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ORIGINAL ARTICLE

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CXCL12-3′A Polymorphism and lung cancer metastases protection: new perspectives in immunotherapy?

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Introduction

Lung cancer remains a major worldwide health problem, being the most common form of cancer in the world, both in terms of incidence, 12.3% of all cancers (with an estimated 1.2 million new cases in 2000), and mortality (1.1 million deaths in 2000), representing 17.8% of the total number of deaths caused by cancer. More than half of the new cases occur in more developed countries (52%), with incidence rates lower in women (11.1 per 1,000,000), than in men (34.9 per 1,000,000) [17].

There are two major groups of lung carcinomas: small-cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). The latter includes epidermoid (squamous cell) carcinoma (which accounts for 25–40% of all lung cancers), adenocarcinoma and large cell carcinoma [5]. During the development of epidermoid carcinoma, oncogenic stimuli leads to a series of conversions of the normal bronchial epithelium, passing from premalignant lesions (hyperplastic, metaplastic, and dysplatic lesions) to carcinoma in situ that progresses to an overt carcinoma [19]. Adenocarcinomas are also thought to develop in part from premalignant precursor lesions such as atypical adenomatous hyperplasia [19].

Non-small cell lung cancer metastases to regional lymph nodes, liver, adrenal glands, contralateral lung, brain, and bone marrow are a key factor in the aggressiveness of this cancer [17] and experimental data have demonstrated that sites of metastases are determined both by the characteristics of neoplastic cells and the microenvironment of the specific organ [4].

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Although tobacco smoking is pointed as the most important cause of lung cancers with 80–90% arising in cigarette smoker individuals [12], the biology of lung cancer is complex and poorly understood, and a variety of biomarkers have been implied in the virulence of this type of cancers [23].

Chemokines have recently emerged as an important family of proteins involved in tumourgenesis and organ-specific metastases [1, 13–15, 17, 21]. The chemokine receptor CXCR4 and its sole ligand SDF-1/CXCL12 (Stromal Cell-Derived Factor-1) play important roles in inflammation and hematopoiesis, by acting as chemoattractant for leukocytes and stem cells [20]. Furthermore, it has been shown that in several types of cancer—including melanoma, ovarian, breast, and lung cancer—CXCL12 can stimulate the proliferation and/or survival of CXCR4-expressing cancer cells when they are grown under sub-optimal conditions, an adaptation that may allow tumour cells to grow in distant sites that would normally be less favourable [1]. The CXCR4-expressing metastatic cells might spread to many sites in the body, but only become established as metastatic locations where high levels of CXCL12 are found [1].

A single nucleotide polymorphism in the 3′ untranslated region of the *CXCL12* gene—*CXCL12-3′A*—was reported as delaying AIDS progression to death [22, 24] and Zafiropoulos et al. associated the allelic frequency of the polymorphism with breast cancer and melanoma [25].

The aim of our study was to evaluate the genetic influence of *CXCL12-3′A* polymorphism in the susceptibility to lung cancer development.

Materials and methods

Patient and control characteristics

We conducted a study among 403 individuals. The case group consisted of 154 Caucasian patients from the

north region of Portugal with lung cancer diagnosed in the Portuguese Institute of Oncology, Porto, Portugal between 1999 and 2001. The median age at diagnosis was 64.0 years with a mean age of 62.5 years (standard deviation = 10.7). Two hundred and forty-nine healthy controls were randomly recruited from the same areas as the cases, all of them unrelated, with a mean age of 50 years (standard deviation = 17.8). The control group represents asymptomatic and apparently clinically disease- free subjects. All samples were obtained with the written informed consent of the participants prior to their inclusion in the study, according to the Helsinki declaration.

DNA extraction and genotyping

Approximately 8 ml of venous blood was obtained with a standard venipuncture technique using EDTA containing tubes and after DNA extraction [11], genotypes were analysed through PCR (annealing temperature: 58°C) followed by RFLP (*MspI*) techniques, as previously described by Kristiansen et al. [8], using the following primers: *CXCL12-3′A* F 5′—CAGTCAACCTGGGCAAAGCC-3′ and *CXCL12-3′A* R 5′—GCTTTGGTCCTGAGAGTCC-3′.

The restriction fragments were then visualised by agarose gel electrophoresis (3%), with ethidium bromide staining, showing three types of band patterns: wild type homozygote (G/G), two bands corresponding to 201 and 101 bp; heterozygote (G/A), three bands corresponding to 302, 201, and 101 bp; and mutated homozygote (A/A), only one band with 302 bp. Whenever there were doubts in the discrimination between partial digests and apparent heterozygotes, genotyping was repeated twice, using higher concentrations of restriction enzyme to rule out partial digestion. Two investigators scored gels independently and unclear positions were repeated.

