



Clinicopathologic significance of CXCR4 and Nrf2 in colorectal cancer

Tinghua Hu^{a,Δ}, Yu Yao^{a,Δ}, Shuo Yu^b, Hui Guo^a, Lili Han^a, Wenjuan Wang^a, Tao Tian^a, Yibin Hao^c,
Zhiyan Liu^d, Kejun Nan^{a,✉}, Shuhong Wang^{a,✉}

Departments of ^aOncology and ^bHepatobiliary Surgery, the First Affiliated Hospital of Medical College,
Xi'an Jiaotong University, Xi'an, Shaanxi 710061, China;

^cDepartment of Pediatrics, Zhengzhou Central Hospital, Zhengzhou, Henan 450007, China;

^dDepartment of respiration, Xi'an Central Hospital, Xi'an, Shaanxi 710003, China.

Received 04 May 2013, Revised 06 June 2013, Accepted 18 June 2013, Epub 6 July 2013

Abstract

The CXCR4 and Nrf2 signaling pathways are abnormally activated in response to cellular stress in various types of human cancers. In this study, we examined the expression of CXCR4 and Nrf2 in colorectal cancer (CRC) tissue specimens and investigated their correlation with patient clinicopathologic characteristics. We determined CXCR4 and Nrf2 expression in 76 CRC tissue specimens and paired normal tissue specimens by immunohistochemistry and real-time PCR. We found that the protein and mRNA transcript levels of CXCR4 were significantly higher in CRC tissue specimens than in paired normal tissues, while the expressions of Nrf2 protein and mRNA were increased in CRC tissues compared to distant non-cancerous tissues. High expression level of CXCR4 was positively correlated with poorly differentiated ($P = 0.031$), more advanced tumor-node-metastasis (TNM) stage ($P = 0.019$), lymph node metastasis ($P = 0.007$) and distant metastasis ($P = 0.018$). However, the expression of Nrf2 protein was positively correlated with larger tumor size ($P = 0.049$), more advanced TNM stage ($P = 0.013$), lymph node metastasis ($P = 0.016$) and distant metastasis ($P = 0.023$). Moreover, there was a strong relationship between CXCR4 and Nrf2 expression in CRC tissues, indicating that high Nrf2 expression may contribute to CXCR4 overexpression. In addition, combined expression of CXCR4 and Nrf2 strongly correlated with lymph node metastasis and distant metastasis ($P = 0.003$). Furthermore, we found that combined high expression of CXCR4 and Nrf2 had stronger correlation with lymph node metastasis and distant metastasis than any single molecule did. This study indicated that the abnormal expression of CXCR4 and Nrf2 contributed to the progression of CRC.

Keywords: CXCR4, Nrf2, colorectal cancer, biomarker

This study was supported by the National Natural Science Foundation of China (No. 81172361, 81001090 and 81201824); Specialized Research Fund for the Doctoral Program of Higher Education of China, the First Affiliated Hospital of Medical School of Xi'an Jiaotong University, the Fundamental Research of Xi'an Jiaotong University (No.20110201120061); Fundamental Research Funds for the Central Universities (No.xjj2010013)

^ΔThese two authors contributed equally to this work.

✉Corresponding author: Kejun Nan, Ph.D., Department of Oncology,

the First Affiliated Hospital of Medical College, Xi'an Jiaotong University, Yanta West Road 766, Xi'an, Shaanxi 710061, China. Tel/Fax: +86-29-85324086/+86-29-85324086, Email: nankj@163.com; Shuhong Wang, Ph.D., Department of Oncology, the First Affiliated Hospital of Medical College, Yanta West Road 766, Xi'an Jiaotong University, Xi'an, Shaanxi 710061, China. Tel/Fax: +86-29-85324086/+86-29-85324086, Email: wsh2003@126.com.

The authors reported no conflict of interests.

INTRODUCTION

Colorectal cancer (CRC) is one of the most prevalent malignancies and the third leading cause of cancer-related death worldwide. The 5-year survival rate is 90% for patients with local CRC, which decreases to 12% for patients with distant metastasis^[1]. At present, besides radical surgery, adjuvant therapies such as chemotherapy and targeted therapy have been widely used. However, there has been no breakthrough in the control of CRC once it develops extra lymph node metastasis, and most patients with CRC develop liver metastasis either synchronously or metachronously^[2,3]. Therefore, it is necessary to identify factors involved in colon cancer metastasis.

