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Tumor-Specific Histo-Blood Group Antigens: Apropos of Two Cases

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Abstract

Cancer cells with immunogenic properties having altered protein glycosylation, modified blood group substances have been widely studied. Due to the genetic instability occurring during carcinogenesis the glycosyltransferases may suffer from posttranslation sequence modification. The author describes 2 autopsy cases, where in the background of the unusual metastatic tumor presentation, incompatible blood group antigenic determinants have been demonstrated using blood group specific lectins and monoclonal antibodies (mAb). In the first case, reported here, a 10-year-old girl developed an acute myeloid leukemia and died in a septic endotoxin shock after successful cytostatic treatment of a juvenile signet ring cell cancer of her colon. At autopsy there were no signs of tumor except bilateral apple-sized mucinous ovarian (Krukenberg) metastases. While she had erythrocyte phenotype of blood group A, the signet ring adenocarcinoma cells expressed blood group B incompatible antigenic determinants with lectin/mAb. In the second case, the autopsy of a 78-year-old female resulted in no macroscopic tumor sign except a moderately enlarged, ham hard spleen. Light microscopy revealed adenocarcinomatous infiltration in the splenic sinusoids. The patient had blood group O, while the metastatic cells in the spleen reacted with Breast Carcinoma Antigen (BioGenex) and incompatible anti-B Banderiaeasimplicifolia agglutinin I and anti-B mAb. It proved to be a case of an occult, completely regressed breast cancer. Based on these observations the expression of tumor specific incompatible blood group antigens might occur from time to time, mostly in adenocarcinomas. Accordingly, blood group-based specific immuno-oncotherapy could be considered in some cancer cases.

Keywords

Occult Breast Cancer, Krukenberg Metastasis, Incompatible Tumor-Specific, Histo-Blood Group Antigens

1. Introduction

Karl Landsteiner identified the ABO blood groups by examining the blood of his colleagues with allohemagglutination at the Institute of Pathology of the Medical University of Vienna [1]. Originally, Landsteiner designated the blood groups with the letters A, B, C, but since the blood group called C did not contain antigens, he named it O, referring to the German word "ohne" (without). His discovery enabled the safe blood transfusion, for which he received the Nobel Prize in 1930. Epidemiological studies suggest that people with blood group AB or B have a higher incidence of pancreatic and stomach cancer than people with blood group O. The connection between blood type A and stomach cancer has been known for a long time. The underlying mechanism of these observations is still unknown [2]. Commemorations have been published in the OrvosiHetilap on the 100th anniversary of Landsteiner's birth and the 20th anniversary of his death [3] [4]. Rex-Kiss [5] [6] summarized a short history of blood group research in 1980, where he described in detail the results of blood group serology up till that time. He reported on phytohemagglutinins from plant extracts and seeds, as well as antibody-like substancesas new findings, which proved to be lectins, found in the protein gland of the Roman snail (H. pomatia). The macromolecules, proteins, that can recognize and bind to the various chain-end or interchain monosaccharides non-covalently and have at least two binding sites are called lectins. In the case of these compounds, since they recognize cell surface carbohydrates, the designation haemagglutinin was replaced by the name "lectin" due to their role in recognition: this comes from the Latin verb "legere", which refers to the role of lectin proteins to "select" or "choose" [7] [8]. The discovery of the first lectin, Ricinus communis agglutinin (RCA), is attributed to Stillmark (1888), who observed that an extract of the seed of the castor plant can agglutinate human red blood cells [9]. It turned out that RCA binds to beta-D-galactose and is not blood group specific. The induction of mitosis and blast transformation of human leukocytes (lymphocytes) with phytohaemagglutin lectin was observed by Nowell in 1960 [10]. It was confirmed also with lectin, (wheat germ agglutinin) that modified cell surface glycosylation is a fundamental characteristic of oncogenic transformation [11]. Many lectins have blood group antigen specificity [12] (**Figure 1**).

