida N, Watanabe M, Takamori H and Baba H. Cancer-associated fibroblast-derived CXCL12 causes tumor progression in adenocarcinoma of the esophagogastric junction. Med Oncol 2015; 32: 618.

[16] Xu CJ, Mikami T, Nakamura T, Tsuruta T, Nakada N, Yanagisawa N, Jiang SX and Okayasu I. Tumor budding, myofibroblast proliferation, and fibrosis in obstructing colon carci-

noma: the roles of Hsp47 and basic fibroblast growth factor. Pathol Res Pract 2013; 209: 69-74.

[17] Tyan SW, Kuo WH, Huang CK, Pan CC, Shew JY, Chang KJ, Lee EY and Lee WH. Breast cancer cells induce cancer-associated fibroblasts to secrete hepatocyte growth factor to enhance breast tumorigenesis. PLoS One 2011; 6: e15313.

[18] Huang HC, Yang M, Li JZ and Wang HY. Connective tissue growth factor promotes the proliferation of myofibroblast through Erk-1/2 signaling pathway. Zhonghua Yi Xue Za Zhi 2005; 85: 1322-1326.

[19] Allinen M, Beroukhim R, Cai L, Brennan C, Lahti-Domenici J, Huang H, Porter D, Hu M, Chin L, Richardson A, Schnitt S, Sellers WR and Polyak K. Molecular characterization of the tumor microenvironment in breast cancer. Cancer Cell 2004; 6: 17-32.

[20] Li YM, Pan Y, Wei Y, Cheng X, Zhou BP, Tan M, Zhou X, Xia W, Hortobagyi GN, Yu D and Hung MC. Upregulation of CXCR4 is essential for HER2-mediated tumor metastasis. Cancer Cell 2004; 6: 459-469.

[21] Mo W, Chen J, Patel A, Zhang L, Chau V, Li Y, Cho W, Lim K, Xu J, Lazar AJ, Creighton CJ, Bolshakov S, McKay RM, Lev D, Le LQ and Parada LF. CXCR4/CXCL12 mediate autocrine cell- cycle progression in NF1-associated malignant peripheral nerve sheath tumors. Cell 2013; 152: 1077-1090.

[22] Feig C, Jones JO, Kraman M, Wells RJ, Deonarine A, Chan DS, Connell CM, Roberts EW, Zhao Q, Caballero OL, Teichmann SA, Janowitz T, Jodrell DI, Tuveson DA and Fearon DT. Targeting CXCL12 from FAP-expressing carcinoma-associated fibroblasts synergizes with anti-PD-L1 immunotherapy in pancreatic cancer. Proc Natl Acad Sci U S A 2013; 110: 20212-20217.

[23] Domanska UM, Timmer-Bosscha H, Nagengast WB, Oude Munnink TH, Kruizinga RC, Ananias HJ, Kliphuis NM, Huls G, De Vries EG, de Jong IJ and Walenkamp AM. CXCR4 inhibition with AMD3100 sensitizes prostate cancer to docetaxel chemotherapy. Neoplasia 2012; 14: 709-718.

[24] Wen J, Matsumoto K, Taniura N, Tomioka D and Nakamura T. Hepatic gene expression of NK4, an HGF-antagonist/angiogenesis inhibitor, suppresses liver metastasis and invasive growth of colon cancer in mice. Cancer Gene Ther 2004; 11: 419-430.

[25] Doherty JR and Cleveland JL. Targeting lactate metabolism for cancer therapeutics. J Clin Invest 2013; 123: 3685-3692.

[26] Witkiewicz AK, Whitaker-Menezes D, Dasgupta A, Philp NJ, Lin Z, Gandara R, Sneddon S, Martinez-Outschoorn UE, Sotgia F and Lisanti MP. Using the "reverse Warburg effect" to identify high-risk breast cancer patients: stromal MCT4 predicts poor clinical outcome in triple-negative breast cancers. Cell Cycle 2012; 11: 1108-1117.

[27] Haubeiss S, Schmid JO, Murdter TE, Sonnenberg M, Friedel G, van der Kuip H and Aulitzky WE. Dasatinib reverses cancer-associated fibroblasts (CAFs) from primary lung carcinomas to a phenotype comparable to that of normal fibroblasts. Mol Cancer 2010; 9: 168.