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Original Article

Expression of Cyclooxygenase-2 and independent factors associated with its over-expression in Uygur and Han breast cancer patients in Xinjiang, China

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Abstract: Background: In breast cancer, Cyclooxygenase-2 (COX-2) plays a role in carcinogenesis and tumor progression, and COX-2 protein expression was higher in malignant tissues than in normal tissues. Studies show that race/ethnicity is associated with breast cancer survival and plays an important role in the biology of invasive breast cancer. Therefore, the mRNA and protein expression of COX-2 were measured in Uygur and Han breast cancer patients in Xinjiang, China and their clinicopathological characteristics were analyzed. Objectives: The aim of the paper was to investigate the difference between Uygur and Han breast cancer and identifying independent factors associated with the over-expression of COX-2. Materials and methods: A total of 198 breast cancer patients, including 98 Uygur and 100 Han, were recruited in Xinjiang, China. The expression of COX-2 mRNA and protein was measured and compared between Uygur and Han, and independent factors associated with COX-2 protein over-expression were identified. Results: The expression of COX-2 mRNA was not different statistically between Han and Uygur breast cancer patients. COX-2 protein expression and its over-expression rate were higher in Han than in Uygur breast cancer patients. The independent factors associated with over-expression of COX-2 protein included Han patients, positive lymph node metastasis, positive HER 2-neu receptor status, and higher histological grade. Conclusions: COX-2 protein expression had race/ethnicity difference in breast cancer patients between Han and Uygur in Xinjiang, China, and Han patients, positive lymph node metastasis, positive HER 2-neu receptor status and higher histological grade could elevate the expression of COX-2 protein in breast cancer.

Keywords: Cyclooxygenase-2, expression, breast cancer, race/ethnicity

Introduction

Cyclooxygenase-2 (COX-2) can trigger prostaglandin synthesis and has a crucial role in inflammatory processes as a rate-limiting enzyme in prostaglandin metabolism, and is tightly associated with the progression of breast cancer through the inflammatory processes [1]. COX-2 protein expression may be detected in many epithelial cancers [2-8]. In breast cancer, COX-2 expression is correlated with poor differentiation, positive lymph nodes, larger tumor size, higher stage at diagnosis, poor prognosis, and so on [2, 9-11]. COX-2 protein expression was much higher in malignant tissues than in normal tissues and in non-invasive MCF-7 breast cancer cells than benign MCF-10F breast cells [12]. In addition, COX-2 is over-expressed in breast tumor-associated macrophages (TAMs), which exerted adverse effects on the prognosis of breast cancer patients by elevating breast cancer cell survival [13]. Studies also show that race/ethnicity is associated with breast cancer survival [14, 15] and plays an important role in the biology of invasive breast cancer [16]. Therefore, the mRNA and protein expression of COX-2 were measured in Uygur and Han breast cancer patients in Xinjiang, China and their clinicopathological characteristics were analyzed in the paper. The aim was to investigate the difference between Uygur and Han breast cancer

Table 1. Clinicopathological characteristics of Han and Uygur breast cancer patients

	Uygur	Han	χ^2/t	*P*
Age (years)	47.26±8.27	48.67±9.55	0.972	0.416
Tumor long diameter (cm)	2.57±1.89	2.38±1.12	1.023	0.325
Lymph node metastasis				
Yes	62	54	1.751	0.186
No	36	46		
ER				
Positive	68	80	2.953	0.086
Negative	30	20		
PR				
Positive	64	74	1.771	0.183
Negative	34	26		
HER 2-neu receptor				
Positive	31	22	2.343	0.126
Negative	67	78		
Ki67				
Positive	92	98		0.168*
Negative	6	2		
Vascular invasion				
Yes	28	24	0.534	0.465
No	70	76		
TNM stage				
I and II	66	72	0.507	0.476
III	32	28		
Histological grade				
I and II	70	81	2.505	0.114
III	28	19		

*Fisher exact test.

and identify independent factors associated with the over-expression of COX-2.

