RESEARCH COMMUNICATION

Effects of ABO and FUT2 Genetic Transcription Absence on ABH Histo-blood Group Antigen Expression in Lung Cancer Patients

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Abstract

Purpose: To investigate the effect of alterations in mRNAs of ABO and FUT2 genes on the expression of ABH histo-blood group antigens in lung cancer patients. Methods: Totals of 18 patients with blood group A, 14 with group B, 8 with group AB and 9 with group O, were assessed for blood group A/B/H antigens by immunohistochemical staining. Expression of A/B enzyme and FUT2 mRNA was detected in tumor tissues and corresponding lung tissues adjacent to tumors from lung cancer patients using RT-PCR. Results: Expression of FUT2 and A/B enzyme mRNA in lung tissue adjacent to tumors was statistically greater than that in tumor tissues (χ²=14.118, P<0.001). Expression of FUT2 mRNA was statistically lower than that of A/B enzyme in tumor tissues from lung cancer patients whose blood group was A/B/AB (χ²=7.813, P=0.005). Only tumor tissues from 9 patients with mRNA expression of A/B enzyme and FUT2 gene lacked blood group antigens. In particular, expression of A/B antigens was not detected in five cases with A/B mRNA expression, a significant association being observed between the expression of enzyme and antigens (Pearson’s R=0.867; kappa’s coefficient =0.858, P<0.001). Conclusion: Expression of A/B/H blood group antigens was not detected in lung cancer tissues, which may have resulted from down-regulation of ABO/FUT2 gene transcription. Furthermore, the FUT2 gene may indirectly regulate expression of A/B blood group antigens by influencing H antigen expression.

Keywords: Lung neoplasms - histo-blood group antigens - ABO blood-group system - China
Table 1. Sequences of the Primers Used in RT-PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Prime sequence</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A transferase</td>
<td>Sense, 5'-GCCCCAGAAGGTCTAATG CCAG-3'</td>
<td>689</td>
</tr>
<tr>
<td></td>
<td>Anti-sense, 5'-CCCCCAGAAGGTCTAATG CCAG-3'</td>
<td>689</td>
</tr>
<tr>
<td>B transferase</td>
<td>Sense, 5'-TGTGGTTGATCGGGGTCTCTA-3'</td>
<td>304</td>
</tr>
<tr>
<td></td>
<td>Anti-sense, 5'-AGCTTCAAGGAAAGCCACGT-3'</td>
<td>304</td>
</tr>
<tr>
<td>FUT2</td>
<td>Sense, 5'-CTAGCGAAGATTCAAGCCATGGT -3'</td>
<td>341</td>
</tr>
<tr>
<td></td>
<td>Anti-sense, 5'-AGCTTCAGGAAAGCCACGT-3'</td>
<td>341</td>
</tr>
<tr>
<td>β-action</td>
<td>Sense, 5'-CACTCCATGAGGATGTTGAT-3'</td>
<td>205</td>
</tr>
<tr>
<td></td>
<td>Anti-sense, 5'-CTIGAACCGAGTCGGAATGC-3'</td>
<td>205</td>
</tr>
</tbody>
</table>

32 y to 72 y, on average (57.9 y). According to The World Health Organization’s (WHO) 1997 classification system, 18 specimens were histopathologically classified as Adenocarcinoma, 26 as Squamous cell carcinoma, and 5 as Small cell lung cancer. Blood group classifications for patients broke down into: 18 type A, 14 type B, 8 type AB, and 9 with type O. Experimental protocols were approved by the review committee of First Hospital, China Medical University and meet the guidelines of the hospital.

Fresh tissues from carcinomas and non-neoplastic fragments from lung tissues surrounding tumors were snap frozen in liquid nitrogen in 30 minutes and stored at −80°C until use in RT-PCR and immunofluorescence microscopy, or fixed with 10% formaldehyde, embedded in paraffin, and stored for immunohistochemical tests.

Immunohistochemistry for antigens A, B, and H.

The carcinoma tissues obtained from patients were fixed in formalin, embedded in paraffin, and then deparaffinized for immunoperoxidase staining. Immunohistochemistry was performed as previously described using Streptavidin peroxidase conjunction method, followed by diaminobenzidine (DAB). All steps were performed at room temperature. Monoclonal antibodies anti-A, anti-B, anti-H (Biomed, USA) were appropriately diluted to the effective concentration of 1:30. Lymphocytes and connective tissue served as negative controls. Red blood cells and endothelial cells in the section always stained for the corresponding blood group antigen, and never for any other, thus serving as a built-in positive control.

