MUC6 Gene Polymorphism in Healthy Individuals and in Gastric Cancer Patients from Northern Portugal

Elsa Garcia, Filipa Carvalho, António Amorim, and Leonor David
Institute of Molecular Pathology and Immunology of the University of Porto, IPATIMUP, 4200 Porto, Portugal

Abstract

Mucins exhibit a high degree of genetic polymorphism because of the presence of a variable number of tandem repeats. The aims of this work were to describe the MUC6 gene polymorphism in the Portuguese population and to evaluate whether MUC6 gene polymorphism was involved in individual susceptibility to gastric cancer development, as observed previously for the MUC1 gene. We found that the 10 alleles identified in the population of blood donors (n = 376), by Southern blot analysis, were also found in gastric cancer patients (n = 157). However, significant differences in allelic frequencies between the two populations were observed for 4 of the 10 alleles, in agreement with those described previously for the MUC1 gene; the largest allele was more frequent in blood donors, and smaller alleles were more frequent in gastric cancer patients. Our results suggest that MUC6 gene polymorphism is involved in the predisposition to gastric carcinoma development.

Introduction

Mucins are high molecular weight glycoproteins with core proteins (apomucins) rich in serine and threonine residues to which O-linked carbohydrate chains are attached. A constant feature of the eight mucins identified thus far is the presence of a VNTR in the coding regions of the genes that are responsible for the highly polymorphic structure of the genes (1–9).

Previous studies of our group showed that MUC1 gene polymorphism was involved in the individual susceptibility to gastric cancer development (10). It was demonstrated that individuals with MUC1 genotypes displaying a small number of tandem repeats, and therefore with small glycoprotein products, were at increased risk for gastric cancer development. It was postulated that such individuals might have a thinner mucin layer, probably leading to a less efficient protection against environmental insults (10).

Another mucin gene, MUC6 gene, is highly expressed in the gastric mucosa (1, 11–13). MUC6 was identified by expression cloning using antibodies raised against deglycosylated mucin isolated from human gastric tissue (1, 11). The individual repeat unit of the MUC6 gene is composed by 507 bp that code for a peptide stretch with 31 and 18% of threonine and serine residues, respectively (1). The aims of this work were: to describe the MUC6 gene polymorphism in the Portuguese population, characterized by a high incidence of gastric cancer; and to evaluate whether MUC6 gene polymorphism was involved in individual susceptibility to gastric cancer development, as demonstrated previously for MUC1 gene.

Materials and Methods

The polymorphism of the MUC6 gene was studied in a control group of 376 blood donors (recruited from the blood bank during 1 month in 1993 and 1 month in 1995) and in 157 patients with gastric cancer (consecutive cases from which frozen material was available, collected from 1988 to 1995). Both groups are from a population of Northern Portugal. Gastric cancer cases were representative from the general distribution of gastric carcinoma in Northern Portugal regarding age and sex as well as localization (antrum, 86; body, 41; cardia, 30), morphology (intestinal carcinomas, 82; diffuse carcinomas, 38; atypical carcinomas, 37), and staging. DNA from blood donors and cancer patients was isolated by a method described previously (14) from pelleted blood samples (10 ml) and nonneoplastic gastric tissue, respectively. Pelleted blood samples and collected tissue samples (frozen in liquid nitrogen immediately after surgical removal) were stored at −70°C until DNA extraction.

Isolated high molecular weight DNA was digested with TaqI, which cuts the DNA outside the tandem repeat region of the MUC6 gene (1), separated on 0.7% agarose gel by electrophoresis for 17–19 h at approximately 35 V, and transferred to nylon membranes (Hybond-N; Amersham) by alkaline blotting (15).

The blots were prehybridized for 3–4 h in a phosphate solution [0.5 M NaHPO4 (pH 7.2), 1 mM EDTA, and 7% SDS] at 65°C, followed by hybridization at 65°C overnight with a MUC6 probe specific for the VNTR region of the gene. Blots were washed under high stringency conditions (first with 40 mM NaHPO4, 1 mM EDTA, 5% SDS, and next with 40 mM NaHPO4, 1 mM EDTA, and 1% SDS at 65°C). Membranes were exposed for autoradiography at −70°C. Autoradiography was developed after 1 day.