According to the “homing” theory, chemokines are able to chemoattract tumor cells into target tissues^[4]. Recent studies have highlighted the role of chemokines and their receptors in cancer metastasis^[5]. CXCR4, a seven-transmembrane G protein-coupled receptor (GPCR), is the receptor of stromal cell-derived factor (SDF-1), which plays an important role in embryonic development, immune and inflammatory response, HIV infection, directional regulation of hematopoiesis, angiogenesis, and migration of metastatic tumor cells^[6,7]. It has been reported that CXCR4 is the most common chemokine receptor expressed in human tumors, such as breast cancer, lung cancer, pancreatic cancer, oral squamous cell carcinoma and ovarian cancer^[8-13], and especially CRC, in which CXCR4 is essential for directional tumor metastasis^[14-16].

Tumorigenesis is accompanied by various cellular stresses; more specifically, CRC cells have totally different phenotypes in response to cellular stress. Some colonies survive by adapting to the local tumor microenvironment while some escape from the primary lesion and become settlers, which is mainly responsible for metastases. Moreover, cellular stress has been reported to induce cellular factors that cause cell invasion and migration by regulating the SDF-1/CXCR4 signaling pathways directly^[17,18].

The transcription factor NFE2-related factor 2 (Nrf2) has been proved to play an irreplaceable role when cells adaptively respond to stress^[19]. Under normal circumstances, Nrf2 in the cytoplasm remains transcriptionally inactive through binding to its inhibitor Kelch-like ECH-associated protein 1 (Keap1), and is quickly degraded by the proteasome to maintain the protein at low levels^[19]. However, in several cancer types, physiological concentrations of reactive oxygen species (ROS) or reactive nitrogen species (RNS) molecules reduce the free sulfhydryl (-SH) groups of

cysteine in Keap1, which disrupts Nrf2 from binding to Keap1^[20]. Dissociated Nrf2 is transported into the nucleus and bound to antioxidant response elements (AREs) or electrophile response elements (EpREs), finally regulating the expression of genes involved in response to cellular stress^[21]. Evidence suggested that mutations in *Nrf2* are common in cancer cells, which could help tumor cells to survive and might be associated with poor survival of cancer patients^[22-24]. Previous studies have shown that the Nrf2 signaling pathway is abnormally activated in CRC^[25].

Based on these studies, we speculate that there may be a direct correlation between CXCR4 and Nrf2 expression and the progression of CRC. Therefore, we collected 76 CRC tissue specimens and paired normal tissue specimens to analyze Nrf2 and CXCR4 expression by using immunohistochemistry and quantitative real time polymerase chain reaction (qRT-PCR) and examined their association with clinicopathologic parameters of CRC patients.

SUBJECTS AND METHODS

Patients and samples

A total of 76 patients with CRC who underwent surgical resection at the authors' affiliated hospital between January 2009 and December 2012 were enrolled in the study. They had received no prior radiotherapy or chemotherapy. Cancer and paired normal tissue (5 cm from the tumor margin) specimen were collected from each patient. Histological diagnosis was performed for all the cases by three independent, experienced pathologists. This study protocol was approved by the ethics committee of the authors' affiliated institution, the procedures were performed in accordance with the Helsinki Declaration and all specimens were obtained from patients with informed consent.

The age of the patients (42 males and 34 females) ranged from 28 to 79 years (median age 61 years). Other clinicopathologic data are shown in **Table 1**. Tumor-node-metastasis (TNM) stages were assigned using the American Joint Committee on Cancer (AJCC) criteria.

The tissue samples were taken from the operating room during surgery and snap frozen in liquid nitrogen and stored at -80°C for RNA isolation. For immunohistochemistry, the tissue samples were fixed in 4% paraformaldehyde for 24 hours, dehydrated and embedded in paraffin.

Immunohistochemistry

Immunohistochemical staining was performed to

detect the expression of CXCR4 and Nrf2 by using hematoxylin/eosin. The antigens were retrieved by deparaffinization and hydration. Each section was blocked with 3% H₂O₂ for 10 minutes to inhibit endogenous peroxidase, and then incubated with primary antibody against CXCR4 (ab2074, Abcam, HK) or Nrf2 (ab62352, Abcam) overnight at 4°C. Negative controls were set up by omitting the primary antibody. Then, they were incubated with biotinylated secondary antibodies for 30 minutes at room temperature. DAB substrate was added to reveal immunoreactive products, and then the tissue sections were counterstained with hematoxylin to reveal nuclei.

To count the number of positive cells, we used a microscope to examine and analyze the sections at 10 random fields. Staining intensity was graded as follows: 0, no staining; 1, weak intensity; 2, moderate intensity; 3, strong intensity. The percentage of positive cells was scored as follows: 1, <25%; 2, 26-

50%; 3, 51-75%; 4, >76%. Scores for expression and percentage of positive cells were added. Based on the total scores, samples were categorized into four groups: the negative group (-), ≤ 5% cells were stained, 0; the weak expression group (+), 1-3; the moderate expression group (++), 4-5; the strong expression group (+++), 6-7. In these groups, the negative and the weak expression groups (- or +) were considered to have lower gene expression and noted as negative results for statistical analysis, while the moderate and the strong expression groups were considered to have higher gene expression and noted as positive results.