Statistical analysis

Analysis of data was performed using the computer software SPSS for windows (Version 11.5). Chi-square analysis was used to compare categorical variables. A 5% level of significance was used in the analysis.

The odds ratio (OR) and its 95% confidence interval (CI) was calculated as a measure of the association between *CXCL12-3′A* genotypes and lung cancer risk.

Multivariate logistic regression analysis was used to calculate the adjusted OR (aOR) and 95% CI for the influence of *CXCL12-3′A* genotypes in the risk for lung cancer with adjustment for age and gender. The Hardy–Weinberg equilibrium was tested by a Pearson goodness-of- fit test to compare the observed versus the expected genotype frequencies.

Results

The *CXCL12-3′A* genotype distribution in the case and control groups was in the Hardy–Weinberg equilibrium ($P = 0.78$ in the case group and $P = 0.65$ in the control group).

Table 1 summarises the characteristics of the lung cancer patients and the controls included in this study.

Allele frequencies (%) of the *CXCL12-3′A* polymorphism in lung cancer patients and controls are shown in Table 2.

No statistically significant association was found when we compared the allelic frequencies of *CXCL12-3′A* in the three different histological types of lung cancer analysed and the control group (SCLC, $P = 0.758$; epidermoid, $P = 0.276$; adenocarcinoma, $P = 0.368$).

Table 3 represents the prevalence and odds ratio of *CXCL12-3′A* genotypes among Epidermoid NSCLC with and without long distance metastases (LDM).

Table 1 Characteristics of lung cancer patients and controls

	Patients ($n = 154$)		Controls ($n = 249$)	
	n	Percentage	*n*	Percentage
Female	20	13.0	169	67.9
Male	134	87.0	80	32.1
Total	154	100.0	249	100.0
Mean ± SD	62.5 ± 10.7		50 ± 17.8	
NSCLC Epidermoid	78	50.6		
NSCLC Adenocarcinoma	50	32.5		
Small-cell lung cancer (SCLC)	14	9.1		
Not stated	12	7.8		
Total	154	100.0		
I	22	114.3		
II	15	9.7		
III	56	36.4		
IV	48	31.2		
Not stated	13	8.4		
Total	154	100.0		
Yes	47	30.5		
No	94	61.0		
Not stated	13	8.5		
Total	154	100.0		

Table 2 Allele frequencies (%) of the *CXCL12-3′A* polymorphism in lung cancer patients and controls

Histology	*CXCL12-3′A*		*CXCL12-3′A*		*P*
	n	Percentage	*n*	Percentage	
Controls ($n = 249$)	95	19.1	403	80.9	–
SCLC ($n = 14$)	6	21.4	22	78.6	0.758
Epidermoid ($n = 78$)	36	23.1	120	76.9	0.276
Adenocarcinoma ($n = 50$)	23	23.0	77	77.0	0.368

SCLC small cell lung cancer

Table 3 Prevalence and odds ratio of *CXCL12-3′A* genotypes among Epidermoid NSCLC with and without long distance metastases (*LDM*)

	Epidermoid without LDM (n = 58)		Epidermoid with LDM (n = 20)		OR	95%CI	P
	n	Percentage	n	Percentage			
Genotypes							
AA	5	8.6	0	0.0			
AG	22	37.9	4	20.0			
GG	32	53.5	16	80.0			
Recessive Model							
A Carrier	27	46.6	4	20.0	1.00	Reference	
GG	31	53.4	16	80.0	0.29	0.09–0.97	0.036

Table 4 Multivariate logistic regression analysis of the presence of A allele, gender and age at diagnosis regarding the susceptibility to develop LDM of epidermoid NSCLC

	P^*	aOR*	95% CI*
A Carrier	0.032	0.221	0.056–0.877
Age≥66	0.013	0.220	0.067–0.728
Gender	0.067	0.109	0.010–1.164

*P, aOR, and 95% CI using logistic regression analysis

The frequencies of GG, GA, and AA genotypes were 53.5, 37.9, and 8.6%, respectively, in the case group without LDM and the frequencies of GG and GA genotypes were 80.0 and 20.0%, respectively, in the case group with LDM, with the genotype AA absent in this group. The analysis of the frequencies of *CXCL12-3′A* genotypes indicates that individuals with genotypes carrying the A allele present almost 3.5 times less probability of developing LDM of Epidermoid NSCLC (OR = 0.29; 95%IC 0.09–0.97; $P = 0.036$).

Multivariate logistic regression analysis of the presence of A allele, gender and age at diagnosis regarding the susceptibility to develop long distant metastases of epidermoid NSCLC is represented in Table 4.

