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 ($\chi 2=14.118$, $P<0.001$). 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 ($\chi 2=7.813$, $P=0.005$). 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

Asian Pacific J Cancer Prev, **12**, 3201-3206

Introduction

Lung cancer is one of the major health issues in China and the burden is getting serious. Aging is the main cause for the increasing incidence and mortality of lung cancer (Chen et al., 2010; Chen et al., 2011). There are more than one-hundred recognized blood group systems (BGA), containing more than 500 antigens. The ABO (including A, B, H antigens) and Lewis (Lea, Leb, Lex, Ley) systems are the most common means used to classify blood. Blood group isoantigens of the ABH system are represented by a variety of glycoproteins and glycolipids, the antigenic specificity of which is determined by variation in their constituent carbohydrate chains. These antigens are not confined to red blood cells but are present on other cell types, such as endothelial cells, many normal/cancerous epithelial cells and in secretions(histo-blood group antigen) (Kay, 1957).

Aberrant expression of the ABH and Lewis antigens has been found in all types of carcinomas and is often associated with prognosis (Hakomori, 1996; Le Pendu et al., 2001), for conditions such as colon cancer (Nakagoe et al., 2000; Tsuboi et al., 2007), breast cancer (Nakagoe et al., 2002), oral cancer (Dabelsteen and Gao, 2005), and lung cancer (Kuemmel et al., 2007). However, the exact mechanism of the aberrant expression is not clear yet. The current investigation was performed principally to determine whether alterations to expression of ABO and FUT2 mRNA influence the ABH- Histo-blood group antigens.

Materials and Methods

Specimens

Primary lung carcinomas were surgically resected from 49 patients at the Department of Thoracic Surgery, First Affiliated Hospital of China Medical University, between April 2004 and October 2004, (31 male, 18 female, ages

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Table 1. Sequences of the Primers Used in RT-PCR

Gene	Prime sequence	Product size (bp)
A transferase	Sense, 5'-GCCCCAGAAGTCTAATG CCAG-3'	689
	Anti-sense'5-CCCCCCCAGGTAGTAGAAATCGCCCTCGTCCTT-3'	
B transferase	Sense, 5'-TGTTTGGTTACGGGGTCCTA-3'	304
	Anti-sense,5'- AGCTTCAGGAAAGCCACGTA-3'	
FUT2	Sense, 5'-CTAGCGAAGATTCAAGCCATGTGG -3'	341
	Anti-sense,5'-GACGTACTCCCCCGGGATGTG -3'	
β-action	Sense, 5'-CACTCCATGCACCGATTGGAT -3'	205
	Anti-sense,5'-CTTGAACCGACTGGAAATGCCGAA -3'	

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 immunochemistry tests.

Immunohistochemistry for antigens A, B, and H.

The carcinoma tissues obtained from patients were fixed in formalin, embedded in paraffin, and then deparaffined 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 (Biomeda, 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 transcri ption 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 5×PCR 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

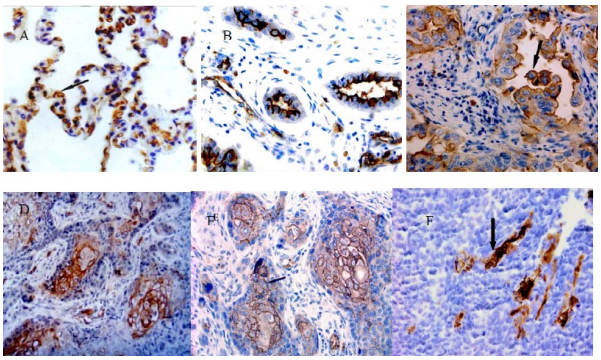

Figure 1. Expression of Histo-blood Group Antigens in Alveolar Epithelium and Lung Cancer. A. The expression of histo-blood group A antigen in alveolar epithelium (200x, the arrow shows the stained alveolar epithelium) B. The expression of histo-blood group A antigen in lung adenocarcinoma(400x) C. The 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