The O blood group is used as a synonym for the H antigen, which has the same biochemical structure as the O antigen and was discovered in connection with the Bombay blood group phenotype. An increasingly deeper understanding of the chemical structure of the bacterial antigens and human blood groups in the 1960s, led to the realization that there was a very high degree of similarity between the bacterial antigens and the blood group antigens. The base chain of the oligosaccharide chains of ABH and Lewis blood group substances shows a high degree of similarity to the tetrasaccharide structure of the XIV antigen of Diplococcus pneumonia [13] [14]. Why anti-B is formed in the serum of patients with blood group A, anti-A in patients with blood group B, and anti-A and

Lectin	Abbreviation	Sugar specificity	Blood group specificity
Ulex europaeus agglutinin	UEA-I	α-L-fuc	0/H
Lotus tetragonolobus Bandeiraea (Griffonia) simplicifolia	LTA BSA-I	α-L-fuc α-D-Gal	0/H B
Dolichos bflorus	DBA	α-D-GalNac	A
Arachis hypogaea (peanut agglutinin)	PNA	β-D-Gal(1-3)-D-GalNac	T (Thomsen-Friedenreich)
Soybean (Glycine max) agglutinin	SBA	α-D-Gal (1-3 v. 1-4) α-D-GalNac	T and praecursor 0/H
Wheat germ agglutinin	WGA	α-D-GlcNac	Not known
Lens culinaris	LCA	α-D-Man	Not known

α-L-fuc=alpha-L-fucose;
α-D-Gal=alfa-D-galactose;
α-D-GalNac=N-acetilated-alpha-D-galactosamine;
β-D-Gal (1-3)-D-GalNac=D-galactose (1-3)-N-acetilated-D-galactosamine;
α-D-Gal (1-3 or 1-4) alpha-D-GalNac=alpha-D-galactose (1-3 or 1-4) N-acetilated-alpha-D-galactosamine;
α-D-GlucNac=N-acetylated-alpha-D-glucosamine;
α-D-Man= alpha-D-mannose

Figure 1. Origin, blood group and sugar specificity of some lectins.

anti-B antibodies in patients with blood group O can also be explained mainly by the antibody reaction against ABH blood group antigens in the capsule of the bacteria in the intestinal flora. It was also clarified that the immunodeterminant groups of the blood group antigens are formed by the terminal monosaccharide units of the oligosaccharide chains on the glycolipid and glycoprotein molecules of the cell membrane [15] [16]. The blood group antigens are lactoseamino-glycans in terms of their biochemical structure, glycoproteins, and glycolipids. Glycoproteins can be O- or N-glycosylated. O-glycosylated glycoproteins are found in the protective mucous membranes of the body. The oxygen (O-bond) of the N-acetylgalactosamine is connected most often to the hydroxyl group of serine or threonine. Its main sugar component is galactose. In the N-glycosidic bond, the glycosidic hydroxyl group is most often bound to the amide nitrogen of the aspartic acid residue. The oligosaccharide side chain has a high mannose content and is involved in signal recognition and cell surface receptor functions.85% of the body's secreted proteins are O-glycosylated. The sugar component is added to the peptides in the Golgi, which is purely a post-translational process. In glycolipids, oligosaccharides are linked to dolichol (lipid "carrier") [17]. **Figure 2** shows the schematic biosynthesis of the ABH and