Materials and methods

Participants

A total of 198 breast cancer patients were recruited in Xinjiang Medical University Affiliated Tumor Hospital from February 2010 to August 2014. All participants were definitely diagnosed through pathology and included 98 Uygur and 100 Han breast cancer patients. Inclusion criteria: 1) breast cancer patients could be treated with an surgical resection; 2) breast cancer patients lived in Xinjiang for more than 15 years; 3) Uygur and Han breast cancer patients had no a history of mixed marriage; 4) breast patients had full information of clinicopathological characteristics including pathological type, vascular invasion, TNM stage, histological grade, estrogen receptor (ER) status, progesterone receptor (PR) status, HER 2-neu receptor status, Ki67 status, tumor long diameter, lymph nodes metastasis status. Exclusion criteria included: 1) breast cancer patients complicated with other cancers; 2) late breast cancer patients could not be treated with an operation; 3) breast cancer patients had a history of mixed marriage; 4) breast cancer patients had received radiotherapy, chemotherapy, or endocrine therapy in the past. The study received the approval of the ethic committee of Xinjiang Medical University Affiliated Tumor Hospital, and all participants provided informed consent.

The participants included 98 Uygur and 100 Han breast cancer patients with an average age of 47.97±8.92 years. The pathological type of all participants was invasive ductal carcinoma. The average age, vascular invasion, TNM stage, histological grade, ER status, PR status, HER2-neu receptor status, Ki67 status, tumor long diameter, and lymph nodes metastasis status were not statistically different between Uygur and Han breast cancer patients (**Table 1**).

Detection methods

Breast cancer tissues were fixed with 4% formaldehyde and embedded in paraffin, and then sliced into 5 μm-thickness sections. COX-2 was stained with immunohistochemistry streptavidin-peroxidase conjudated method after antigen retrieval. Antibodies were purchased from ZSGB-BIO, Beijing, China.

RNA was extracted from 50 ug fresh frigorific tissues, and cDNA was then transcribed reversely with Reverse Transcription System (Promega, USA). Quantitative reverse transcription polymerase chain reaction (QRT-PCR) was performed in ABI 7500 Fast PCR System