Real-time reverse transcription polymerase chain reaction (RT-PCR)

Total mRNA was extracted using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and reverse transcription was performed using an RT-PCR kit (TaKaRa, Dalian, China). Complementary DNA syn-

Table 1 Association of CXCR4 and Nrf2 expressions with clinicopathological features of colorectal cancer patients

Characteristic	n (%)	CXCR4		P	Nrf2		P
		Positive	Negative		Positive	Negative	
Age							
< 60 y	37 (48.68)	23	14	0.516	22	15	0.853
≥ 60 y	39 (51.32)	27	12		24	15	
Gender							
Male	42 (55.26)	30	12	0.249	26	16	0.785
Female	34 (44.74)	20	14		20	14	
Tumor Location							
Colon	50 (65.79)	32	18	0.648	31	19	0.751
Rectum	26 (34.21)	18	8		15	11	
CEA							
< 3.4 ng/mL	21 (27.63)	11	10	0.128	10	11	0.155
≥ 3.4 ng/mL	55 (72.37)	39	16		36	19	
Tumor size							
< 5 cm	35 (46.05)	25	10	0.338	17	18	0.049*
≥ 5 cm	41 (53.95)	25	16		29	12	
Histological grade							
Well/moderate	34 (44.74)	17	14	0.031*	18	16	0.224
Poor	42 (55.26)	33	9		28	14	
TNM stage							
I + II	30 (39.47)	15	15	0.019*	13	17	0.013*
III + IV	46 (60.53)	35	11		33	13	
Lymph node metastasis							
Positive	48 (63.16)	37	11	0.007**	34	14	0.016*
Negative	28 (36.84)	13	15		12	16	
Distant metastasis							
Positive	18 (23.68)	16	2	0.018*	15	3	0.023*
Negative	58 (76.32)	34	24		31	27	

*P < 0.05, **P < 0.01.

thesis was conducted using the SYBR ExScript RT-PCR kit (TaKaRa) according to the manufacturer's instructions. Real-time PCR was conducted using the iQ5 Multi-color Real-time PCR Detection System (Bio-Rad, Hercules, CA, USA) and SYBR Premix Ex Taq™ II (TaKaRa). The primers of CXCR4, Nrf2 and GAPDH were designed and synthesized by TaKaRa, and the sequences were as follows: CXCR4, forward 5'-TCTGTGACCGCTTCTACC-3', and reverse 5'-AGGATGAGGATGACTGTGG-3' Nrf2, forward 5'-CCAACACACGGTCCACAGCT-3', and reverse 5'-TCCGTCGCTGACTGAAGTCAA-3'; GAPDH, forward 5'-ACCACAGTCCATGCCATCAC-3', and reverse 5'-TCCACCACCCTGTTGCTGTA-3'. Each measurement was performed at least three times. A dissociation curve analysis was conducted for each quantitative PCR. The expression of the target gene was evaluated using a relative quantification approach ($2^{-\Delta\Delta Ct}$ method) with GAPDH as the internal control.

Statistical analysis

Data were evaluated by using Student's *t*-test and the chi-squared (χ^2) test. Spearman analysis was performed to analyze correlation between CXCR4 and Nrf2 expression. Statistical Package for Social Science (SPSS) version 17.0 (SPSS Inc., Chicago, IL, USA) was used. All reported *P* values were two-sided. $P < 0.05$ was considered statistically significantly different and $P < 0.01$ was considered highly statistically significantly different.

RESULTS

CXCR4 gene expression according to patient characteristics

Patient demographic and clinicopathologic data are shown in **Table 1**. Firstly, we assessed the expression of CXCR4 protein by immunohistochemistry. Compared to the positive rate of CXCR4 expression in paired normal tissues (28/76, 36.84%), higher expression of CXCR4 was detected in 50 of the 76 tumors (65.79%). The difference was significant ($\chi^2 = 12.75$, $P < 0.01$, **Fig. 1**). Moreover, to analyze the expression of CXCR4 mRNA, we performed real-time PCR on 76 paired CRC tissues and adjacent non-cancerous tissues. The results showed that the mRNA transcript levels of CXCR4 in the CRC tissues were significantly higher than those in the adjacent non-cancerous tissues (4.13 ± 0.66 vs. 1.24 ± 0.30 , $t = 29.25$, $P < 0.01$, **Fig. 2A**). Then, we analyzed the association of CXCR4 protein expression with clinicopathological data of CRC patients. We found that the expression of CXCR4 was significantly associated with poorly differentiated ($P = 0.031$), more advanced TNM stage ($P = 0.019$), lymph node metastasis ($P = 0.007$) and distant metastasis ($P = 0.018$) (**Table 1**).

