The – 173 G/C Polymorphism of the Promoter Region Macrophage Migration Inhibitory Factor Gene is not Associated with Incidence of Pulmonary Hamartoma

Ewa Kaznowska¹, Karolina Przybyłowska², Andrzej Kulig³

Hamartomas are the third most common cause of solitary pulmonary nodule and the most common benign tumors of lung. Recent study indicated that hamartoma may be associated with a chronic inflammatory diseases. Histochemical analysis of the expression profile of growth-relevant was shown the upregulation of macrophage migration inhibitory factor (MIF) in hamartomas and surrounding lung parenchyma.

We investigated polymorphism G/C at position –173 promoter gene of MIF, pro-inflammatory cytokine in pulmonary hamartoma. This polymorphism of the MIF gene are association with increased production of MIF and have been found to confer increased risk of susceptibility to chronic inflammatory diseases.

DNA samples were obtained from hamartoma tissue fixed with formalin, embedded in paraffin, from 52 patients and from blood samples of 123 sex and age matched healthy person served as control. The G/C polymorphism of MIF gene was determined by PCR-based AluI restriction fragment length polymorphism.

The frequencies of the C allele did not differ significantly between pulmonary hamartoma patients and healthy controls (18% vs 15%, OR 1.26 CI95% 0.68-2.40). The obtained results suggest no association between G/C polymorphism at promoter gene of MIF and the incidence of pulmonary hamartoma, but our study has a preliminary character and should be extended on larger population.

Introduction

Pulmonary hamartoma is the most common type of benign lung tumours reaching on incidence of about 5% among lung tumours [9,16]. Pulmonary hamartomas can be seen in the lung parenchyma and within tracheobronchial tree and occasionally in endobronchial localisation [14, 23]. Parenchymal hamartoma has a more complex structure and consists of fibrous connective tissue, cartilage, fat, bone and various types respiratory epithelium [3, 15]. From to clinical point of view, pulmonary hamartomas can mimic primary malignant lesion or an already metastatic progressive malignancy and thus be misclassified as an advanced tumour stage. Clinical interests in this unusual tumour centres to clarified whether they are true neoplastic origin or represent chronic inflammatory lesions with distinct abnormalities mimicking a neoplasm [19]. Recent study indicated that hamartoma may be associated with a chronic inflammation disease. This hypothesis is supported by histochemical analysis of the expression profile of growth-relevant was shown the upregulation of macrophage migration inhibitory factor (MIF) in hamartomas and surrounding lung parenchyma [17].

MIF was originally described as a T cell–derived lymphokine [6, 11], and this protein has been re-evaluated as a pluripotent cytokine involved in broad-spectrum functions within and beyond the immune system [7, 21]. MIF has been recognized as a pituitary hormone released in response to an array of stimuli [5, 13], a proinflammatory cytokine released mainly by macrophages [8], and a T-cell activator essential for immune responses [2]. Data show that MIF can bypass p53-mediated growth arrest, suppress p53-dependent transcriptional activation and apoptosis, as well as prolong senescence of primary mouse fibroblasts. Moreover, the function of MIF seems to correlate with its ability to suppress transcription of p53 target genes. MIF action in inflammation and loss of p53 function during the
acute inflammatory response may be beneficial to permit local cell proliferation and tissue repair. If these were to persist chronically, then the prolonged loss of p53 as a cell cycle regulator may lead to hyperplasia of tissue, genomic instability and ultimately malignant transformation [22].

Promoter polymorphism G/C at −173 position of the MIF gene is associated with increased production of MIF and has been found to confer increased risk of susceptibility to chronic inflammatory diseases [1,4,12,20]. We investigated whether there is association between promoter polymorphism of the MIF gene and incidence of pulmonary hamartomas.

Materials and Methods

Formalin-fixed, paraffin-embedded of hamartoma tumor were obtained from patients with pulmonary hamartoma treated in 2000 and 2005 in Tuberculosis and Pulmonary Diseases Specialistic Hospital in Rzeszów, Poland. The study group consisted of 52 patients with pulmonary hamartoma represented in most as solitary pulmonary nodule and distributed even equally in the pulmonary lobes (26 in the left lung and 26 in the right lung) with mean transverse diameter 2 cm (range 1.0 to 7.0 cm), 32 men and 20 women, mean age years 53 (range 24 to 74 years). Among them were 5 subjects with chronic inflammation diseases and 14 smokers (but 23 had no data). Blood samples were obtained from 123 sex and age matched healthy person served as control.

