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Author(s): Tomita, Masao; Nakagoe, Toru; Kawahara, Katsunobu; Ayabe, Hiroyoshi; Tagawa, Yutaka

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Immunohistochemical Study on Blood Group Related Carbohydrate Antigens in Primary Breast Carcinoma and Its Metastasis

Masao Tomita, Toru Nakagoe, Katsunobu Kawahara, Hiroyoshi Ayabe, and Yutaka Tagawa

The First Department of Surgery, Nagasaki University School of Medicine

The immunohistochemical study was performed in comparison with the expression of blood group related A/B/H/Lea /Lex antigens between primary and metastatic cancer cells.

The disappearance of blood group-related antigen expression has become much more apparent in metastatic cancer cells rather than in primary one.

It is strongly suggested that cancer cells which tend to easily metastasize should be showing an aberration of oncogenes in which carbohydrate converting enzymes are coded.

Introduction

The development of monoclonal antibodies (MoAbs) in which antigenic determinant is clearly defined shed light on the research of cancer antigenicity. It has been clarified that most of MoAbs recognize the terminal carbohydrate chains of glycolipids or glycoproteins in cancer cell membrane. With advances in a research of sialylated carbohydrate antigens, the antigenic determinant of MoAb NS19-9, reported by Koprowski et al.\(^1\) has been identified as a sialyl derivative of human Lewis\(^a\) (Le\(^a\)) blood group antigen (Ag)\(^b\).

In contrast, although the significance of cancer antigen in the terminal chain of sialylated glycoprotein or glycolipid is clearly elucidated, the functional roles of molecules on the surface of cell membrane are not yet certain\(^9\).

There are a few reports regarding a relationship between the expression of sialylated carbohydrate antigens and metastasis or its expression in the metastatic lesions.

The purpose of this study is to clarify the correlation in the expression of A/B/H, Lewis\(^a\) and Lewis\(^b\) between primary lesions and metastatic lymphnodes in the metabolic course of carbohydrate antigens.

Materials and Methods

During the time from May 1985 to December 1985, 74 patients underwent surgical resection of breast glands at the First Department of Surgery, Nagasaki University School of Medicine and affiliated hospital. Tissues were taken from those patients who included primary carcinoma in 41 mastitis in 17 and benign tumor in 16 (fibroadenoma in five and dysplasia in 11) and 32 metastatic lymphnodes in 11 patients.

All tissue samples were fixed in formalin, embedded in paraffin and cut into serial section for immunoperoxidase staining.

Antibodies

Antigens evaluated in this study were as follows, blood group substance A B H2, Lewis\(^a\) (Le\(^a\)) Lewis\(^b\) (Le\(^b\)) blood group-related antigen. MoAbs which recognize above antigens were Anti A, Anti B, anti H type 2 (DAKO Co. Ltd. Coppenhagen, Denmark). CLEA 1 and CLEX 1 (UCLA Paul I Terasaki)

Peroxidase-conjugated goat F(ab')\(_2\) anti-mouse IgG and IgM were purchased from Capple (West chester PA USA).

Immunoperoxidase staining

Indirect immunoperoxidase staining was performed as follows: paraffin-embedded sections were heated to 60 °C for 30 min, treated for five min with 3% hydrogen peroxide in 0.05 MTBS (to block endogenous peroxidase), then rinsed three times with TBS. The sections were incubated for one hour at room temperature with the appropriately diluted. MoAbs listed in Table 1. After three washing in TBS, peroxidase conjugated goat f (ab')\(_2\) antimonous IGG diluted 1:100 in TBS, containing 3% normal goat serum was added to the previously labeled sections and incubated for 45 min at room temperature. After being rinsed with TBS, slices were blooded for 5 min with a freshly mixture of 0.05% 3, 3'- diaminobenzidine and 0.03% hydrogen peroxide. Sides were washed again with TBS and counter stained with hematoxylin. Sections were evaluated using a light microscope with results expressed as a score based on the percentage of total field staining positively with the various MoAbs. Scores were based on the following scales: for 30 to 50% + for 5 to 30% and - for less than 5%.
**ABO blood typing**

The determination of ABO blood typing was made by means of erythrocyte agglutinine test using anti A and anti B serum (Ortho Co)

**Statistical analysis**

Results were analyzed by the $X^2$ test and Fischer exact probability test was used for determination of statistical significance between both groups. The classification regarding breast cancer was followed by the rule set forth by Japanese Research Society for Breast Cancer.

