–2518 A/G *MCP-1* but not –403 G/A *RANTES* gene polymorphism is associated with enhanced risk of basal cell carcinoma

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Abstract

Introduction: Polymorphic variants of *MCP-1* and *RANTES* genes and their protein serum levels have been implicated in the increased risk and severity of several malignancies. However, the subject has not been explored in basal cell carcinoma (BCC) patients so far.

Aim: To investigate the association between monocyte chemoattractant protein 1 (MCP-1) (–2518 A/G) and *RANTES* (–403 G/A) polymorphism and risk and clinical course of BCC.

Material and methods: The study group consisted of 150 unrelated patients with BCC and 140 healthy, unrelated, age- and sex-matched volunteers. The polymorphisms were analysed using the amplification refractory mutation system polymerase chain reaction method (ARMS-PCR) and single specific primer-polymerase chain reaction (SSP-PCR). Serum cytokine levels were measured with ELISA.

Results: The presence of the *MCP-1* –2518 GG genotype was statistically more frequent in BCC patients and it increased the risk of BCC (OR = 2.63, p = 0.003). Genotype –330 GG was statistically more common in patients with less advanced tumours (OR = 2.8, p = 0.017). Monocyte chemoattractant protein 1 serum level was statistically higher with GG genotype. In the BCC group MCP-1 serum levels were decreased. Neither polymorphic variants of *RANTES* nor the chemokine serum concentration differed significantly between the study groups.

Conclusions: These findings suggest that –2518 A/G *MCP-1* polymorphism may be involved in BCC pathogenesis.

Key words: monocyte chemo-attractant protein 1, monocyte chemoattractant protein 1, CCL2, regulated upon activation normal T-cell expressed and secreted, RANTES, CCL5, gene polymorphism, basal cell carcinoma, BCC.

Introduction

The immune response plays an important role in the development and progression of cancer. The tumour microenvironment consists of stromal and inflammatory cells which secrete a plethora of cytokines, chemokines and growth factors. It was demonstrated that chemokines have a fundamental role not only in inflammation and immune surveillance but also in cancer progression. Several malignant solid tumours have been found to be associated with marked alterations in chemokine patterns, reflecting the tumours' biological aggressiveness, clinical progression and prognosis [1–4].

The pathogenesis of basal cell carcinoma (BCC), the most common malignancy in Caucasian populations, is complex and still not fully unravelled. It seems to be strongly associated with environmental and genetic factors. Previous studies have indicated a polygenic background of the disease. Basal cell carcinoma is an immunogenic neoplasm; the tumour tissue is infiltrated by CD4+, CD25+, Foxp3+ T regulatory lymphocytes (Treg) and immature dendritic cells (DCs). The imbalance of Th1 and Th2 cytokine expression was also reported. The immune response seems to play a major role in spontaneous and pharmacologically induced (imiquimod) BCC regression [5–7].

Neoplastic tissues have been demonstrated to contain a variety of chemokines and their receptors. The monocyte chemoattractant protein 1 (MCP-1), also known as chemokine ligand 2 (CCL2), and regulated upon activation normal T-cell expressed and secreted (RANTES), also known

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as CCL5, are among the best investigated ones. Both are members of the CC chemokine subfamily and are involved in the chemotaxis of monocytes, T-lymphocytes, and DCs. MCP-1 and RANTES can act as growth factors, facilitate angiogenesis and promote metastasis formation. However, their functions in *in-vivo* cancer biology remain unclear, as available data suggest that they present with both pro- and anti-tumour properties [4, 8–11].

Polymorphic variants of *MCP-1* and *RANTES* genes and serum levels of MCP-1 and RANTES proteins are associated with increased risk and severity of several malignancies. However, to our best knowledge, the subject has not been explored in BCC patients so far [4, 12–14].

In this study, polymorphisms in the *MCP-1* gene (–2518 A/G) and *RANTES* gene (–403 G/A) as well as the serum concentrations of these two chemokines were assessed in relation to BCC incidence and its clinical course in a population from northern Poland.