continuous line of even and light color (Figure 1). For lung carcinoma, blood group phenotypes A, B, and AB matched with tissue immunoreactivity for AB in 47.5% of cases (19/40, of which 4 cases had coexpression of AB antigen), there was positive incidence for H antigen in 83.67%(41/49), and in 63.41%(26/41) with cytoplasm stained. The overall match of blood group phenotype A, B, and AB with tissue immunoreactivity for AB blood group plus phenotype O with tissue immunoreactivity for H was 53.06%(26/49), and the miss rate was 46.94%(23/49). Compared to H antigen, the miss rate of A, B antigen was higher ($\chi2=13.12$,$P<0.001$, table2). The match of corresponding antigen was 100% for non-neoplastic fragments from surrounding lung tissues, and blood group antigen for fragments presented in higher amounts than in lung carcinomas ($\chi2=30.01$, $P<0.001$).

Immunofluorescence technique was employed to indentify the quality and location of antigens. The results were consistent with those of immunochemistry. For some poorly differentiated squamous cell carcinoma, either cytoplasm or the membrane combined with stained cytoplasm (B, C, D), reflecting antigen accumulation, but for higher differentiated bronchioloalveolar carcinoma membranes were the only partially stained (A).

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

Blood group	A antigen	B antigen	H antigen	Rate* (%)
A=18	8	0	17	
B=14	0	7	11	47.50
AB=8	4	5	7	
O=9	0	0	6	83.67

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

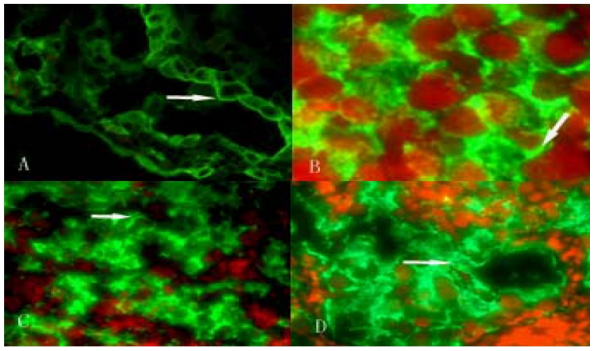

Figure 2. Expression of Transferase A in Lung Cancer Tissues and Corresponding Lung Tissues Adjacent to the Cancer A The expression of histo-blood group A antigen in bronchioloalveolar carcinoma of the lung (Immunofluorescence IF,400x,the arrow shows the stained cell membrane). B The expression of histo-blood group B antigen in kytoplasm 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 ($\chi2=7.813$, $P=0.005$). In tumor-adjacent lung tissues, expression of A/B/FUT2 mRNA was statistically higher than in tumor tissues ($\chi2=14.118$, $P<0.001$). There was no significant difference in expression of A/B/FUT2 mRNA between all lung cancer tissues ($\chi2=2.219$, $P=0.546$) (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

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)

Blood group	A transferase gene	B transferase gene	FUT2 gene[b]	Rate[a] (%)
A=18	11(8)	0	17	61.11
B=14	0	10(7)	11	71.43
AB=8[c]	4(4)	7(5)	7	50.00/87.50
O=9	0	0	7(6)	77.78

[a]No significant difference between blood groups was demonstrated for the corresponding enzyme mRNA expression ($\chi2=2.219$, $P=0.546$); [b]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 ($\chi2=7.813$, $P=0.005$); [c]Three cases with the mRNA expression of A/B enzyme simultaneously

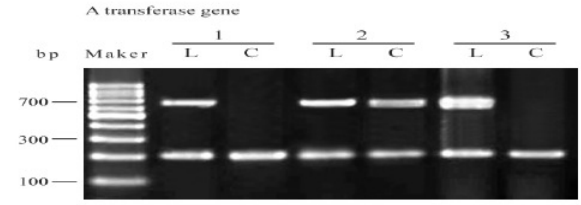

Figure 3. Expression of Transferase A Gene in Lung Cancer Tissues and Lung Tissues Adjacent to the Cancer (1C and 3C expression absent in lung cancer tissues; 1-3L expression in corresponding lung tissues: 687bp)