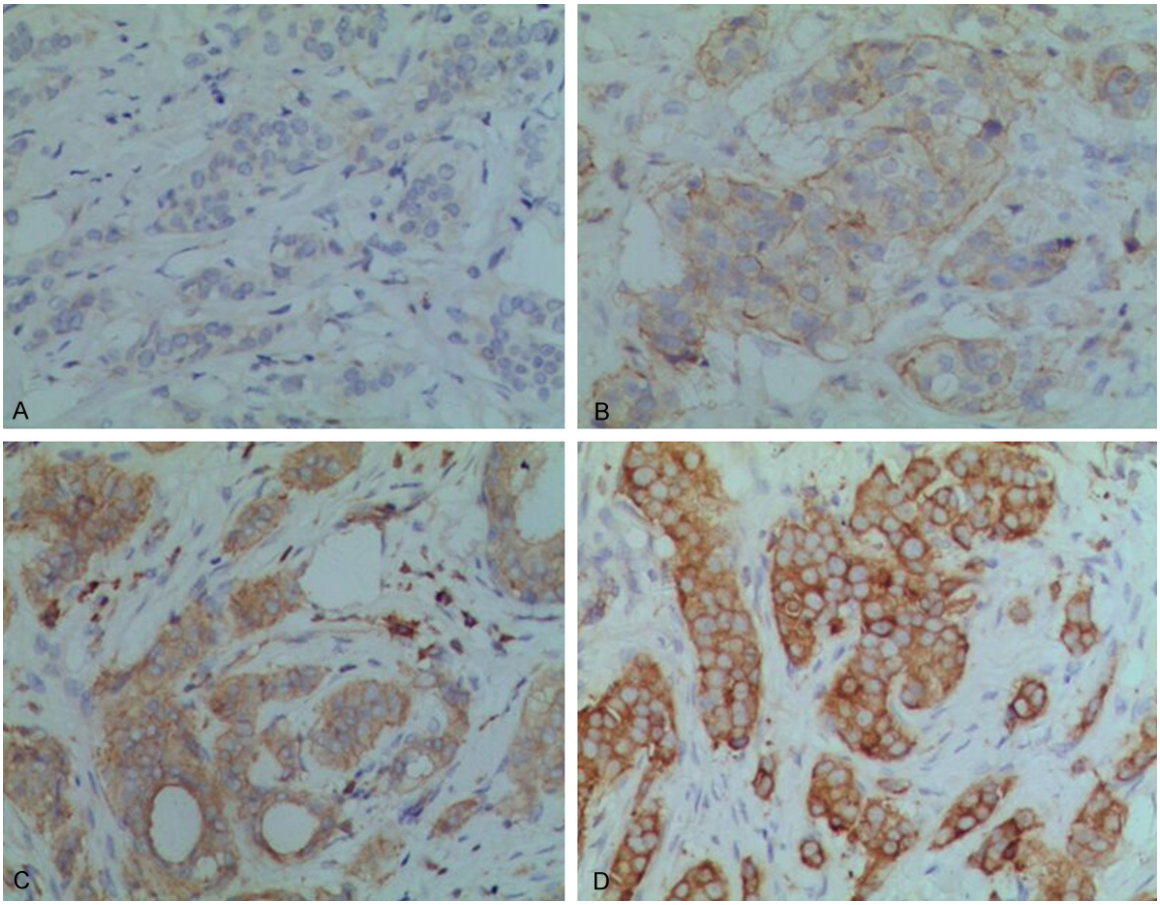

Figure 1. Staining intensity of COX-2 protein. A was for the score = 0, B for the score = 1, C for the score = 2, and D for the score = 3.

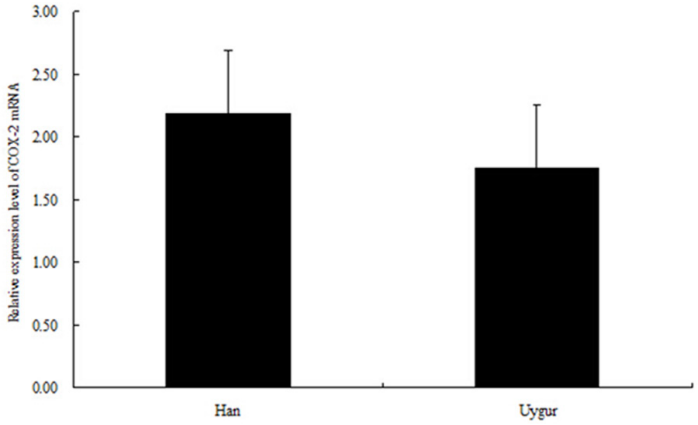

Figure 2. Relative expression level of COX-2 mRNA in Han and Uygur breast cancer patients.

(Applied Biosystems). Primer pairs were 5'-TGCCTGGTCTGATGATGT-3' (forward) and 5'-TAGCCACTCAAGTGTTGC-3' (reverse) for COX-2, and 5'-GAAGGTGAAGGTCGGAGTC-3' (forward) and 5'-GAAGATGGTGATGGGATTTC-3' (reverse) for GAPDH. The products were 180 and 225 bp, respectively. Thermal cycling conditions were 95°C for 30 sec, followed by 5 sec at 95°C, 1 min at 60°C for 35 cycles. QRT-PCR was repeated three times for each specimen, and semi-quantitative analysis of products were performed with $2^{-\Delta\Delta CT}$.

Interpretation methods

The relative expression level of COX-2 mRNA was evaluated with the result of $2^{-\Delta\Delta CT}$. COX-2 protein was expressed in cytoplasm, and its expression level was evaluated with the product of the percentage of positive cells accounting for total cells and staining intensity [17]. Briefly, the percentage of positive cells was defined as 0, 1, 2, 3 and 4 scores when the percentage was less than 5%, between 5% and 25%, between 26% and 50%, between 51% and 75%, and more than 75%, respectively. The