The results indicate the association of the A allele presence (aOR = 0.221; 95%IC 0.056–0.877; $P = 0.032$) and age at diagnosis above 66 years (aOR = 0.220; 95%IC 0.067–0.728; $P = 0.013$) with the development of long distant metastases. There was no statistically significant association regarding gender (aOR = 0.109; 95%IC 0.010–1.164; $P = 0.067$).

We present only uncorrected *P*-values because applying Bonferroni correction of *P* for multiple tests, only the parameter age ≥66 years remains significant.

Discussion

The aim of the present study was the investigation of a potential role of the *CXCL12-3′A* polymorphism in the susceptibility to lung cancer development.

In this study, the analysis of the results suggests a protective association between *CXCL12-3′A* polymorphism and the development of LDM of Epidermoid NSCLC. Individuals carrying the A allele from *CXCL12-3′A* polymorphism seem to present almost 3.5 times less probability of developing LDM of Epidermoid NSCLC (OR = 0.29; 95%IC 0.09–0.97; $P = 0.036$). However, when applying the Bonferroni correction, a more conservative approach, these associations did not remain statistically significant, except for the parameter age ≥66 years.

Stromal cell-derived factor 1 (CXCL12) is a member of the CXC sub family of chemokines, constitutively expressed by stromal cells. Chemokines are chemotactic cytokines that cause the direct migration of leukocytes, and are induced by inflammatory cytokines, growth factors and pathogenic stimuli. Directed migration of cells that express the appropriate chemokine receptor occurs along a chemical gradient of ligand—known as the chemokine gradient—allowing cells to move towards high local concentrations of chemokines [1].

The main cause of treatment failure and death for cancer patients is metastases—the formation of secondary tumours in organs far away from the original cancer [10]. Studies on the contribution of chemokine receptors to organ-specific metastases provided important clues why some cancers metastasize to specific organs.

The work of Muller et al. [14] showed that breast cancer cells express chemokine receptors in a defined, rather than a random manner and provided direct evidence that the CXCL12/CXCR4 biological axis is involved in breast cancer metastases to specific organs, similar in character to NSCLC metastases.

Later, Phillips et al. [17] examined the interrelationship between primary NSCLC tumour growth and metastases and the pair CXCL12/CXCR4. Their studies revealed that although NSCLC primary tumour and plasma have measurable levels of CXCL12, higher levels of the chemokine are observed in organs that are known to be the preferred sites of NSCLC tumour metastases. Furthermore, in vivo neutralization of CXCL12 in a SCID mouse system of spontaneous metastases of human NSCLC resulted in a marked attenuation of NSCLC metastases to several organs, including the adrenal glands, liver, lung, and bone marrow, suggesting that a chemotactic gradient could be established between

the site of primary tumour and those organs that develop NSCLC tumour metastases.

Taking in to account where CXCL12 is expressed, the CXCL12/CXCR4 axis appears to be the most important pair regulating metastases to the lung, liver, bone marrow, adrenal glands, and perhaps brain [26] and the prevailing theory for this metastases regulation is that the ability of chemokine receptor to mediate cancer metastases is similar to its normal function in regulating cell migration [2].

CXCL12-3′A is a single nucleotide polymorphism, consisting of a G to A transition at the position 801 (counting from the ATG starting codon) in the 3′ untranslated region (UTR3′) of the CXCL12 gene. Although our results suggest a protective role of the *CXCL12-3′A* polymorphism in the development of epidermoid NSCLC with LDM, the mechanism remains unclear, because very little is known about the effects of the polymorphism in CXCL12 function.

Association of CXCL12 with CXCR4 activates the receptor, leading to the activation of multiple signalling 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 polymorphism, this activation may not occur or simply be diminished.

Immunotherapy is a promising approach for the development of integrative therapies for cancer. In combination with strategies such as surgery, chemotherapy and radiation therapy, immunotherapy could provide a tool to efficiently attack residual disease and provide prolonged tumour-specific survival [6]. Recent literature points chemokines and their receptors as possible therapeutic targets in adjuvant cancer therapies [1, 3, 18].

In spite of being suggestive of and consistent with our hypothesis, the present results must be considered cautiously. Further studies are needed to confirm the role of a functional *CXCL12* gene SNP regarding the relationship between NSCLC and host. This is a very complex matter involving an incredible high number of variables each of which may influence this relationship to some degree. Should our data be confirmed, in the future *CXCL12-3′A* could be a reliable candidate for inclusion in a panel of genetic factors conditioning the course of the disease and serve as a model to the development of an immunotherapy, leading to a better prognosis in patients suffering from lung cancer.

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