Material and methods

Patients and controls

The study group consisted of 150 unrelated patients with BCC (96 women, 84 men; mean age: 68.7 ±11.6 years) and 140 healthy, unrelated age- and sex-matched volunteers. Characteristics of patients and controls are presented in Table 1. Patients were treated with surgery for primary or recurrent BCC, in the Department of Dermatology, Venereology and Allergology, Medical University of Gdansk, Poland, between 2009 and 2010. Individuals with histopathological features of tumour regression were excluded. Organ transplant recipients, patients on immunosuppressive therapy, and those suffering from any systemic inflammatory disease or other malignancy were excluded from the study participation. All subjects were exclusively of Eastern European/Polish descent.

Table 1. Characteristics of the 150 BCC patients investigated

Variables	Number (%)
Gender	
Males	88 (58.7)
Females	62 (41.3)
Tumour size [cm]	
≤ 1	69 (46)
> 1	81 (54)
Recognition	
BCC	108 (72)
BCC recurrent	42 (28)
Number of tumours	
Single	109 (72.7)
Multiple	41 (27.3)
Location	
Area exposed to UV	111 (74)
Area not exposed to UV	39 (26)

The study was approved by the local research ethics committee of the Medical University of Gdansk.

MCP-1 and *RANTES* genotyping

The polymorphism of *MCP-1* (–2518 A/G) was analysed using the amplification refractory mutation system polymerase chain reaction method (ARMS-PCR), while the *RANTES* (–403 G/A) polymorphism was assessed with single specific primer-polymerase chain reaction (SSP-PCR) according to the methods described previously [15, 16].

MCP-1 and RANTES serum levels

Serum concentrations of MCP-1 and RANTES were measured in 120 patients with BCC and in 60 controls using ELISA tests (Human ELISA kit, Diaclone SAS, France and the Quantikine Human RANTES Immunoassay, R&D Systems, Inc., Minneapolis, USA) following the manufacturer's instructions. Concentrations of the proteins were not affected by the age or sex of examined individuals, either in the BCC or in the control group.

Statistical analysis

χ^2 analysis was used to compare the observed number of genotypes with that expected for a population in Hardy-Weinberg equilibrium. χ^2 analysis was employed to test the significance of differences in the observed alleles and genotypes between groups. A logistic regression model was used to calculate the odds ratios (ORs) and the 95% confidence intervals (CIs). The Mann-Whitney *U*-test was used to compare the median values, and the correlation was determined using mean Spearman coefficient values. Analyses were performed with the Statistica 10.0 software package (StatSoft, Inc., 2011). *P*-value < 0.05 was considered statistically significant.

Results

MCP-1 and *RANTES* polymorphisms

The *MCP-1* and *RANTES* genotype frequencies in BCC patients and in controls are shown in Table 2. The distribution of genotypes was consistent with the Hardy-Weinberg equilibrium for *MCP-1* only in the control group and for *RANTES* in both analysed groups.

The presence of the *MCP-1* 2518 GG genotype was statistically more frequent in patients and it increased the risk of BCC (OR = 2.63, $p = 0.003$). The presence of allele G (GG or GA) in the 2518 *MCP-1* polymorphism was associated with an increased risk of developing BCC (OR = 1.67, $p = 0.0034$). Genotype GG was statistically

more common in a group of patients with less advanced tumours (diameter less than 1 cm) (33.33% vs. 15.15%) (OR = 2.8; $p = 0.017$).

At the –403 G/A locus of the *RANTES* polymorphism, the frequencies of genotypes and alleles did not differ significantly between the patients and controls.

No statistically significant correlations were demonstrated between the *RANTES* polymorphisms and the chemokine serum levels.

MCP-1 and RANTES serum levels

Monocyte chemoattractant protein 1 serum levels were significantly lower in BCC patients compared with healthy controls (median: 419.13; mean: 711.41 ±305.52 pg/ml; range: 220.00–1631.10 pg/ml vs. median: 661.53, mean: 446.74 ±182.419 pg/ml; range: 147.50–1583.30 pg/ml; $p < 0.0000001$) (Figure 1). Monocyte chemoattractant protein 1 serum levels were statistically higher in patients bearing the GG genotype (median: 475.30 pg/ml vs. 394.00 pg/ml; $p = 0.03$).