**Results**

The expression of normal ductal epithelium of breast glands and benign diseases (Table 1).

| Table 1. Le* and Le* antigen expression in normal ductal epithelium and benign diseases |
|-----------------|-----------------|-----------------|
|                  | Le*             | Le*             |
| normal duct      | 15 (88.2%)      | 16 (94.1%)      |
| benign disease   |                 |                 |
| fibroadenoma     | 5 (100%)        | 5 (100%)        |
| dysplasia        | 8 (72.7%)       | 11 (100%)       |

In normal ductal epithelium of the breast glands, the expression of Blood A/B antigens in normal ductal expression was quite the same as that in blood A/B types and H type 2 antigen expression which was a precursor of blood A/B antigens accounted for 41.7 to 66.7%. The positive rates of immunohistochemical expression of blood A/B antigens in benign breast tumor tissues were almost equivalent to those in 1 normal ductal epithelium of the breast.

On the other hand, the positive Le* /Le* expression rates of normal ductal epithelium of the breast were shown as being 88.2% (15/17) and 94.1% (16/17), respectively. And also both of Le* and Le* expressions were a high positive rate.

In reference to breast cancer the expression rates blood A antigen were 33.3% in blood A type patients those for B antigen 11.1% in blood B type patients. There was no positive response to A or B antigen in blood AB type patients with breast cancer. In contrast, the expression of H type 2 antigen was 12.5% in blood 0 type patient.

On the other hand, the expressions of Le* and Le* were seen in 30.0% and 47.5% as shown in Table 2. A compatible expressions of A/B/H type 2 antigens with blood types in breast cancer were 22.2% and 33.3%. On the contrary, negative rates for A/B/H type 2 antigens were 32.3% and 51.6% with a high expression rate. Pathohistologic types between original breast and metastatic node (Table 3).

It was characteristic of an increase in solid tubular and scirrhous types and a decrease in papillotubular and mucinous types in metastatic nodes as compared with original cancer. There was no significant differences in the expression of A/B/H type 2 antigens of patients with or without node metastasis among blood A, B and AB types. In comparison the expression of A/B/H type 2 antigens between primary and metastatic node cancer cells 54.5% were identical each other.

The other 45.5% showed a diminution of the expression of own blood type antigen in metastatic node. In case of exhibiting the expression of H type 2 antigen in primary cancer, it was diminished in metastatic node.

In patients with blood A type, who showed the expression of incompatible blood B type antigen in primary cancer, an incompatible blood antigen failed to detect in metastatic node.

On the other hand, the identical expressions of Le* and Le* of primary cancers to metastatic nodes indicated in 53.1% (17/32) and 43.8% (14/32) respectively. The negative expression of Le* and Le* in metastatic nodes was shown in 43.8% (14/32) and 15.6% (5/32).

There was a lower tendency in type I sialylated carbohydrate antigen expression in metastatic nodes rather than type II.

**Discussion**

The blood ABO types are in association with the three different antigens of A/B/H type and the blood Lewis types are in relation to the two antigens of Le* and Le*, which is constituting 2 molecules of glycoprotein and glycolipid, containing in erythrocytes, saliva and gastric juice.

The blood groups are based on antigen determinant which is fated to be the terminal carbohydrate chains of glycoprotein or glycolipid. A lacto-series type I chain consists of Gal B1-3GlcNAc unit. In addition, a lacto-series type II chain comprises of Gal B1-4GlcNAc. As a result,

| Table 2. A/B/H type 2 antigen expression in breast cancer patients of A, B, and Ab blood groups with or without node metastasis |
|-----------------|-----------------|-----------------|
|                  | without node metastasis | with node metastasis |
| compatible expression | 5 (23.8%) | 3 (25.0%) |
| deletion of A/B with H accumulation | 4 (19.0%) | 2 (16.7%) |
| deletion of A/B/H | 12 (57.1%) | 6 (50.0%) |

<table>
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<th>Table 3. Blood group-related antigen express</th>
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<td>primary carcinoma</td>
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<tr>
<td>Histology Le<em>Le</em> ABH</td>
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A/B/H type 2 antigens include two kinds of carbohydrate chains, type 1 type 2 chain.

