POLSKI
PRZEGLĄD CHIRURGICZNY
2010, 82, 8, 459–463
10.2478/v10035-010-0066-1

THE – 801 G/A POLYMORPHISM OF *CXCL12* PROMOTER GENE AS UNFAVORABLE GENETIC PROGNOSIS FACTOR INVOLVED IN COLORECTAL CANCER*

EWA LANGNER[1], KAROLINA PRZYBYŁOWSKA[2], ANDRZEJ SYGUT[1], MICHAŁ MIK[1], ŁUKASZ DZIKI[1], MIROSŁAW DZIEKIEWICZ[3], IRENEUSZ MAJSTEREK[2], ADAM DZIKI[1]

Department of General and Colorectal Surgery, Medical University in Łódź[1]
Kierownik: prof. dr hab. *A. Dziki*
Department of Clinical Chemistry and Biochemistry, Medical University in Łódź[2]
Kierownik: prof. dr hab. *I. Majsterek*
Department of General, Oncological and Vascular Surgery, Military Institute of Medicine in Warsaw[3]
Kierownik: gen. rez. prof. dr hab. *M. Maruszyński*

CCXL12 also called stromal derived factor-1 (SDF-1), a protein related to angiogenesis and inflammation, has been correlated with the progression of a number of malignancies. Single nucleotide – 801G/A polymorphism of *CXCL12* gene has been described and is regarded as a target for cis-acting factor that has the ability to up-regulate CXCL12 expression.
The aim of the study. Based on the suggested role of CXCL12 in the pathogenesis of cancer we examined the association of the gene variant *CXCL12-A* with colorectal cancer.
Material and methods. We genotyped – 801G/A polymorphism of *CXCL12* gene in 164 colorectal patients and 184 age-matched healthy subjects. Genotyping was done with PCR-RFLP.
Results. There were no significant differences in the frequencies of SDF1-3'A allele, between patients and controls. The frequency of CXCL12 G/A and G/A plus A/A genotype was significantly higher in a group of patients with lymph node metastasis compared with those without metastasis.
Conclusions. The *CXCL12* gene G/A polymorphism was not related to colorectal cancer risk but is associated with the induction of lymph-node metastasis of colorectal cancer disease in Polish.
Key words: colorectal cancer, – 801 G/A polymorphism, *CXCL 12* gene

Chemokines are small cytokines, structurally characterized by conserved cysteine residues and first described for their ability to control leukocyte migration under basal and inflammatory conditions (1, 2). Whereas chemokines and their receptors not only regulate the development of T and B lymphocytes but also promote and regulate neoplastic progression including metastasis and angiogenesis (3, 4). The chemokine CXCL12, also known as stromal cellderived factor-1 (SDF-1), and its unique receptor CXCR4, have been implicated in cancer metastasis of many different neoplasms where the CXCL12/CXCR4 pathway is able to stimulate survival and growth of neoplastic cells in a paracrine fashion, which can be further enhanced by CXCL12-induced angiogenesis (5). Additionally, CXCL12 is present in lymph nodes, lungs, bone and liver, suggesting that this distribution could contribute to the tropism of cancer metastases for these sites (6, 7). Both *CXCR4* and *CXCL12* are normally expressed in the intestinal epithelium but in colorectal cancer cells CXCR4 is overexpressed while CXCL12 seems to be partially or irregularly expressed (8, 9). Recently, Kim J group identified the significance of CXCR4 expression in patients with colorectal cancer and demonstrated a significant association between high CXCR4 gene expression of primary CRC tumors and poor clinical outcomes (10). Moreover, Kollmar O group

identified that CXCL12 promotes a dose-dependent migration of colorectal cancer cells in vitro and tumor growth of solid metastasis in vivo (11). These findings suggest that high plasma CXCL12 levels in the blood will serve to retain tumor cells within the circulation and out of the metastatic organ site, and thus, low plasma CXCL12 levels will serve as a predictive marker for distant metastasis. CXCL12 mRNA and protein expression may be regulated by a common polymorphism in promoter region of *CXCL12* gene. A single nucleotide polymorphism (SNP) at position 801 (G to A) in the 3-untranslated region (3'UTR), whose A allele is regarded as a target of cis-acting factors, has been shown to have the ability of upregulating the expression of *CXCL12* (12, 13). Based on these hypothesis we examined the association of the *CXCL12* G801A promoter polymorphism gene with colorectal cancer.