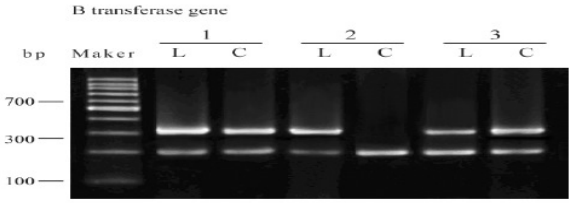

Figure 4. Expression of Transferase B Gene in Lung Cancer Tissues and Lung Tissues Adjacent to the Cancer (2C absent expression in lung cancer tissues; 1-3Lexpression in corresponding lung tissues: 304bp)

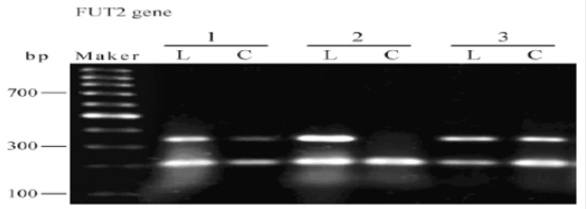

Figure 5. Expression of FUT2 Gene in Lung Cancer Tissues and Lung Tissues Adjacent to the Cancer (2C absent expression lung cancer tissues; 1-3L expression in corresponding lung tissues: 341bp)

expression of A/B/FUT2 mRNA and A/B/H blood group antigen in tumor tissues ($\chi 2=3.517$, P=0.061). Expression of A/B/FUT2 mRNA was consistent with the expression of A/B/H blood group antigens in 40 cases. In only 9 cases was mRNA of A/B enzyme and FUT2 expressed while blood group antigen was absent, of which, A/B antigen was absent in 5 cases (3 for A, 2 for B). This may have resulted from the absence of precursor H antigen and corresponding glycosyltransferase FUT2; in the other 4 cases, mRNA of blood group antigen enzyme and precursor antigen was expressed, but corresponding blood group antigens were absent. There was significant association and consistency between the expression of enzyme and antigens (Pearson's R=0.867; kappa's coefficient =0.858, P<0.001) (see Figure 5 and Table 4).

Table 4. Correlation mRNA Expression of A, B Transferase Gene AND FUT2 to Antigen ABH in Lung Cancer (McNemar test and spearman correlation analysis)

Enzyme mRNA	ABH antigen positive	negative	r	P
positive	30	4	0.867	0.125
negative	0	23		

McNemar test P=0.125, Pearson's R=0.867; Consistency test Kappa coefficient =0.858

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-acetylgalactosaminyltransferase (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 mucosae 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 de-synthesis 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.

Acknowledgements

The Project Supported By young scientists in Jiangxi Province (Jinggangmycin Star) of cultivation program funded, the Natural Science Foundation of Jiangxi (2010GZY0132), the Research Fund of Shandong Provincial Health Bureau(2007QW036), the Doctor Fund Project of Shandong Province (BS2009YY012 and NSFC30700196). The funding source had no involvement in design; collection, analysis, or interpretation of data; in the writing of the report; or in the decision to submit this article for publication. This manuscript has been kindly edited by Paul Stanton (University of Michigan). The author(s) declare that they have no competing interests.

References

Bianco-Miotto T, Hussey DJ, Day TK, et al (2009). DNA methylation of the ABO promoter underlies loss of ABO allelic expression in a significant proportion of leukemic patients. *PLoS One*, **4**, e4788.

Chen WQ, Zhang SW, Zou XN (2010). Evaluation on the incidence, mortality and tendency of lung cancer in China. *Thoracic Cancer*, **1**, 35-40.

Chen WQ, Zheng R S, Zeng HM (2011). Bayesian age-period-cohort prediction of lung cancer incidence in China. *Thoracic Cancer*, **2**, 149-55.