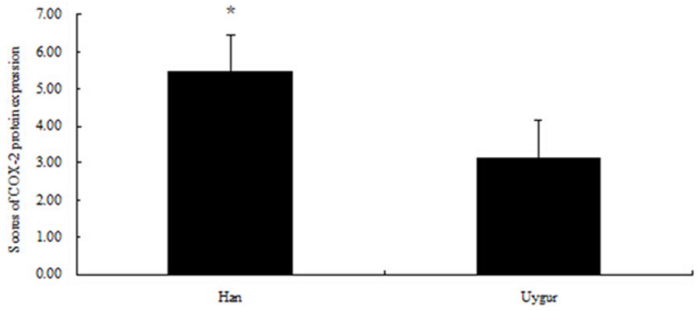

Figure 3. Scores of COX-2 protein expression in Han and Uygur breast patients. *P<0.05, Han vs Uygur.

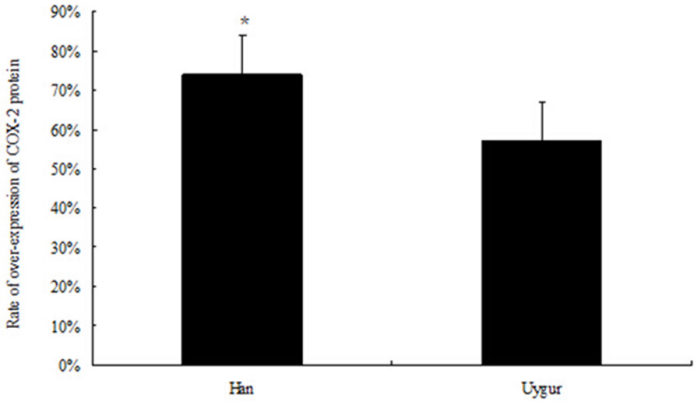

Figure 4. Rate of over-expression of COX-2 protein in Han and Uygur breast patients. *P<0.05, Han vs Uygur.

staining intensity score was defined as 0 for no color (**Figure 1A**), 1 for mild staining (**Figure 1B**), 2 for moderate staining (**Figure 1C**), and 3 for intensive staining (**Figure 1D**). Low expression was defined for the total scores of 0-4, and over-expression for 5-12.

Statistical analysis

All statistical analyses were performed with the SPSS version 17.0 for Windows (SPSS Inc., USA). Quantitative variables were expressed as mean ± SD and qualitative variables as percentage. Quantitative variables were analyzed with Student's t test, and qualitative variables with chi-square test or Fisher exact test. The variables with a P value less than 0.10 in univariate analysis were included in the multivariate analysis with a backward stepwise logistic regression model. Multivariate logistic regression analyses were then carried out to identify the independent factors influencing over-expression of COX-2. Significance was set at P<0.05.

Results

Expression of COX-2 in Han and Uygur breast cancer patients

Clinicopathological characteristics of Han and Uygur breast cancer patients were not statistically different (**Table 1**). The relative expression level of COX-2 mRNA was not different statistically between Han and Uygur breast cancer patients (2.189±3.052 *vs* 1.720±0.892, t=1.264, P=0.209, **Figure 2**). The total score of COX-2 protein expression was higher in Han than in Uygur breast cancer patients (6.05±3.124 *vs*. 3.14±2.843, t=2.264, P=0.019, **Figure 3**). The rate of over-expression of COX-2 protein was higher in Han than in Uygur breast cancer patients (74.00% *vs*. 57.14%, χ^2=6.237, P=0.013, **Figure 4**).

Independent factors associated with COX-2 over-expression

According to the results of univariate analysis, the factors associated with over-expression of COX-2 protein included nationality, age, lymph node metastasis, HER2-neu receptor status, vascular invasion, and histological grade (**Table 2**). According to the results of multivariate analysis, the independent factors associated with over-expression of COX-2 protein included Han patients, positive lymph node metastasis, positive HER 2-neu receptor status, and higher histological grade (**Table 3**).