All bands from the autoradiograms were scored visually. The size of the different alleles of the MUC6 gene was determined by two independent observers by comparison with the size of the fragments of the marker λ HindIII (Amersham). All
of the cases were analyzed twice, and reference alleles were included in every blot.

MUC6 probe was constructed using primers to the tandem repeat region of the gene (primer sense, 5'-GCAGGCTAAC-CACACCTTCAA-3'; primer antisense, 5'-ATGTTGCGAT-CATAGGACCGTG-3'). Thirty cycles of amplification were undertaken at 95°C for 45 s (denaturing) and at 61°C for 1 s (annealing) as well as at 72°C for 1 min (extension). The purified PCR product of 603 bp (Prep-A-Gene DNA purification kit; Bio-Rad) was digested with EcoRI and subcloned in the vector pT7T3 (Pharmacia) digested previously with EcoRI. The ligation product was then used to transform Epicurian Coli Sure Competent Cells (Stratagene). Selection of recombinant clones was made on plates supplemented with 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (galactose) and isopropylthio-f3-D-galactosidase. The total plasmid was extracted using the Qiagen Plasmid Mini kit (Qiagen, Inc., Chatsworth, CA), labeled with [α-32P] dCTP (Amersham) using the oligo-labeling extension method (16) and purified from non-incorporated nucleotides by passage through Sephadex-G50 columns.

Allelic frequencies were estimated by gene counting, and population conformity with Hardy-Weinberg expectations was tested according to Guo and Thompson (17), using the statistical software package GENEPOP (18).

The statistical analysis of the results was made by Student’s t and χ² tests. The results were considered significantly different when P < 0.05. Comparison of gene frequencies between patients and controls was made separately for each allele using 2 × 2 contingency χ² analysis with 1 df.

Results

MUC6 Gene Polymorphism in the Portuguese Population (Blood Donors). We have studied a Portuguese population of 376 blood donors 39.7 ± 11.2 years of age with a male:female sex ratio of 3.8:1. Ten different alleles with sizes ranging from 8.5 to 12.5 Kb were identified (Fig. 1). The alleles were numbered according to their molecular weight (allele 1 is the heaviest, and allele 10 the lightest), and the most frequent were: allele 4, 11.2 Kb (37.5%); allele 7, 10.0 Kb (20.9%); and allele 2, 12.0 Kb (17.2%).

Genotype distribution was in accordance with Hardy-Weinberg equilibrium, and the observed heterozygosity was 75.8%. No differences were found in allelic pattern distributions regarding sex, age, and histo-blood group (data not shown).

Comparison between Controls and Gastric Cancer Patients. Blood donors and gastric cancer patients were significantly different regarding sex (P = 0.0001) and age (P < 0.0001) distributions. The control population was younger than the group of gastric cancer patients (61.3 ± 12.3); the male:female sex ratio was lower in the group of gastric cancer patients (1.6:1). In both populations, MUC6 allele distribution was independent of age and sex (Table 1). The ABO histo-blood group was found in all blood donors and in 111 gastric cancer patients, and the distributions were not significantly different. We considered the samples appropriate for a case-control study of the MUC6 gene polymorphism based also on the same origin of both populations.

In gastric cancer patients, the same 10 different alleles of the control group were identified, and the modal alleles were also the same in both populations: allele 4, allele 7, and allele 2 (Table 2 and Fig. 2). Significant differences in allelic frequencies between blood donors and gastric cancer patients were observed for alleles 1, 5, 8, and 10 (Table 2). Allele 1, the largest allele, was more frequent in blood donors; alleles 5, 8, and 10, of smaller sizes, were more frequent in gastric cancer patients (Table 2 and Fig. 2). Allele distribution in gastric cancer patients was identical in cases with different localizations (P = 0.229) and different morphological types (P = 0.628; data not shown).