The cells were viewed using an Olympus BH2 microscope and yellow-brown granules in the plasma or on the membrane were regarded as positive results. 10 fields were selected on every slide, and 100 cells were counted in every field. Positive cells totaling less than 5% was considered a negative result, while more was considered a positive result.

Location of A, B, H antigen by immunofluorescence microscopy

In brief, fresh frozen specimens embedded by OCT were cut into slices of 5µm width. Then fixation was performed with 4% paraformaldehyde plus PBS for 30 min at 4°C, followed by 10 minutes of incubation with 0.2% TritonX-100 plus PBS at room temperature. After blocking with 3xBSA(Sigma)/PBS for 2 hours at 37°C, coverslips were washed with PBS, and incubated with anti-A, B, and H monoclonal antibodies (dilution, 1:30) at room temperature overnight followed by FITC-conjugated goat anti-rabbit antibodies (dilution, 1:100; Santa Cruz Biotech) for 1 h at 37°C. For control, parallel coverslips were incubated with anti-A, B, and H (dilution, 1:30) and FITC-conjugated goat anti-rabbit antibodies as secondary antibody (dilution, 1:100; Santa Cruz Biotech) for 1 h at 37°C. Glycerin was used to cover the slides. Images were then observed using a fluorescence microscope.

Reverse-transcriptase polymerase chain reaction analyses of mRNA of A/B enzyme and FUT2 gene

Total RNA was prepared with Trizol Reagent (Invitrogen) according to the manufacturer’s instructions. The concentration and purity of RNA was determined using ultraviolet spectrophotometry and RNA was stored at −70°C until analyzed.

For reverse transcription of extracted RNA into complementary DNA (cDNA), a TaKaRa RNA PCR Kit(AMV)Ver.3.0 was applied, and the procedure was carried out following the manufacturer’s instructions. Subsequently, PCR reactions were performed in a thermal cycler (Gene Amp PCR System, Bioetra, Germany): 5 μl RT products were amplified into a volume of 25 μl containing 5xPCR buffer 5μl, 0.5 μL each primer, and 0.2μl Taq DNA polymerase and distilled water. The primers (TaKaRa) used are described in table 1. For enzyme A the thermal cycle profile consisted of denaturing at 94°C for 5 minutes, followed by 94°C for 1 min, 56°C for 30s, and 72°C for 90s for 35 cycles. Enzyme B was denatured at 94°C for 2 minute, followed by 94°C for 1 min, 57°C for 30s, and 72°C for 1min for 30 cycles, then extension at 72°C for 7 min. FUT2 gene was denatured at 94°C for 2 minute, followed by 94°C for 1 minute, 58°C for 30s, and 72°C for 1min for 30 cycles, with extension at 72°C for 7 min. The integrity of messenger RNA in all samples was confirmed by amplification of β-actin.

PCR products were separated on 2% agarose gels and photographed.

Results

Immunohistochemical and immunofluorescence investigation of A, B, H antigens

Immunohistochemical investigation revealed that A, B, and H blood group antigens were present in lung carcinomas, A and B were located at the cell membrane almost like coarse particles, and H antigens were located in both the membrane and cytoplasm, in a so-called antigen accumulation(Nakagoe et al., 2000; Le Pendu et al., 2001; Guzman-Bistoni et al., 2008); normal lung tissues were also stained for the antigens, which were expressed as a
ABO and FUT2 Genetic Transcription Effects on ABH Histo-blood Group Antigens in Lung Cancer Patients

Table 2. The Expression ABH Histo-blood Group Antigens in Lung Cancer

<table>
<thead>
<tr>
<th>Blood group</th>
<th>A antigen</th>
<th>B antigen</th>
<th>H antigen</th>
<th>Rate* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A=18</td>
<td>8</td>
<td>0</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>B=14</td>
<td>0</td>
<td>7</td>
<td>11</td>
<td>47.50</td>
</tr>
<tr>
<td>AB=8</td>
<td>4</td>
<td>5</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>O=9</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>83.67</td>
</tr>
</tbody>
</table>

*The positive rate of antigen A and B was 45.70%, the positive rate of antigen H was 83.67%.