Nrf2 gene expression according to patient characteristics

By using immunohistochemistry to examine the

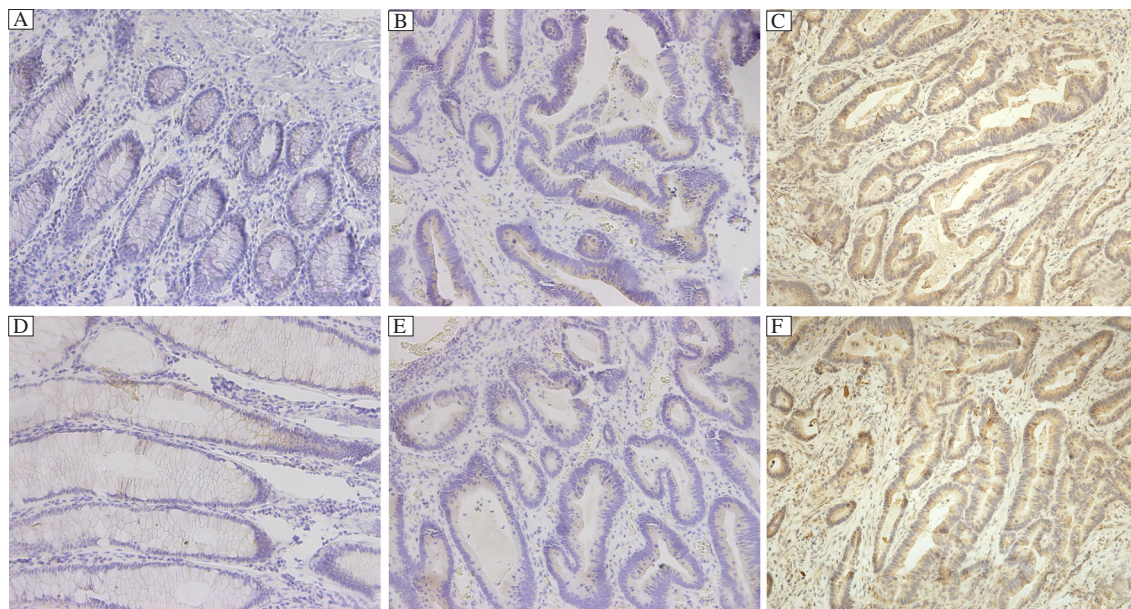


Fig. 1 Immunohistochemistry of CXCR4 and Nrf2 in colorectal (CRC) and paired adjacent non-tumors tissues. A: Negative expression of CXCR4 in adjacent non-tumors tissues ($\times 200$); B: Negative expression of CXCR4 in CRC tissues ($\times 200$); C: Positive expression of CXCR4 in CRC tissues ($\times 200$); D: Negative expression of Nrf2 in adjacent non-tumors tissues ($\times 200$); E: Negative expression of Nrf2 in CRC tissues ($\times 200$); F: Positive expression of Nrf2 in CRC tissues ($\times 200$).

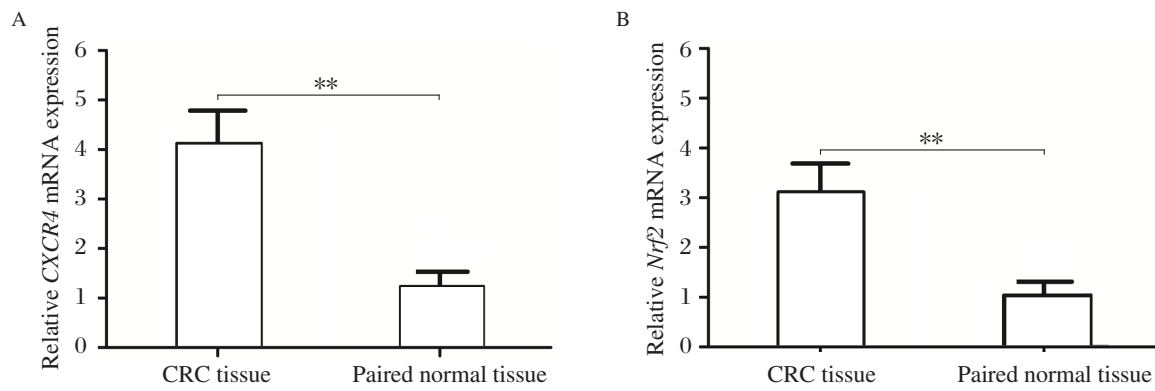


Fig. 2 *CXCR4* and *Nrf2* mRNA levels were quantified by real-time PCR in 76 patients. A: The relative expression of *CXCR4* mRNA in CRC compared to paired normal tissues; B: The relative expression of *Nrf2* mRNA, which compared to adjacent non-tumors tissues, was quantified by Real-time PCR in 76 CRC tissues. (** $P < 0.01$).