As the blood-related antigens, the main alloantigens of a man, exist in erythrocytes and various kinds of epithelium, so it is of great value to know changes in the levels of these antigens in order to diagnose a presence of carcinoma and the results inform physicians of cancer extension and recurrence on oncology since carcinoma arises from the sites were these antigens are present.

In carcinomas, it is characteristic of the disappearance of A and B blood group antigen and accumulation of H antigen which is a precursor of A and B antigens.

Lee reported that immunohistochemical expressions of A/B/H antigens by using of MoAb were revealed in erythrocytes, vascular endothelium and normal ductal epithelium of the breast and a disappearance of A/B/H antigen in patients with A, B and AB blood type accounted for 64%, 77% and 73%, respectively in the analysis of a result of immunohistochemical study on 233 patients with breast cancer.

In this study, there was seen a 100% expression of A/B/H antigen in normal ductal epithelium and benign diseases of the breast. In contrast, negative rates of own blood group antigen was 61.9% in A type breast cancer patients, 88.9% in B type and 100% in AB type with a high percentage and also the accumulation of H type 2 antigen, precursor of A, B antigens, was seen in 9.5% of A type, 22.2% of B type and 66.7% of AB type.

It is accepted that causes of disappearance of blood group antigens are associated with elimination of glucose-converting enzym activity. The interaction of H enzym, converting enzym to fucose, A enzym, converting enzym to N-acetylgalactosamine and B enzym, converting enzym to galactose, is essential on the course of synthetic pathway of carbohydrate antigens related to blood groups. The structural genes, which produces enyzms are in accordance with H/A/B genes.

It is generally taken into consideration that a phenomenon such as disappearance of blood group antigens and accumulation of precursor material is based on changes in oncogen on the course of cancer cell proliferation. There has been a controversy as to whether changes in blood group antigens in cancer cells are closely associated with the intensity of malignancy and metastasis. Detailed study has been made on transitional carcinoma of the urogenital organs. Coon reported that the expression of A/B/H blood group-related antigens has been kept positive as far as non-infiltrative carcinoma may be present. It is very rare in occurrence that non-infiltrative carcinoma involves the wall of the urinary bladder. In case of negative expression of ABH blood group-related antigens, the risk of involving the urinary bladder is considered to be high.

In contrast, it is reported that the immunohistochemical expression of ABH blood-related antigen does not correlate with cell differentiation, disease stages and disease free interval to recurrence as reported by Lee in 233 patients with breast cancer as well as by Enblad in 68 patients with rectal cancer.

It is assumed that there is no close correlation between the expression of blood-related antigens and tumor malignancies. On the other hand, the report about metastatic tumor is scant in conjunction with the expression of blood group-related antigens. Schoentag reported that the expression of ABH blood group antigens in primary cancer cells were almost similar to those in metastatic one, although there were the reports on disappearance of blood group antigen expression in liver metastasis and appearance of H antigen in metastatic nodes which has not been revealed in primary cancer cells in one, respectively.

Enblad also reported the fact that the expression of A antigen disappeared in metastatic nodules from colorectal cancer and there was no close correlation between the immunohistochemical expression of ABH blood related antigens and histologic cell differentiation/disease stages/disease free interval as well as between the intensity of malignancy and the expression of blood-related antigens.

On the other hand, there were few reports about the expression of blood-related antigen in primary and metastatic tumor cells. Schoentag reported that the expression of ABH blood-related antigen in primary tumor cells was almost the same as that in metastatic tumor cells except the disappearance of ABH blood related antigen expression in one of hepatic metastases and the appearance of abnormal H antigen expression in metastatic node which had not been revealed in primary cancer cells.

Enblad also indicated that the A antigen expression was disappeared in metastatic nodes from rectal cancer.

In this series, 45.5% of metastatic nodes from breast cancer showed disappearance of A/B/H antigen which had been revealed in primary malignant cancer cells. It is suggested that oncogenes coded carbohydrate conveting enzym should be highly affected.

It is not clear as to whether an etiologic relationship between aberration of oncogen in cancer cells and metastasis is present or not. Further accumulated study is necessary to solve this question.

References