Table 3. Correlation mRNA Expression of A, B Transferase Gene AND FUT2 to Antigen ABH in Lung Cancer (Brackets for the blood group antigen expression Unit: Cases)

<table>
<thead>
<tr>
<th>Blood group</th>
<th>A transferase gene</th>
<th>B transferase gene</th>
<th>FUT2 gene</th>
<th>Rate* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A=18</td>
<td>(11) (8)</td>
<td>0</td>
<td>17</td>
<td>61.11</td>
</tr>
<tr>
<td>B=14</td>
<td>0</td>
<td>(10) (7)</td>
<td>11</td>
<td>71.43</td>
</tr>
<tr>
<td>AB=8</td>
<td>(4) (4)</td>
<td>7 (5)</td>
<td>7</td>
<td>50.00 (87.50)</td>
</tr>
<tr>
<td>O=9</td>
<td>0</td>
<td>0</td>
<td>7 (6)</td>
<td>77.78</td>
</tr>
</tbody>
</table>

*No significant difference between blood groups was demonstrated for the corresponding enzyme mRNA expression (χ2=2.219, P=0.546); **mRNA expression of A/B enzyme in cancer tissues with A, B and AB blood groups was significantly lower than FUT2 mRNA expression of corresponding prosoma H antigen (χ2=7.813, P=0.005); †Three cases with the mRNA expression of A/B enzyme simultaneously.

Figure 1. Expression of Histo-blood Group Antigens in Alveolar Epithelium and Lung Cancer. A. The expression of histo-blood group A antigen in alveolar epithelium (400x), the arrow shows the stained alveolar epithelium. B. The expression of histo-blood group A antigen in lung adenocarcinoma (400x). C. The expression of histo-blood group B antigen in lung adenocarcinoma (400x). D. Accumulative expression of histo-blood group H antigens in lung squamous cell carcinoma (400x), cell membrane and cytoplasm stained simultaneously. E. Expression of histo-blood group H antigen in lung squamous cell carcinoma (400x), arrow shows stained cell membrane. F. Negative expression of H antigen in small cell undifferentiated carcinoma.

Figure 2. Expression of Transferase A in Lung Cancer Tissues and Corresponding Lung Tissues Adjacent to the Cancer. A. The expression of histo-blood group A antigen in bronchialalveolar carcinoma of the lung (Immunofluorescence IF, 400x, the arrow shows the stained cell membrane). B. The expression of histo-blood group B antigen in cytoplasm of lung adenocarcinoma cell (nucleus was red stained, PI staining, 400x). C. The accumulative expression of histo-blood group H antigen in small cell lung cancer (cell membrane and cytoplasm were stained simultaneously, 200x). D. The expression of H antigen in lung squamous cell cancer, cell membrane was stained and there was sporadic expression in cytoplasm (200x).

The mRNA expression of A/B enzyme and FUT2 gene

In primary lung cancer tissues whose blood groups were A, B, or AB, expression of A/B and FUT2 mRNA was 60% (24/40) and 90% (35/40) respectively, and the difference was statistically significant (χ2=7.813, P=0.005). In tumor-adjacent lung tissues, expression of A/B/FUT2 mRNA was statistically higher than in tumor tissues (χ2=14.118, P<0.001). There was no significant difference in expression of A/B/FUT2 mRNA between all lung cancer tissues (χ2=2.219, P=0.546) (see Table 3 and Figures 2, 3, 4).

The correlation between mRNA expression of A/B/FUT2 and expression of blood group antigen ABH

There was no significant correlation between...
Figure 3. Expression of Transferase A Gene in Lung Cancer Tissues and Lung Tissues Adjacent to the Cancer (1C and 3C expression absent in lung cancer tissues; 1-3L expression in corresponding lung tissues: 687bp)

Figure 4. Expression of Transferase B Gene in Lung Cancer Tissues and Lung Tissues Adjacent to the Cancer (2C absent expression in lung cancer tissues; 1-3L expression in corresponding lung tissues: 304bp)

Figure 5. Expression of FUT2 Gene in Lung Cancer Tissues and Lung Tissues Adjacent to the Cancer (2C absent expression in lung cancer tissues; 1-3L expression in corresponding lung tissues: 341bp)

Discussion

The gene of ABO histo-blood antigen system located at 9q34.1—9q34.2: the gene of A/B enzyme encodes A enzyme (transferase A, alpha 1-3-N-acetylgalactosaminyltransferase) and B enzyme (transferase B, alpha 1-3-galactosyltransferase) separately. Some base transposition of ABO gene lead to transcription of two different amino acids located at 266 and 268, which can produce special alpha-N-acetylgalactosaminytransferase (transferase A) and alpha D-galactosyltransferase (transferase B). The gene encoding O is kept silent, it cannot be translated into glycosyltransferase or encode an inactive enzyme protein(Roubinet et al., 2004). The formation of antigen H can be regulated by α1,2-fucosyltransferase, which is encoded by FUT1(fucosyltransferase 1) and FUT2(fucosyltransferase 2) genes located at 19q13.3 together with other FUTs. FUT1 gene (H gene) encodingα1,2-fucosyltransferase (H enzyme) controls the formation of antigen H disposition on the surface of erythrocytes and vascular endothelial cells. FUT2 gene encoding Se enzyme is responsible for the formation of antigen H disposition on mucosas epithelium and Glandular epithelium(Oriol et al., 2000). In conclusion, antigen H of primary lung cancer may be regulated by the Se enzyme encoded by the FUT2 gene.