DNA from pulmonary hamartoma was isolated by protease K digestion and phenol/chloroform extraction. Genotypes of the G/C polymorphism in the MIF promoter were determined by the PCR-based AluI restriction fragment length polymorphism [20]. The following primers were used: 5’-ACT AAG AAA GAC CCG AGG C-3’ (forward primer) and 5’-GTT AAC AAC TTT TGT GTG CC-3’ (reverse primer). The PCR reaction were carried out by using MJ Research, INC thermal cycler, model PTC-100 (Waltham, MA, USA), in a total volume of 25 μl, containing 20 ng genomic DNA, 20 pmol each primer (Sigma, Germany), 200 mM each dATP, dCTP, dGTP and dTTP (Qiagen, Germany), 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM MgCl₂, and 1 unit Taq polymerase (Qiagen, Germany). The thermal cycling conditions were 5 min at 95°C, followed by 35 cycles of 30 s at 95°C, 30 s at 51°C and 30 s at 72°C. PCR-amplified DNA was digested with 1 U AluI in a 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 10% horizontal polyacrylamide gel and visualized by silver staining. Genotypes were defined as follows: 268, 98-bp for the G al-

Fig. 1. Typical results of the restriction endonuclease (AluI) digestion of PCR products performed with genomic DNA isolated from pulmonary hamartoma and analysed by 10% polyacrylamide gel electrophoresis, silver stained. Lanes 3, 7, 8 display bands for a heterozygotes G/C; lanes 1, 4, 5, 6 display bands for homozygote G/G; lane 2– homozygotes C/C. Lane M – DNA molecular marker.
The –173 G/C Polymorphism of the MIF Gene in Pulmonary Hamartoma

Results

According to the data of RFLP analysis, all the patients and controls were divided into three genotypes of the MIF promoter region: G/G, G/C and C/C (Fig. 1). Table 1 shows genotype distribution between pulmonary hamartoma samples from patients and blood samples from healthy individuals.

There were no significant differences ($\chi^2 = 1.0436; p > 0.05$) between the genotypes distribution and allele frequencies of the G/C polymorphism MIF promoter gene in patients compared with controls.

The overall genotypes frequency distribution was in agreement with Hardy-Weinberg equilibrium expectations. Additionally, there were no differences in the genotypes distribution and frequencies of alleles for groups of patients with different histological type of pulmonary hamartoma (data not shown).

There is no difference in distribution of G/C polymorphism in MIF gene for different age groups or gender.

Discussion

Macrophage migration inhibitory factor (MIF) is a pleiotrophic lymphocyte and macrophage cytokine; it is likely to play an important role in innate immunity [7, 21]. A recent investigation patients with pulmonary hamartoma indicated overexpression of MIF in hamartoma and surrounding lung tissue. In this patients group chronic lung diseases were frequently reported [17]. Thus it is possible that chronic inflammation in lung tissue may lead to development of pulmonary hamartoma.

Polymorphism with potential functional relevance has also been identified in the MIF promoter: single nucleotide polymorphism at position –173 G to C has been found to be associated with altered levels of MIF transcription in vitro. The present of C at position –173 of MIF promoter creates the binding motif of activator protein 4 [11]. Further evidence of the functional importance of the allele C includes findings of significant association with immunomediated inflammatory diseases such as alopecia areata [12], psoriasis [4], rheumatoid arthritis [1], and sarcoidosis [20]. Given the role of MIF regulation of inflammatory response and innate immune response, we hypothesised that C allele at position -173 of MIF may be involved in the genetic-environmental interaction underlying the pathophysiology of pulmonary hamartoma.

In our preliminary study conducted on other population of patients we demonstrated that the percentage of the C allele was higher in our investigated group than another previous investigated group (43% vs ≈20%) [23]. In the present work we investigated 52 patients with pulmonary hamartoma among them were 10% of lung chronic diseases and 48% smokers. There were no significant differences ($\chi^2 = 1.0436; p > 0.05$) between the genotypes distribution and allele frequencies of the G/C polymorphism MIF promoter gene in patients compared with controls. Our results suggest that the G/C polymorphism may not be associated with incidence of pulmonary hamartoma, but further studies of large group patients and control group subjects with diseases free-lung are needed to clarify this point.

<table>
<thead>
<tr>
<th>Genotype Allele</th>
<th>Pulmonary hamartoma patients (n = 52)</th>
<th>Controls (n = 123)</th>
<th>OR (95% PU)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number (Frequency)</td>
<td>Number (Frequency)</td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>34 (0.65)</td>
<td>89 (0.72)</td>
<td>0.72 (0.36; 1.44)</td>
</tr>
<tr>
<td>G/C</td>
<td>17 (0.33)</td>
<td>31 (0.25)</td>
<td>1.44 (0.71; 2.93)</td>
</tr>
<tr>
<td>C/C</td>
<td>1 (0.02)</td>
<td>3 (0.02)</td>
<td>0.78 (0.07; 7.84)</td>
</tr>
<tr>
<td>G</td>
<td>85 (0.82)</td>
<td>209 (0.85)</td>
<td>0.79 (0.43; 1.45)</td>
</tr>
<tr>
<td>C</td>
<td>19 (0.18)</td>
<td>37 (0.15)</td>
<td>1.26 (0.68; 2.40)</td>
</tr>
</tbody>
</table>

$\chi^2 = 0.466704$, $\chi^2 = 0.023549$, $p > 0.05$ as compared with Hardy-Weinberg distribution