Table 2. Frequencies of genotypes and alleles for *MCP-1* –2581 A/G and *RANTES* –403 G/A in patients with BCC and control subjects

Genotypes and alleles	Controls	BCC	OR (95% CI) *P*-value
MCP-1 –2581 A/G	*N* = 140	*N* = 150	
AA	73 52.14%	64 42.67%	NS
AG	51 36.43%	48 32.00%	NS
GG	16 11.43%	38 25.33%	2.63 (1.39–4.97) 0.003
	N = 280	*N* = 300	
A	197 70.36%	176 58.67%	1.67 (1.18–2.36) 0.0034
G	83 29.64%	124 41.33%	
RANTES –403 G/A	*N* = 140	*N* = 150	
GG	84 60.00%	78 52.00%	NS
GA	50 35.71%	65 43.33%	NS
AA	6 4.29%	7 4.67%	NS
	N = 280	*N* = 300	
G	218 77.86%	221 73.67%	NS
A	62 22.14%	79 26.33%	

BCC – basal cell carcinoma, OR – odds ratio, CI – confidence interval, NS – not significant.

RANTES levels did not differ significantly between groups. No association between the tumour stage and the chemokines analysed was found.

Discussion

Exploration of the potential link between polymorphisms of chemokine genes and cancer risk or its clinical course has attracted growing interest over recent decades.

To date, studies addressing this issue have returned inconsistent results, reflecting variations in the selection of patients and controls and their numbers, or opposite effects in different carcinomas [17].

Several *MCP-1* and *RANTES* polymorphisms have been reported in association with cancer risk and clinical course. Here, the functional promoter polymorphisms in the *MCP-1* and *RANTES* genes were chosen, because of their potential impact on chemokine expression [18, 19].

The results of the present study are the first demonstration of the association between 2518 A/G *MCP-1* polymorphism and BCC. We demonstrated that individuals with GG genotype present with more than two-fold higher risk of BCC. Further analysis in BCC subgroups revealed that GG genotype was also connected with less advanced tumours. The current results indicate the involvement of 2518 A/G *MCP-1* polymorphism in the development and clinical course of BCC.

The true impact of the 2518 A/G *MCP-1* polymorphism on cancer susceptibility and progression is still controversial. No statistically significant association between cancer risk and the 2518 A/G *MCP-1* polymorphism was found in the meta-analysis of 19 case-control studies, in-

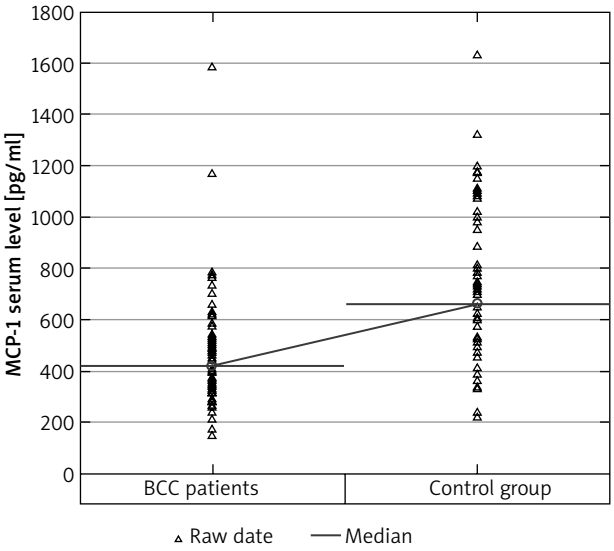

Figure 1. MCP-1 serum levels in BCC patients in relation to healthy controls. Statistically lower median concentration in BCC group (419.13 vs. 661.53 pg/ml; $p < 0.0000001$)

cluding 4162 cases and 5173 controls [12]. However, a significantly increased frequency of digestive system cancer was found in Caucasian individuals with GG genotype. Based on the available data, we hypothesize that 2518 A/G *MCP-1* polymorphism may influence the manifestation of specific cancer types and populations. Unfortunately, data on mechanisms by which 2518 A/G *MCP-1* polymorphism and the chemokine itself are involved in cancerogenesis are still insufficient.

We have also confirmed a positive influence of GG genotype on the chemokine serum expression, which had been reported previously [18, 20].