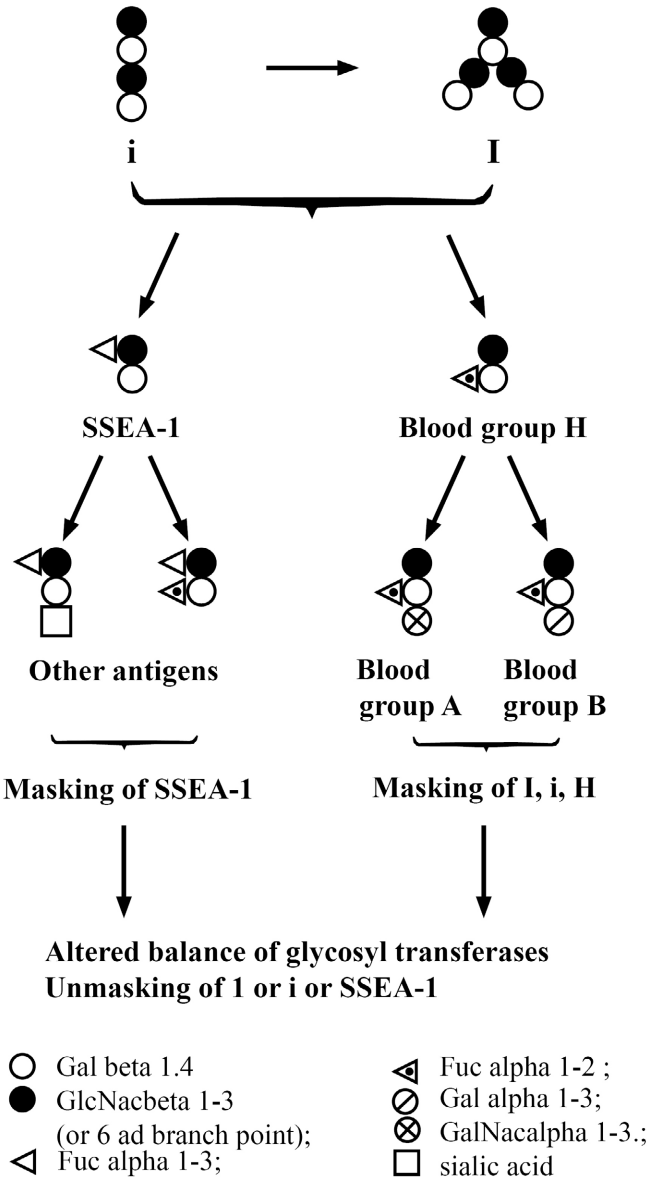

Figure 2. Schematic presentation of the interrelation of the I, i, SSEA-1 and the blood group ABH antigens [Feizi, 1981].

related blood group antigens. The common precursor of blood group A and B antigens is the O/H antigen. In the membranes of red blood cells, a significant proportion of blood group antigens are not bound to proteins, but to lipids (ceramide). The first truly pure ABH glycosphingolipid was isolated from red blood cell membrane only in 1968 [18]. Due to the perception that blood group antigens can occur in the various bodily secretions of so-called secretory individuals (ovarian cyst fluids, mucus, saliva, tears, urine, etc.) as well as due to the fact that the high degree of similarity of the ABO and Lewis antigens ofsome bacteria, such as the capsular antigens of the previously mentioned XIV pneumococcus, the biochemical discovery of the ABH precursor antigens (I and i) was greatly facilitated [19]. Based on the erythrocyte phenotype, the population consists of

about 70% secretory Lewis 'a'-'b'+, 25% non-secretory Lewis 'a'+'b'-, 5% Lewis 'a'-'b'-individuals. The secretory status is under the control of the Lewis genes [20] [21]. Using oligosaccharides (of known structure) isolated from glycoproteins of blood group antigens from chemically well-defined human milk (colostrum) in hapten inhibition reactions showed that between the H and A, B and respectively the Lewis "a" and Lewis "b" structures there is a precursor—end product relationship [21]. The branched I antigen is synthesized from the more ancient, straight chain I antigen. Structures i and I are precursors of the O/H antigen, which on the other hand, is a common precursor of the A and B antigens. It turned out that Lewis "b" is formed from Lewis "a" by antigenic fucosylation. It also became clear that the terminal monosaccharide units are added stepwise to the preceding structures. This is done according to a genetically determined sequence by glycosyltransferases, which, as enzyme proteins are primary gene products, *i.e.* they are transcribed directly from DNA, similar to other protein antigens. Carbohydrates are otherwise generally weak antigens, unlike blood group antigens. Carbohydrate antigens are synthesized as secondary gene products (**Figure 3**.).

An example of a carbohydrate antigen is the hexaglycosylceramide. The biosynthesis of the 6 sugars containing antigen requires 6 different genetically determined enzymes (glycosyltransferases) which are highly specific for the precursor structure. These enzymes are coded on chromosomes that are far from each other, and due to the genetic instability of tumors, their dysfunction and