protein expression of Nrf2, we found that the positive rate of Nrf2 expression was 60.53% (46/76) in CRC tissues and 26.32% (20/76) in paired normal tissues, respectively. Nrf2 protein expression in CRC tissues was significantly higher than that in the adjacent non-cancerous tissues ($\chi^2 = 4.90$, $P < 0.05$, **Fig. 1**). Meanwhile, by using real-time PCR to measure *Nrf2* mRNA expression in 76 cases of CRC tissues and the corresponding non-cancerous tissues, we found that the average expression level of *Nrf2* mRNA transcripts in CRC tissues was significantly higher than that in the adjacent non-cancerous tissues (3.11 ± 0.58 vs. 1.03 ± 0.28 , $t = 21.82$, $P < 0.01$, **Fig. 2B**). Furthermore, Nrf2 expression levels were found to be significantly higher in CRCs with larger tumor size ($P = 0.049$), more advanced TNM stage ($P = 0.013$), lymph node metastasis ($P = 0.016$) and distant metastasis ($P = 0.023$) (**Table 1**).

Correlation between CXCR4 and Nrf2 expression in CRC tissues

CXCR4 promotes tumor cell migration and directional metastasis, which may be associated with Nrf2 activation. Therefore, we investigated the association

between CXCR4 and Nrf2 levels in these CRC and matched normal tissues. By combining the expression of CXCR4 and Nrf2, we obtained the following four combinations in 76 cases: CXCR4+/Nrf2-, CXCR4+/Nrf2+, CXCR4-/Nrf2- and CXCR4-/Nrf2+. We found a strong correlation between CXCR4 and Nrf2 expression. Notably, the co-expression of CXCR4 and Nrf2 was detected in 36 of the 76 tumors (47.37%) and significant difference was observed ($r = 0.326$, $P < 0.01$; **Table 2**). These results indicated that the upregulation of Nrf2 correlates very well with the overexpression of CXCR4 in tumor tissue. In addition, no statistically significant correlation was found between CXCR4 and Nrf2 expression in distant normal tissues ($r = 0.101$, $P > 0.05$; **Table 2**).

Association of CXCR4 and Nrf2 expression with lymph node metastasis

A total of 48 CRC cases showed metastasis to their draining lymph nodes. Among these tumors metastasized to the lymph nodes, 37 samples (37/48, 77.08%) showed a positive expression of CXCR4. A significant association was observed between the expression of CXCR4 and the lymph node-positive tumor status

Table 2 Correlation between CXCR4 and Nrf2 expression in colorectal cancer and normal tissues

Nrf2	CXCR4		<i>r</i>	<i>P</i>
	Positive	Negative		
Tumor				
Positive	36 (47.37%)	10 (13.16%)	0.326	< 0.01
Negative	14 (18.42%)	16 (21.05%)		
Normal				
Positive	9 (11.84%)	11 (14.47%)	0.101	> 0.05
Negative	19 (25.00%)	37 (48.69%)		

Table 3 Correlation of co-expression of CXCR4 and Nrf2 with lymph node status and distant metastasis in colorectal cancer patients

CXCR4 and Nrf2	Lymph node metastasis		<i>P</i>	Distant metastasis		<i>P</i>
	Positive	Negative		Positive	Negative	
Both positive	29 (38.16%)	7 (9.21%)	0.003	14 (18.42%)	22 (28.95%)	0.003
Others	19 (25.00%)	21 (27.63%)		4 (5.26%)	36 (47.37%)	

($P = 0.007$; **Table 1**). Of the 48 cases, 34 cases (34/48, 70.83%) were positive for Nrf2 expression, and statistical analysis showed that the incidence of lymph node metastasis tended to be higher in CRC patients with high expression of Nrf2 ($P = 0.016$; **Table 1**). Upon combining the expression of CXCR4 and Nrf2, particularly, our data showed that the incidence rate of lymph node metastasis in patients with tumors co-expressing CXCR4 and Nrf2 (29/48, 60.42%) was significantly higher than other combinations (19/48, 39.58%). Therefore, statistical analysis showed that the co-expression of CXCR4 and Nrf2 was significantly correlated with lymph node metastasis in CRC patients ($r = 0.342$, $P < 0.01$; **Table 3**).