It was suggested that serum chemokines may direct inflammatory cells to specific sites throughout the body where they participate in the initiation and progression of a tumour. Chemokines are responsible for actions such as leukocyte recruitment, proliferation and survival of malignant cells, invasion, metastasis and neo-angiogenesis [21–23]. In this study, we demonstrated, for the first time, reduced MCP-1 serum levels in BCC patients. The results of studies in other neoplasms provide inconsistent data. Ding *et al.* [24] noted significantly lower MCP-1 and CCL3 serum concentrations in patients with oral squamous cell carcinoma and leukoplakia. Similar results were obtained for ovarian and colorectal cancer [25–27]. Tsaur *et al.* [28] reported down-regulation of *MCP-1* and gene expression of other chemokines in renal cell cancer. In contrast, chemokine over-expression was reported in some other cancers [29–31]. The nature of MCP-1 involvement in oncologic disorders is still unknown. Increased expression of this chemokine in the tumour may recruit more macrophages that accelerate either tumour destruction or progression depending on the type of macrophage recruited. These mechanisms seem to be complex and involve a lot of chemokines and their receptor axes. The source of the MCP-1 in the serum of BCC patients has not been defined. The host and tumour origin should be considered.

The role of MCP-1 in cutaneous malignancies is poorly explored. Welss *et al.* [32] reported *MCP-1* gene over-expression in 5 of 10 analysed BCC. Fan *et al.* [33] observed elevated chemokine expression in BCC in a mouse model. The authors revealed a novel signalling network of hedgehog-transforming growth factor (TGF)-β-MCP-1/CCR2 in the recruitment of myeloid-derived suppressor cells. They concluded that a high concentration of MCP-1 at the tumour site enhanced recruitment of myeloid-derived suppressor cells to the tumour site, and created immunosuppression in the tumour microenvironment.

Nakasone *et al.* [34] assessed the role of MCP-1 and macrophage inflammatory protein-1α (MIP-1α/chemokine ligand 3) in primary and metastatic B16 F10 melanoma. Melanoma growth and metastasis potential was augmented in mice lacking MCP-1 or MIP-1α. This animal model also showed a decreased percentage of infiltrating $CD4^+$ T cells, $CD8^+$ T cells and natural killer cells as well as reduced local expression of interferon-γ, IL-6, tumor necrosis factor (TNF)-α and TGF-β. Oppositely, local administration of MCP-1 and MIP-1α significantly inhibited the primary tumour growth in wild type mice. These results indicated that host-derived MCP-1 and MIP-1α regulate the protective anti-tumour immune response to B16 F10 melanoma by promoting lymphocyte infiltration into the tumour and subsequent cytokine production.

We hypothesize that lower MCP-1 serum expression may enable BCC progression via decreasing the density of infiltrating $CD4^+$ and $CD8^+$ T cells and natural killers cells.

According to the available data, a dichotomous role (i.e. pro- and anti-tumour) of MCP-1 in cancer pathogenesis is proposed. Our findings suggest that patients bearing the GG genotype have higher chemokine serum levels and increased risk of BCC. However, total serum MCP-1 concentration in BCC is decreased. Moreover, higher chemokine serum concentration in the group with less advanced tumours (less than 1 cm in diameter) was noted, but it was not statistically significant ($p = 0.45$). It is possible that the reduction in the MCP-1 serum expression in BCC depends on various factors such as MCP-1 polymorphism, tumour immunologic activity, expression of other cytokines, chemokines and growth factors as well as the host immune response.

Although the connection between *RANTES* polymorphism and chemokine serum concentration and some malignancies has been demonstrated previously, we failed to confirm that phenomenon in BCC [35, 36]. Little is known about the role of RANTES in BCC. Aoki *et al.* [37] found an increased number of mast cells in BCC lesions as well as increased expression of VEGF, IL-8 and RANTES on their surface. They concluded that mast cells may play an active role in the angiogenesis in BCC via production of VEGF and IL-8. Furthermore, mast cells may also regulate lymphocytic infiltration via RANTES and IL-8 production.

In the light of our findings, we suggest that 2518 A/G *MCP-1* polymorphism is involved in BCC pathogenesis. Basal cell carcinoma is characterized by broad and complex changes in levels of serum cytokines, chemokines and growth factors. Monocyte chemoattractant protein 1 requires more detailed studies in this context. The role of chemokines in BCC pathogenesis seems to be complex. Potential associations of BCC with genes of other chemokines and their receptors cannot be ruled out. Further, larger studies involving different populations are needed to confirm these results.

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Conflict of interest

The authors declare no conflict of interest.

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