Genotype distribution in gastric cancer patients was as expected from Hardy-Weinberg equilibrium, and the observed heterozygosity was 79.8%.

Discussion

The first aim of this work was to describe the MUC6 gene polymorphism in the Portuguese population, characterized by a high incidence of gastric cancer. Ten different alleles with sizes ranging from 8.5 to 12.5 Kb were identified in a group of 376 healthy individuals. The modal alleles had 11.2 Kb (allele 4), 10.0 Kb (allele 7), and 12.0 Kb (allele 2). Observed genotype distribution was in accordance with Hardy-Weinberg equilibrium expectations, and the observed heterozygosity was 75.8%. No associations were found between the MUC6 gene polymorphism and sex, age, and ABO histo-blood group.

Our results are in agreement with those reported previously by Toribara et al. (1) in a population in the United States. They studied the MUC6 gene polymorphism in DNA samples of nine individuals, digested with TaqI, and observed a high degree of polymorphism.

The second aim of our work was to evaluate whether MUC6 gene polymorphism was involved in individual susceptibility to gastric cancer development. The rationale of this approach was based on a previous study of our group showing that the polymorphism of MUC1 gene was involved in the
individual susceptibility to gastric cancer development (10). It was demonstrated that individuals with MUC1 genotypes displaying a small number of tandem repeats, and therefore with small glycoprotein products, were at increased risk for gastric cancer development. It was postulated that such individuals might have a thinner mucus layer, probably due to a less efficient protection against environmental insults. It was demonstrated previously that the protein product of the MUC1 gene has a molecular weight that correlates to the size of mRNA and to the size of the polymorphic region, VNTR, of the gene (2, 3, 9, 19). Regarding the MUC6 gene, it has not been demonstrated that alleles showing a small VNTR region originate comparably small gene products, i.e., there is no evidence that the polymorphism at the DNA level (VNTR) accounts for a polymorphism at both mRNA and protein levels. In fact, MUC6 like other secretory mucins typically demonstrates a polydisperser mRNA pattern on Northern blot analysis. Polydispersity of mucin mRNA is a characteristic of most mucins, with MUC6 being a notable exception to this rule. The reasons for this polydispersity are unknown but may be due to either rapid turnover of mucin mRNA or increased susceptibility of the long mucin mRNAs to degradation (20). It is also possible that the repetitive structure of mucin mRNAs renders them physically unstable or more susceptible to RNase degradation (20).

Although our patient and control samples were significantly different regarding sex and age, no differences were observed in allelic frequencies of MUC6 polymorphism regarding these parameters. Both populations were originally from Northern Portugal and were at Hardy-Weinberg equilibrium in regard to the ABO system. We, therefore, assumed that the samples were appropriate for a case-control study of the MUC6 gene polymorphism.

Significant differences in allelic frequencies of blood donors and gastric cancer patients were observed for alleles 1, 5, 8, and 10, suggesting that MUC6 gene polymorphism is involved in the susceptibility to gastric cancer development. The differences of MUC6 allele frequencies between controls and patients are in agreement with those observed previously for MUCI gene (10). Allele 1, the largest allele, was more frequent in blood donors; alleles 5, 8, and 10, which are smaller alleles, were more frequent in gastric cancer patients. Interestingly, the distribution of MUC6 alleles in patients with gastric carcinomas from different localizations and with different morphological types was identical.

Altogether, our data suggest that MUC6 gene polymorphism is involved in the predisposition to gastric carcinoma development, regardless of the histotype. For a full understanding of the role of mucins in the protection of gastric mucosa MUC5AC mucin, widely expressed in the gastric mucosa, should also be taken into consideration (21, 22). Finally, one should not disregard that the amount of protein produced and the final glycoprotein composition of secreted mucins, like MUC5AC and MUC6, may be more important than the gene structure per se.
References


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