A/B antigen was synthesized by adding an oligosaccharide chain to H antigen to form new carbohydrate molecules catalyzed by A/B glycosyltransferase(Smolarek et al., 2008). Recently, some research demonstrated that in carcinoma of mouth, breast cancer, cancer of the colon and endometrial cancer, A/B antigens associated with tumors were absent and the precursor accumulated (Nakagoe et al., 2000; Le Pendu et al., 2001; Guzman-Bistoni et al., 2008). Our study found that the percentage of A/B blood group antigen absent was 52.50%, and precursor antigen H accumulated. Specimens with stained cytoplasm accounted for 63.41%, suggesting that H antigen accumulation combined with absence of A/B antigen was common in lung cancer tissues, a finding which was supported by results of the Immunofluorescence technique and our previous study(Li J, 2004). The molecular mechanism is still unknown, Mandel et al presumed that the activity loss of glycosyltransferase encoded by A/B gene leads to A/B antigen absence(Mandel et al., 1992), Oontoft found mRNA repression of ABO gene was associated with loss of ABO glycosyltransferase activity in bladder carcinoma cell lines(Orlow et al., 1998). The mechanism of A/B antigen regulation in cancer may be complex. Recent research suggests that abnormal activity of Se enzyme in colon carcinoma may explain the phenomenon of antigen loss and precursor antigen H accumulation, but the interaction between enzymes A, B and Se remains incompletely defined. This difference will determine the personalized diagnosis and treatment of lung cancer in the future.(Xu et al., 2010)

In this study, we found loss of A/B enzyme mRNA in 16 of 40 A/B cancer tissues, and A/B antigen absence in corresponding lung cancer tissues, suggesting that the
loss of ABO mRNA led to protein division and finally, loss of A/B antigen. Notably, A/B/H antigen was lost in 9 cases where A/B/FUT2 mRNA was expressed. In 5 cases, A/B mRNA was expressed while precursor H antigen and FUT2 mRNA (regulator for H antigen) was absent, (in 3 cases with A blood group antigen and 2 cases with B blood group antigen).

The results indicated that not only transcription of glycosyltransferase gene but also precursor FUT2 gene can affect the expression of A/B antigen. Comparing the mRNA expression of A/B/FUT2 with A/B/H antigen expression, we found that there was no difference between them and that significant association and consistency existed between the expression of enzyme and antigen. This statistical data indicated that the transcription of A/B/FUT2 enzyme mRNA played an important part in expression of A/B/H antigen and that the synthesis of precursor antigen H can also affect the expression of A/B antigen.

In another 4 cases, A/B enzyme mRNA was expressed, while the corresponding antigen was absent without precursor loss. Mas et al found similar phenomenon while studying FUT1-7 gene transcription enzyme activity and Lewis series antigen expression. Mas postulated this phenomenon may result from low activity of fucosyl transforase regulated by FUT enzyme genes. We considered that different activity of A/B/Se enzyme or contraindication and competition in carbohydrate antigen synthesis was the reason. Yoshihiko presumed that the transcription and regulation mechanism of ABH antigen-related glycosyltransferase gene were still not completely understood, and that the synthesis of ABH antigen in malignant tissues was a multistep process involving multiple genes. Intensive research about the expression and regulation mechanisms of the ABO-related fucosyl transforase gene explored molecular pathways for oligosaccharide glycosylation(Kominato et al., 2005). In this study, we detected ABH histo-blood type status, A/B glycosylase gene, precursor H antigen and the regulator FUT2 gene to analyze related factors affecting expression of antigens. Based on above results we concluded that down regulation of ABO gene was one of the main mechanisms for down regulation of A/B antigen; the desynthesis of H antigen can also affect the expression of A/B antigen; and FUT2 gene can affect the expression of A/B antigen indirectly by regulating synthesis of H antigen.

Regarding the mechanism of down regulation of the ABO gene, research about oral cancer (Gao et al., 2004) and bladder carcinoma (Chihara et al., 2005) demonstrated heterozygous loss of allele in the ABO gene was responsible for the down regulation of ABO gene; Furthermore, Bianco-Miotto found promoter methylation of ABO gene played an important part in loss of ABO gene allele in leukemia patients (Bianco-Miotto et al., 2009). In lung cancer, the mechanism for down regulation of ABO gene, the role of glycosylase, the interaction between them, and how they both affect the synthesis of histo-blood group antigens, are still unexplained, and require further investigation.

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References