MATERIAL

We analyzed 164 patients with sporadic colorectal cancers (67 females and 97 males, mean ages 56±10.8) who underwent surgery in the Department of General and Colorectal Surgery at the Medical University of Łódź between 2005 – 2008. The main familial syndrome like FAP and Lynch Syndrome were excluded from the research. Tumour stage according to TNM'classification was T1 in 2 patients, T2 in 30, T3 in 129 and T4 in 3 patients. The histological grades of tumours were determined according to a three degrees' scale as follows; 11 grade I°, 145 grade II° and 8 grade III° tumours. The control group consists of 184 individuals (82 females and 102 males mean age 51±10.1) who were confirmed without colorectal cancer or polyps by colonoscopy and without any apparent cancer phenotype or history. We did not observe any characteristic differences in association between cases and controls.

METHODS

DNA was extracted from normal colorectal tissue from colorectal cancer patients. DNA was isolated by proteinase K digestion and phenol/chloroform extraction. Genotypes of the – 801 G/A polymorphism in the *CXCL12* gene promoter were determined by the PCR-based *Msp*I restriction fragment length polymorphism (14). The following primers were used: CXCL12-F – 5'-CTGGGCAAAGCTAGTGAAG-3' (forward primer) and CXCL12-R – 5'-AGAACGTGGAGGATGTGGAG-3' (reverse primer). The PCR was carried out in MultiGene, Labnet International Inc thermalcycler, in a total volume of 50 µl, containing 50 ng genomic DNA, 10 pmol each primer (Eurogentec, Seraing, Belgium), 200 mM each dATP, dCTP, dGTP and dTTP (Qiagen, Germany), 20 mM Tris-HCl (pH 8.4) 50 mM KCl, 1.5 µl $MgCl_2$, and 1 unit Taq polymerase (Qiagen, Germany). Cycling conditions were a single predenaturation step at 94°C for 5 minutes followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing 57°C for 30 seconds, and elongation at 72°C for 30 seconds, and a final incubation at 72°C for 5 minutes. PCR-amplified DNA was digested with 3 U *Msp*I in total volume of 18 µl. The solution was incubated at 37°C for 16 h. 15 µl aliquots of the digest were electrophoresed on a 12% polyacrylamide gels and visualized by ethidium bromide staining. After *Msp*I digestion, if the allele G was present, amplified DNA (209 bp) produced two fragments 117 bp and 92 bp. When the A allele was present only one fragment 209 bp were observed. The heterozygote had three bands.

Statistical analysis

The significance of the differences of observed alleles and genotypes between groups was tested using the chi-square test. Odd ratios (ORs) and corresponding 95% confidences intervals (CI) were calculated to quantify the association between the clinicopathological features of colorectal cancer and all genotypes and alleles.

RESULTS

According to the data of RFLP analysis, all the patients and controls were divided into three genotypes of the *CXCL12* promoter region: G/G, G/A and A/A. Table 1 shows genotype distribution between colorectal cancer (CRC) patients and healthy individuals. There was no statistical difference in the genotypes frequencies of the CXCL12 G/A polymorphism between cases and controls. The genotype frequencies of the G/A polymorphism in patients (n = 164), and healthy controls (n = 184) were consistent with the Hardy-Weinberg equilibrium distribution (p value = 0.57071

Table 1. The – 801 G/A polymorphism of *CXCL12* gene promoter in CRC patients and the controls

Genotype Allele	CRC patients (n = 164)a number (frequencies)	Controls (n = 184)b number (frequencies)	OR (CI 95%)
G/G	98 (0,60)	119 (0,65)	Ref.
G/A	59 (0,36)	61 (0,33)	1,17 (0,75; 1,84)
A/A	7 (0,04)	4 (0,02)	1,70 (0,52; 5,52)
GA/AA	66 (0,40)	65 (0,35)	1,23 (0,80; 1,90)

a ($\chi 2$= 0,57071) b($\chi 2$= 1,427208) p> 0,05 as compared with Hardy-Weinberg distribution

and 1.42721 respectively). We investigated the relationship between the distribution of genotypes and frequency of alleles and the clinicopathological features of colorectal cancer. We did not find differences in distribution of genotypes and frequencies of alleles in groups of patients with different histological type. Patients groups with grading 2 and 3 were compared with grading 1 served as control (tab. 2). Table 3 shows the distribution of genotypes and frequencies of alleles in groups of patients with different tumor stages (T). There was no difference between the two groups with regard to pT criteria. The distribution of genotypes and frequencies of alleles in patients groups with positive and negative lymph node are displayed in tab. 4. We shown the higher risk of metastasis development in lymph node for G/A and G/A plus A/A genotypes (OR = 2.09; CI 95% 1.08; 4.03 and OR = 2.23; CI 95% 1.18; 4.23 respectively).