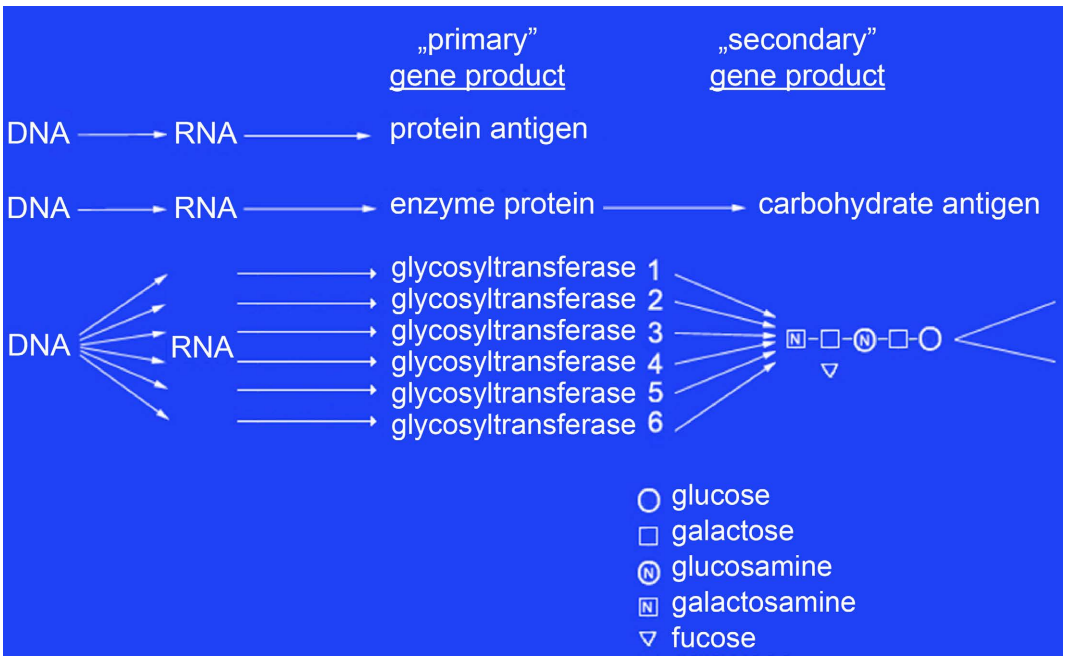

Figure 3. Schematic comparison of the biosynthesis of a protein and a carbohydrate antigen. An example of a carbohydrate antigen is blood group A, hexaglycosylceramide. The biosynthesis of the antigen containing 6 sugars re quires 6 different, genetically determined enzymes (glycosyltransferases) highly specific for the precursor structure. These enzymes are coded on chromosomes that are far from each other, and due to neoplastic genetic instability, their dysfunction and sequence changes can easily occur.

sequence changes can easily occur.

In the blood group antigen research, the recognition and interpretation of specific mixed agglutination has brought significant progress. Wiener and Herman [22] discovered that the antibody (horse serum) produced against type XIV pneumococci agglutinates human red blood cells. Coombs hypothesized that agglutinating antibodies create cell-microorganism and cell-cell connections when they carry identical, common antigens. Coombs *et al.* [23] observed for the first time that the epithelial cells in a normal human epithelial cell suspension are agglutinated by sera against the blood group antigen. In this way, it was proven that normal epithelial cells carry so-called isoantigens that are equivalent to the antigen of the same blood group of red blood cells, and which are specifically linked via bivalent antibodies. Davidsohn [24] used the red blood cell adsorption method based on specific mixed agglutination to detect ABO isoantigens in epithelial tissues (sections), which became known as the SRCA (specific red cell adherence) test.

Köhler and Milsten [25] developed a genetic method for mapping immunoglobulins based on the methodology of somatic cell hybridization with which an isotype *i.e.*, monoclonal antibody can be produced against a single immunodeterminant of a wide variety of antigens and receptors. A number of mAbs produced by the hybridoma method are available against for both ABH and Lewis blood groups, etc. antigens. Overriding the red blood cell adsorption based specific red blood cell adherence test, monoclonal antibody immunoperoxidase methods against different blood group antigens were developed.

During the phylogenesis, ABH-Lewis antigens first appeared in tissues in amphibians and reptiles in their mucus-producing cells. In rodents, they were expressed in epithelial tissue and nerve receptors. In primates (baboons), they were also detected on vascular endothelial cells, and in the course of evolution, the "Landsteiner" antigens appeared on human red blood cells only at the latest.

Classical immunohistochemical methods (SRCA test, immunofluorescence) have already proven that the expression of ABH blood group antigens is closely related to embryonic differentiation and in many organs (gastrointestinal tract, bladder, prostate, breast, cervix, lung, etc.) the loss of isoantigens A and B was observed during the development of carcinoma, while in some organs, e.g. thyroid, liver cells, the epithelial cells of the colo-rectal transition in adults, mature tissues do not contain blood group antigens, while on tumorous, carcinomatous epithelial cells of these organs (papillary thyroid carcinoma (**Figure 4**.), on the cells of hepatocellular carcinoma, colon adenocarcinoma—similarly to embryonic life—they can appear again during malignant transformation (neoexpression) [26] [27] [28] [29].