Association of CXCR4 and Nrf2 expression with distant metastasis

As shown in **Table 1**, the incidence of distant metastasis in CRC patients was significantly associated with high expression of CXCR4 (16/18, 88.89%; $P = 0.018$) or Nrf2 (15/18, 83.33%; $P = 0.023$). Moreover, upon combining the expressions of CXCR4 and Nrf2, we found that the incidence rate of distant metastasis in patients with tumors co-expressing CXCR4 and Nrf2 (14/18, 77.78%) was significantly higher than other combinations (4/18, 22.22%). Spearman correlation analysis showed that the co-expression of CXCR4 and Nrf2 was significantly correlated with distant metastasis in CRC patients ($r = 0.339$, $P < 0.01$; **Table 3**).

DISCUSSION

In this study, we investigated CXCR4 and Nrf2 expression in human CRC samples by using immunohistochemistry and real-time PCR. Our results demonstrated that the expression of CXCR4 and Nrf2 was significantly higher in CRC tissue than that in paired normal tissue. The high expression of CXCR4 was associated with poor differentiated, advanced TNM stages, lymph node metastasis and distant metastasis. However, among the clinicopathological features, we found that the expression of Nrf2 protein was only positively associated with larger tumor size, advanced TNM stages and metastasis. In addition, the data

showed that there was a statistically significant correlation between CXCR4 and Nrf2 expression in CRC tissues. We also found that high expression of CXCR4 and Nrf2 was associated with increased metastatic potential in human CRC.

The tumor microenvironment has become a research hotspot in recent years, which is closely related to the malignant progression of tumor. It has been shown that CXCR4, activated by its ligand SDF-1 α (CXCL12), is critical for the adhesion, migration and invasion of tumor cells. The expression of CXCR4 has been shown to be associated with tumorigenesis and metastasis of many types of cancer^[8-16]. Previous studies found that high expression of CXCR4 in CRC was significantly associated with advanced tumor stages and lymphatic or hematogenic metastasis^[26]. Our result is in accordance with the previous studies. The association between lymph node metastasis and CXCR4 in CRC was the most notable among them ($P = 0.007$). Considering that CXCR4 is involved lymphocyte homing^[27], high expression of CXCR4 may act on some factors to induce the migration of cells, not only lymphocytes, but also cancer cells.

HIF-1 α has emerged as an important tumor-secreted factor in response to hypoxia, and CXCR4 has been reported to be a target gene of HIF-1 α ^[7, 28]. Their results point to a key role of the HIF-1 α -CXCR4 pathway during cell migration^[29]. However, as the tumor grows, apart from hypoxia, tumor cells will face with cell stresses such as oxidative stress and the accumulation of toxic substances. At the same time, Nrf2 activated by ROS and RNS in response to cellular stress also plays a key role. Previous studies showed that inhibition of NRF2 results in the failure of HIF-1 α to accumulate under hypoxic conditions and further limits the progression of tumor^[30]. Some evidence suggested that Nrf2 is related with anti-carcinogenesis and chemotherapeutic resistance at the same time^[31]. But recent report showed that mice lacking *Nrf2* are more susceptible to colitis and colorectal carcinogenesis^[32]. Thus, the activation of Nrf2 plays an irreplaceable role in cellular dense, not only in normal tissue, but also in cancer cells^[33]. Strikingly, Tsai et al. found that Nrf2 modulates both the migration and

retention of haematopoietic stem cells in their niche by binding to the *CXCR4* promoter and activates its expression^[34]. These findings together directed us to investigate the interaction between Nrf2 and CXCR4 in tumors. Our results revealed that Nrf2 was upregulated in human CRC compared to normal tissues. We found that high expression of Nrf2 in CRC was significantly associated with tumor size ($P = 0.049$). The bigger the tumor is, the higher the cell stress level will be. Moreover, the expression of CXCR4 protein in our study was significantly associated with TNM stage. These data suggested that the cell stress-ROS/RNS-Nrf2 pathway played a crucial role in advanced stage of the tumor. Interestingly, the expression of Nrf2 had a significant correlation with lymph node metastasis and distant metastasis.

Although the process by which Nrf2 protein promoted CRC progression was unknown, we did find that the upregulation of Nrf2 correlated very well with the overexpression of CXCR4 protein. Co-expression of CXCR4 and Nrf2 were detected in 36 cases of the 76 tumors. Furthermore, the co-expression of CXCR4 and Nrf2 was significantly associated with lymph node metastasis and distant metastasis in CRC patients. This suggests that the combination of high Nrf2 expression and elevated CXCR4 has a synergistic effect on CRC progression. We speculate that Nrf2, as a transcription factor, may regulate the expression of CXCR4 in CRC when facing with cell stress, which can help CRC cells to escape from the severe tumor microenvironment and speed up the progression of the tumor. However, the precise mechanism by which Nrf2 regulates the expression of CXCR4 in CRC cells needs further investigation.

In conclusion, the overexpression of CXCR4 and Nrf2 apparently plays key roles in CRC progression and CXCR4 and Nrf2 may become new candidate targets for targeted therapy of CRC.