DISCUSSION

CXCL12 and its exclusive receptor, CXCR4, are reported to play important roles in tumor growth, angiogenesis and metastasis of different types of tumors. Therefore, the – 801 G/A polymorphism *CXCL12* gene studies have been done in several cancers and have shown that it is associated with an increased susceptibility to develop prostate, breast, lung, oral, and hepatocellular cancer (14-18). The epidemiologic data involving, the – 801 G/A functional

Table 2. The – 801 G/A polymorphism of *CXCL12* gene promoter in CRC patients with different histological types

Genotype Allele	G3 (n = 8) number (frequencies)	G2 (n = 145) number (frequencies)	G1 (n = 11) number (frequencies)	OR (PU 95%) G3/ G1	OR (PU 95%) G2/G1
G/G	4 (0,50)	89 (0,61)	5 (0,45)	Ref.	Ref.
G/A	4 (0,50)	50 (0,35)	5 (0,45)	1,11 (0,15; 6,42)	1,75 (0,30; 2,37)
A/A	0 (0,00)	6 (0,04)	1 (0,10)	----------	2,93 (0,29; 29,28)
GA/AA	4 (0,50)	56 (0,39)	6 (0,55)	1,22 (0,19; 7,44)	1,85 (0,54; 6,36)

Table 3. The – 801 G/A polymorphism of *CXCL12* gene promoter in CRC patients with different T stage

Genotype Allele	T3+T4 (n = 132) number (frequencies)	T1+T2 (n = 32) number (frequencies)	OR (CI 95%)
G/G	76 (0,58)	22 (0,69)	Ref.
G/A	49 (0,37)	10 (0,31)	1,42 (0,62; 3,25)
A/A	7 (0,05)	0 (0,00)	----------
GA/AA	56 (0,42)	10 (0,31)	1,62 (0,71; 3,69)

Table 4. The – 801 G/A polymorphism of *CXCL12* gene promoter in CRC patients with different lymph node status

GenotypeAllele	N1 (n = 75) number (frequencies)	N0 (n = 89) number (frequencies)	OR (PU 95%)
G/G	37 (0,49)	61 (0,69)	Ref.
G/A	33 (0,44)	26 (0,29)	2,09 (1,08;4,03)
A/A	5 (0,07)	2 (0,02)	4,12 (0,76;22,33)
GA/AA	38 (0,51)	28 (0,31)	2,23 (1,18;4,23)

transition of *CXCL12* gene in colorectal cancer risk are still discussed. In a previous report, Dimberg et al. analyzed the effect of – 801 G/A polymorphism in colorectal cancer risk. They found no statistical difference between colon cancer patients and controls from a Swedish series (19). However, when patients were stratified according to the tumor location (colon vs rectum), they found a significant increased frequencies of the allele A among rectal cancer patients when compared to the general population. Lack of association with risk cancer was determined in independent analysis of – 801 G/A polymorphism in a larger colorectal cancer series of Spanish origin (20). The data in this report showed that the genotype distribution and allelic frequencies were not significantly associated with colorectal cancer patients compared with controls. We conclude that this polymorphism is not a general susceptibility factor for colon cancer in the Polish population and are considered with previous studies. Nevertheless, the absence of an association with CRC risk does not exclude the possibility that – 801 G/A polymorphism may influence cancer progression. For this reason we performed different analyses to elucidate whether this polymorphism may be conditioning the tumor aggressiveness profile. According to our results, no correlation has been found among the typical progression markers such as histological grade or tumor size but we shown the higher risk of metastasis development in lymph node for G/A and G/A plus A/A genotypes. Association of CXCL12 with CXCR4 activates the receptor, leading to the activation of multiple signaling pathways, which regulates locomotion, chemotaxis, adhesion and secretion of tumour CXCR4 positive cells (9) and according to our results, we may speculate that in the presence of the allele G, this activation may be diminished. Comparable results were obtained in several cancer including breast, oral, prostate (14-18, 21).

The genetic data presented so far suggest that – 801 G/A polymorphism of *CXCL12* gene does not play a role in colorectal cancer risk, but might important in progression and metastasis formation.

Ethics

Written informed consent had been obtained from all participating subjects and the study had been approved by the local Ethics Committee of Medical University of Łódź.