Discussion

COX-2 has been detected in breast cancer tissues and indicated to play a role in carcinogenesis and tumor progression [1-2, 9-11, 18-21]. The potential mechanisms included: 1) COX-2 induces tumorigenesis by reducing tumor cell apoptosis [22]; 2) COX-2 promotes tumor cell proliferation by increasing the transcription of aromatase [23]; 3) COX-2 promotes neoangiogenesis by elevating the expression of angiogenic factors, including basic fibroblast growth factor (bFGF), transforming growth factor 1 (TGF-1), VEGF, endothelin and platelet-derived growth factor (PDGF) [9]. In the paper, both the

Table 2. Results of univariate analysis for factors associated with Cyclooxygenase-2 over-expression

	Over-expression	Lower expression	χ^2/t	*P*
Nationality				
Uygur	56	42	6.237	0.013
Han	74	26		
Age (years)	47.15±9.37	43.56±6.73	2.038	0.043
Tumor long diameter (cm)	2.80±1.84	2.65±0.95	0.440	0.661
Lymph node metastasis				
Yes	86	30	8.936	0.003
No	44	38		
ER				
Positive	102	46	2.426	0.112
Negative	28	22		
PR				
Positive	93	45	0.608	0.436
Negative	37	23		
HER 2-neu receptor				
Positive	40	13	4.528	0.021
Negative	90	55		
Ki67				
Positive	127	63		0.126*
Negative	3	5		
Vascular invasion				
Yes	32	20	4.197	0.032
No	98	48		
TNM stage				
I and II	89	49	0.274	0.601
III	41	19		
Histological grade				
I and II	91	60	8.201	0.004
III	39	8		

*Fisher exact test.

expression level of COX-2 protein and its over-expression rate were higher in Han than in Uygur breast cancer patients. The results indicated that the pathogenesis of breast cancer might be different between Han and Uygur, and the potential difference would be further studied in the next work. It is noteworthy that the relative expression level of COX-2 mRNA was not different statistically between Han and Uygur breast cancer patients. The difference in protein and mRNA expression levels is probably caused by post-transcriptional processing and alterations [1]. COX-2 mRNA can undergo complicated modifications to yield the active protein [12].

Studies show that many clinicopathological factors may be associated with the expression of COX-2 in breast cancer, including HER 2-neu receptor status, Ki67 status, hormone receptor status, histological grade, lymph nodes metastasis status, distant metastasis status, vascular invasion status, stage at diagnosis, and tumor size [2, 9-11]. Singh-Ranger G *et al.* report that the expression of COX-2 is significantly associated with metastasis [9], and Ranger GS *et al.* also report that the expression of COX-2 is significantly associated with distant metastasis through a small sample of 29 patients [19]. The expression of COX-2 is also significantly correlated with HER 2-neu status [9], and HER2 can up-regulate COX-2 expression through direct transcriptional mechanisms [24]. Besides, the expression of COX-2 is also significantly associated with hormone receptor status [9], and can promote tumor growth by elevating oestrogen levels in hormone receptor-positive breast cancer. In the paper, we showed that Han patients, positive lymph node metastasis, positive HER2-neu receptor status and higher histological grade were independent factors associated with the over-expression of COX-2 in breast cancer patients in Xinjiang.

The strengths of the work included: 1) the work showed a higher expression level of COX-2 in Han than in Uygur breast cancer patients in Xinjiang, China; 2) the work identified independent factors associated with COX-2 over-expression in breast cancer patients in Xinjiang.

Table 3. Results of multivariate analysis for independent factors associated with Cyclooxygenase-2 over-expression

Risk factors	Wald	*P* value	OR	95% CI	
Nationality (Han patients)	4.823	0.035	3.206	1.029	4.927
Age	2.435	0.119	2.142	0.855	1.297
Positive lymph node metastasis	6.274	0.011	3.300	1.032	3.689
Positive HER 2-neu receptor	4.258	0.040	3.177	1.023	2.729
Vascular invasion (Yes)	0.179	0.672	2.919	0.175	3.032
Histological grade (III)	7.627	0.006	3.496	1.046	7.494

The limitations of the work included: 1) the measurement of COX-2 mRNA and protein expression was semi-quantitative; 2) the sample size was small; 3) instead of tumor size, tumor long diameter was measured. The difference between our results and previous studies might be caused by the above limitations.

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Disclosure of conflict of interest

None.

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