It was noticed that the deletion and disappearance of ABH antigens from the surface of tumor epithelial cells may indicate a more aggressive biological behavior of the urinary bladder tumors and a less favorable prognosis [30] [31] [32] (**Figure 5**).

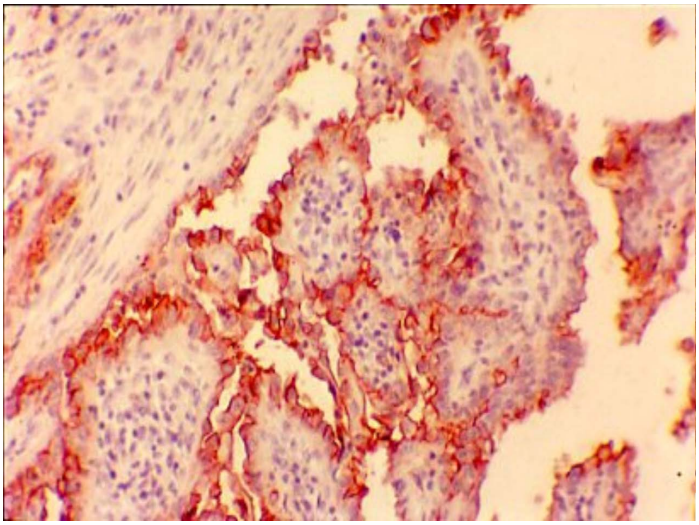

Figure 4. In papillary carcinoma of the thyroid in a patient with blood group A, blood group antigen can be detected, which is not present on mature normal thyroid epithelial cells, while it is found on embryonic thyroid cells. Neoplastic neoexpression, (Anti-A monoclonal antibody, immunoperoxidase reaction).

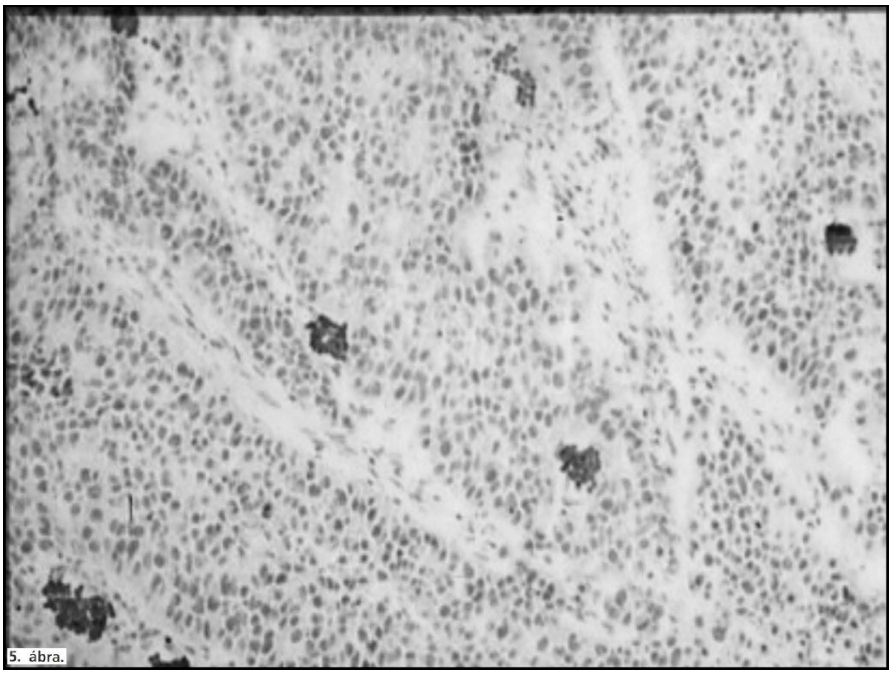

Figure 5. Papillary uroepithelial carcinoma, grade III. Antigens of the same blood group as the patient's blood group (B) cannot be detected on immature carcinoma cells. (deletion). Endothelial cells, which also serve as endogenous controls, react intensely (anti-B mAb, immunperoxydase reaction immunoperoxidase reaction).