Chihara Y, Sugano K, Kobayashi A, et al (2005). Loss of blood group A antigen expression in bladder cancer caused by allelic loss and/or methylation of the ABO gene. *Lab Invest*, **85**, 895-907.

Dabelsteen E, Gao S (2005). ABO blood-group antigens in oral cancer. *J Dent Res*, **84**, 21-8.

Gao S, Worm J, Guldberg P, et al (2004). Genetic and epigenetic alterations of the blood group ABO gene in oral squamous cell carcinoma. *Int J Cancer*, **109**, 230-7.

Guzman-Bistoni C, Pinillos B, Blanco-Arias MC, et al (2008). Expression of blood group-related antigens in neoplastic uterine cervix. *Clin Transl Oncol*, **10**, 227-30.

Hakomori S (1996). Tumor malignancy defined by aberrant glycosylation and sphingo(glyco)lipid metabolism. *Cancer Res*, **56**, 5309-18.

Kay HE (1957). A and B antigens of normal and malignant cells. *Br J Cancer*, **11**, 409-14.

KominatoY, Hata Y, Matsui K, Takizawa H (2005). Regulation of ABO gene expression. *Leg Med (Tokyo)*, **7**, 263-5.

Kuemmel A, Single K, Bittinger F, et al (2007). The prognostic impact of blood group-related antigen Lewis Y and the ABH blood groups in resected non-small cell lung cancer. *Tumour Biol*, **28**, 340-9.

Le Pendu J, Marionneau S, Cailleau-Thomas A, et al (2001). ABH and Lewis histo-blood group antigens in cancer. *APMIS*, **109**, 9-31.

Li J, Li Y, Liu HX, et al (2004). Correlation between expression of ABH and Lewis A histo-blood group antigens and biological behavior of primary pulmonary adenocarcinoma. *Chin J Cancer Prev Treat*, **11**, 1046-50.

Mandel U, Langkilde NC, Orntoft TF, et al (1992). Expression of histo-blood-group-A/B-gene-defined glycosyltransferases in normal and malignant epithelia: correlation with A/B-carbohydrate expression. *Int J Cancer*, **52**, 7-12.

Nakagoe T, Fukushima K, Itoyanagi N, et al (2002). Expression of ABH/Lewis-related antigens as prognostic factors in patients with breast cancer. *J Cancer Res Clin Oncol*, **128**, 257-64.

Nakagoe T, Nanashima A, Sawai T, et al (2000). Expression of blood group antigens A, B and H in carcinoma tissue correlates with a poor prognosis for colorectal cancer patients. *J Cancer Res Clin Oncol*, **126**, 375-82.

Oriol, R., Candelier, J. J., and Mollicone, R (2000). Molecular genetics of H. *Vox Sang*, **78**, 105-8.

Orlow I, Lacombe L, Pellicer I, et al (1998). Genotypic and phenotypic characterization of the histoblood group ABO(H) in primary bladder tumors. *Int J Cancer*, **75**, 819-24.

Roubinet F, Despiau S, Calafell F, et al (2004). Evolution of the O alleles of the human ABO blood group gene. *Transfusion*, **44**, 707-15.

Smolarek D, Krop-Watorek A, Wasniowska K, et al (2008). [Molecular background of the ABO blood group system]. *Postepy Hig Med Dosw*, **62**, 4-17.

Tsuboi K, Asao T, Ide M, et al (2007). Alpha1,2fucosylation is a superior predictor of postoperative prognosis for colorectal cancer compared with blood group A, B, or sialyl Lewis X antigen generated within colorectal tumor tissues. *Ann Surg Oncol*, **14**, 1880-9.

Xu Y, Liu HY, Chen J, Zhou QH (2010). Comparisons between the National Comprehensive Cancer Network (NCCN) non-small-cell lung cancer (NSCLC) Clinical Practice Guidelines (Chinese version), the NCCN original edition, and the European Society for Medical Oncology NSCLC Guidelines in 2009. *Thoracic Cancer*, **1**, 83-6.