Acknowledgements

The authors thank Prof. Chen Huang of Xi'an Jiaotong University (Xi'an, China) for providing experimental platform and expert opinions, and Ms. Xin Wei for preparation of this manuscript.

References

- [1] Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. *CA Cancer J Clin* 2013; 63: 11-30.
- [2] Mano MS, Duhoux F. Colon cancer: update on adjuvant therapy. *Clin Colorectal Cancer* 2008; 7: 178-83.
- [3] Gallagher DJ, Kemeny N. Metastatic colorectal cancer: from improved survival to potential cure. *Oncology* 2010; 78: 237-48.
- [4] Fidler IJ. The pathogenesis of cancer metastasis: the "seed and soil" hypothesis revisited. *Nat Rev Cancer* 2003; 3: 453-8.
- [5] Stetler-Stevenson WG, Kleiner DE Jr. Molecular biology of cancer: invasion and metastasis. In: DeVita VT Jr, Hellman S, Rosenberg SA, Editors. *Cancer: Principles and Practice of Oncology*. Philadelphia, PA: Lippincott Williams. 2001: 123-36.
- [6] Yao-Chun Wang, Xing-Bin Hu, Fei He, Feng F, Wang L, Li W, et al. Lipopolysaccharide-induced Maturation of Bone Marrow-derived Dendritic Cells Is Regulated by Notch Signaling through the Up-regulation of CXCR4. *J Biol Chem* 2009; 284: 15993-6003.
- [7] J. A. Burger, T. J. Kipps. CXCR4: a key receptor in the crosstalk between tumor cells and their microenvironment. *Blood* 2006; 107: 1761-7.
- [8] Egawa T, Kawabata K, Kawamoto H, Amada K, Okamoto R, Fujii N, et al. The earliest stages of B cell development require a chemokine stromal cell-derived factor/pre-B cell growth-stimulating factor. *Immunity* 2001; 15: 323-34.
- [9] Muller A, Homey B, Soto H. Involvement of chemokine receptors in breast cancer metastasis. *Nature* 2001; 410: 50-6.
- [10] Hao L, Zhang C, Qiu Y, Wang L, Luo Y, Jin M, et al. Recombination of CXCR4, VEGF, and MMP-9 predicting lymph node metastasis in human breast cancer. *Cancer Letters* 2007; 253: 34-42.
- [11] Saur D, Seidler B, Schneider G. CXCR4 expression increases liver and lung metastasis in a mouse model of pancreatic cancer. *Gastroenterology* 2005; 129: 1237-50.
- [12] Kato M, Kitayama J, Kazama S and Nagawa H. Expression pattern of CXC chemokine receptor-4 is correlated with lymph node metastasis in human invasive ductal carcinoma. *Breast Cancer Res* 2003; 5: 144-50.
- [13] Uchida D, Begum NM, Almofti A, Nakashiro K, Kawamata H, Tateishi Y, et al. Possible role of stromal-cell-derived factor-1/CXCR4 signaling on lymph node metastasis of oral squamous cell carcinoma. *Exp Cell Res* 2003; 290: 289-302.
- [14] Kim J, Takeuchi H, Lam ST, Turner RR, Wang HJ, Kuo C, et al. Chemokine receptor CXCR4 expression in colorectal cancer patients increases the risk for recurrence and for poor survival. *J Clin Oncol* 2005; 23: 2744-53.
- [15] Matsusue R, Kubo H, Hisamori S, Okoshi K, Takagi H, Hida K, et al. Hepatic stellate cells promote liver metastasis of colon cancer cells by the action of SDF-1/CXCR4 axis. *Ann Surg Oncol* 2009; 16: 2645-53.
- [16] Zeelenberg IS, Ruuls-Van Stalle L, Roos E. The chemokine receptor CXCR4 is required for outgrowth of colon carcinoma micrometastases. *Cancer Res* 2003; 63: 3833-9.
- [17] R. H. Wenger, D. P. Stiehl, G. Camenisch. Integration of oxygen signaling at the consensus HRE. *Sci STKE* 2005; 2005: re12.
- [18] Ishikawa T, Nakashiro K, Klosek SK, Goda H, Hara S,