REFERENCES

1. *Burger M, Glodek A, Hartmann T* et al.: Functional expression of CXCR4 (CD184) on small-cell lung cancer cells mediates migration, integrin activation, and adhesion to stromal cells. *Oncogene* 2003; 22: 8093-01.
2. *Klein RS, Rubin JB, Gibson HD* et al.: SDF-1_ induces chemotaxis and enhances Sonic hedgehog-induced proliferation of cerebellar granule cells. *Development*. 2001; 128: 1971-81.
3. *Raman D, Baugher PJ, Thu YM* et al.: Role of chemokines in tumor growth. *Cancer Lett* 2007; 256(2): 137-65.
4. *Zeelenberg IS, Ruuls-Van Stalle L, Roos E:* The chemokine receptor CXCR4 is required for outgrowth of colon carcinoma micrometastases. *Cancer Res* 2003; 63: 3833-39.
5. *Burger JA, Kipps TJ:* CXCR4: a key receptor in the crosstalk between tumor cells and their microenvironment. *Blood* 2006; 107(5): 1761-67.
6. *Fernandis AZ, Prasad A, Band H* et al.: Regulation of CXCR4-mediated chemotaxis and chemoinvasion of breast cancer cells. *Oncogene* 2004; 23: 157-67.
7. *Taichman RS, Cooper C, Keller ET* et al.: Use of the stromal cellderived factor-1/CXCR4 pathway in prostate cancer metastasis to bone. *Cancer Res* 2002; 62: 1832-37.
8. *Wendt MK, Johanesen PA, Kang-Decker* N et al.: Silencing of epithelial CXCL12 expression by DNA hypermethylation promotes colonic carcinoma metastasis. *Oncogene* 2006, 25: 4986-97.
9. *Yoshitake N, Fukui H, Yamagishi H* et al.: Expression of SDF-1 alpha and nuclear CXCR4predicts lymph node metastasis in colorectal cancer. *Br J Cancer* 2008; 98(10): 1682-89.
10. *Kim J, Mori T, Chen SL* et al.: Chemokine receptor CXCR4 expression in patients with melanoma and colorectal cancer liver metastases and the association with disease outcome. *Ann Surg* 2006; 244(1): 113-20.
11. *Kollmar O, Rupertus K, Scheuer C* et al.: Stromal cell-derived factor-1 promotes cell migration and tumor growth of colorectal metastasis. *Neoplasia* 2007; 9(10): 862-70.
12. *Winkler C, Modi W, Smith MW* et al.: Geneticr estriction of AIDS pathogenesis by an chemokine

gene variant. ALIVE Study, Hemophilia Growth and Development Study (HGDS), Multicenter AIDS Cohort Study (MACS), Multicenter Hemophilia Cohort Study (MHCS), San Francisco City Cohort (SFCC). *Science* 1998; 279: 389-93.
13. *Watanabe MA, de Oliveia Cavassin GG, Orellana MD* et al.: SDF-1 gene polymorphisms and syncytia induction in Brazilian HIV-1 infected individuals. *Microb Pathog* 2003; 35: 31-34.
14. *Hirata H, Hinoda Y, Kikuno N* et al.: CXCL12 G801A polymorphism is a risk factor for sporadic prostate cancer susceptibility. *Clin Cancer Res* 2007; 13(17): 5056-62.
15. *Razmkhah M, Talei AR, Doroudchi M* et al.: Stromal cell-derived factor-1 (SDF-1) alleles and susceptibility to breast carcinoma. *Cancer Lett* 2005; 225: 261-66.
16. *Razmkhah M, Doroudchi M, Ghayumi SM* et al.: Stromal cell-derived factor-1 (SDF-1) gene and susceptibility of Iranian patients with lung cancer. *Lung Cancer* 2005; 49: 311-15.
17. *Teng YH, Liu TH, Tseng HC* et al.: Contribution of genetic polymorphisms of stromal cell-derived factor-1 and its receptor, CXCR4, to the susceptibility and clinicopathologic development of oral cancer. *Head Neck* 2009; 31(10): 1282-88.
18. *Chang CC, Chen SC, Hsieh YH* et al.: Stromal cell-derived factor-1 but not its receptor, CXCR4, gene variants increase susceptibility and pathological development of hepatocellular carcinoma. *Clin Chem Lab Med* 2009; 47(4): 412-18.
19. *Dimberg J, Hugander A, Löfgren S* et al.: Polymorphism and circulating levels of the chemokine CXCL12 in colorectal cancer patients. *Int J Mol Med* 2007; 19(1): 11-15.
20. *Hidalgo-Pascual M, Galan JJ, Chaves-Conde M* et al.: Analysis of CXCL12 3'UTR G>A polymorphism in colorectal cancer. *Oncol Rep* 2007; 18(6): 1583-87.
21. *Vairaktaris E, Vylliotis A, Spyridonodou S* et al.: A DNA polymorphism of stromal-derived factor-1 is associated with advanced stages of oral cancer. *Anticancer Res* 2008; 28(1A): 271-75.

Received: 3.07.2010 r.
Adress correspondence: 90-647 Łódź, Plac Hallera 1