- Uchida D, et al. Hypoxia enhances CXCR4 expression by activating HIF-1 in oral squamous cell carcinoma. *Oncol Rep* 2009; 21: 707-12.
- [19] Kensler TW, Wakabayashi N, Biswal S. Cell survival responses to environmental stresses via the Keap1-Nrf2-ARE pathway. *Annu Rev Pharmacol Toxicol* 2007; 47: 89-116.
- [20] DeNicola GM, Karreth FA, Humpton TJ, Gopinathan A, Wei C, Frese K, et al. Oncogene-induced Nrf2 transcription promotes ROS detoxification and tumorigenesis. *Nature* 2011. 475: 106-9.
- [21] Sporn MB, Liby KT. NRF2 and cancer: the good, the bad and the importance of context. *Nat Rev Cancer* 2012; 12: 564-71.
- [22] Ma Q, He X. Molecular basis of electrophilic and oxidative defense: promises and perils of Nrf2. *Pharmacol Rev* 2012; 64: 1055-81.
- [23] Singh A, Misra V, Thimmulappa RK, Lee H, Ames S, Hoque MO, et al. Dysfunctional KEAP1-NRF2 interaction in non-small-cell lung cancer. *PLoS Med* 2006; 3: e420.
- [24] Shibata T, Kokubu A, Gotoh M, Ojima H, Ohta T, Yamamoto M, et al. Genetic alteration of Keap1 confers constitutive Nrf2 activation and resistance to chemotherapy in gallbladder cancer. *Gastroenterology* 2008; 135: 1358-1368, 1368.e1-4.
- [25] Li CQ, Kim MY, Godoy LC, Thiantanawat A, Trudel LJ, Wogan GN. Nitric oxide activation of Keap1/Nrf2 signaling in human colon carcinoma cells. *Proc Natl Acad Sci USA* 2009; 106: 14547-51.
- [26] Schimanski CC, Schwald S, Simiantonaki N, Jayasinghe C, Gönner U, Wilsberg V, et al. Effect of chemokine receptors CXCR4 and CCR7 on the metastatic behavior of human colorectal cancer. *Clin Cancer Res* 2005; 11: 17437-50.
- [27] Juarez JG, Thien M, Dela Pena A, Baraz R, Bradstock KF, Bendall LJ. CXCR4 mediates the homing of B cell progenitor acute lymphoblastic leukaemia cells to the bone marrow via activation of p38MAPK. *Br J Haematol* 2009; 145: 491-9.
- [28] Zagzag D, Lukyanov Y, Lan L, Ali MA, Esencay M, Mendez O, et al. Hypoxia-inducible factor 1 and VEGF upregulate CXCR4 in glioblastoma: implications for angiogenesis and glioma cell invasion. *Lab Invest* 2006; 86: 1221-32.
- [29] Wang X, Li C, Chen Y, Hao Y, Zhou W, Chen C, et al. Hypoxia enhances CXCR4 expression favoring microglia migration via HIF-1 α activation. *Biochem Biophys Res Commun* 2008; 371: 283-8.
- [30] Kim TH, Hur EG, Kang SJ, Kim JA, Thapa D, Lee YM, et al. NRF2 blockade suppresses colon tumor angiogenesis by inhibiting hypoxia-induced activation of HIF-1 α . *Cancer Res* 2011; 71: 2260-75.
- [31] Akhdar H, Loyer P, Rauch C, Corlu A, Guillouzo A, Morel F. Involvement of Nrf2 activation in resistance to 5-fluorouracil in human colon cancer HT-29 cells. *Eur J Cancer* 2009; 45: 2219-27.
- [32] Li W, Khor TO, Xu C, Shen G, Jeong WS, Yu S, et al. Activation of Nrf2- antioxidant signaling attenuates NF κ B-inflammatory response and elicits apoptosis. *Biochem Pharmacol* 2008; 76: 1485-9.
- [33] Arlt A, Bauer I, Schafmayer C, Tepel J, Mürköster SS, Brosch M, et al. Increased proteasome subunit protein expression and proteasome activity in colon cancer relate to an enhanced activation of nuclear factor E2-related factor 2 (Nrf2). *Oncogene* 2009; 28: 3983-96.
- [34] Tsai JJ, Dudakov JA, Takahashi K, Shieh JH, Velardi E, Holland AM, et al. Nrf2 regulates haematopoietic stem cell function. *Nat Cell Biol* 2013; 15: 309-16.
- [35] Smith MC, Luker KE, Garbow JR, Prior JL, Jackson E, Piwnica-Worms D, et al. CXCR4 regulates growth of both primary and metastatic breast cancer. *Cancer Res* 2004; 64: 8604-12.
- [36] Gros SJ, Kurschat N, Drenckhan A, Dohrmann T, Forberich E, Effenberger K, et al. Involvement of CXCR4 Chemokine Receptor in Metastatic HER2-Positive Esophageal Cancer. *PLoS One* 2012; 7: e47287.
- [37] Shibata T, Ohta T, Tong KI, Kokubu A, Odogawa R, Tsuta K, et al. Cancer related mutations in NRF2 impair its recognition by Keap1-Cul3 E3 ligase and promote malignancy. *Proc Natl Acad Sci USA* 2008; 105: 13568-73.