In recent decades, tissue blood group antigens have come to the forefront of interest because it has been clearly proven that the majority of the tumor-specific believed antigens, isolated from the membrane of tumor cells, show a structural affinity with blood group antigens expressed in embryonic-fetal age (oncodevelopmental blood group associated antigens) [33] [34] [35] [36]. Stage-specific

embryonic antigens were recognized by monoclonal antibodies produced against teratocarcinoma stem cell lines, because they selectively react with antigens that appear in a specific developmental stage of ontogenesis [37]. The cells of the 8-cell mouse embryo reacted with CD15 (Leu M1) serum react against the stage-specific embryonic antigen 1 (SSEA 1) [38]. Interestingly, the SSEA-1 structure (3-fucosyl-N-acetyl-lactosamine) is related to the O/H blood group antigen (**Figure 2**) and can be detected on many normal human cells, including cells of the central nervous system, as well as on tumor cells and tissues [39], also on myelomonocytic cells and in some Sternberg-Reed cell variants in Hodgkin's disease [40]. In secretory individuals, together with the ABH antigens, some Lewis blood group antigens also enter the body fluids and are incorporated into the red blood cell membrane from the blood plasma. Those serological, biochemical, immunohistochemical, and molecular genetic tests which compared the antigenic status of the normal Lewis blood group in secretions (saliva, tears and red blood cells) to the expression of Lewis antigens found on the surface of tumor cells began in the second half of the 1980s [41] [42]. More and more observations indicate that in the early phase of tumor transformation, Lewis antigens that do not correspond to the Lewis antigens found on the surface of red blood cells appear on the surface of tumor cells. The Lewis "a" antigen immunodeterminant is synthesized by the alpha-1-4-fucosyltransferase enzyme encoded by the Lewis gene, which can be detected in the body secretions of non-secretory individuals together with the Lewis "x" antigen. The Lewis "b" antigen determinant (fucose) appearing in secretory individuals is built on the Lewis structure by the alpha-1-2-fucosyltransferase encoded by the secretory (Se/) gene [43]. The secreted (ABH, Lewis "b" and "y") blood group antigens can be detected in more than 70% of the population.

Modification of the cell surface or tissue ABH blood group antigens may occur in the most common malignant tumors of epithelial origin (carcinomas), during the malignant transformation. The most common of these is the deletion of the A, B antigen, the accumulation of the precursor O/H antigen, the appearance of fetal neoantigen and, rarely, on the tumor cells, the presence of an incompatible (not matching the patient's blood group) antigen can be detected.

The specific function of tissue blood group antigens and cell surface oligosaccharides is unclear. They play a role in embryonic development, differentiation, recognition, cell adhesion, migration, and oncogenesis [44] [45].

It is to be separately mentioned the Thomsen-Friedenreich (T) blood group and onco-fetal antigen, which is found on the surface of carcinoma cells in about 90% of the cases [46]. The T-antigen is linked to glycophorin and is MN blood group-associated. It has been shown that it has an active role in the progression and metastasis of carcinomas. The T-antigen is a disaccharide, Gal-beta-(1-3-GalNac). It is often masked with sialic acid (Neu Ac/alpha2-6/) and can be revealed by neuraminidase digestion (Tn antigen).The terminal galactose can then be detected by immunohistochemical method with Arachis hypogaea (peanut

agglutinin /PNA/) lectin. The very short carbon chain O-GalNac glycans were tested on melanoma, cervix, stomach, etc. cell lines. Clinical trials have also taken place. Despite many efforts, most vaccines against tumor antigens have not induced effective, strong T-cell responses. In tumor cells, the modified cell surface glycosylation can open new possibilities in the anti-tumor strategy [47], as well as in active, specific immunotherapy against blood group (carbohydrate) antigens [48].

Regarding tumor-specific incompatible blood group antigens, the best-known case was reported by Levine in 1976 [49].

It was a female patient with stomach cancer with blood group O, in whom a rare variation /pp/ of the antigen of blood group P was identified. The patient was transfused, which caused a severe hemolytic reaction with an elevated /8-512/ anti PP, Pk IgG antibody. Considering the tumor inoperable, a partial gastric resection was performed. The tumor patient lived for another 22 years without a detectable tumor. P and P1 antigens were identified in the resected frozen gastric carcinoma tissue during a subsequent examination. The high-titer antibody immune response—presumably together with other mechanisms—selectively destroyed the tumor cells containing incompatible P and P1 antigens.

2. Material and Methods

Tissue samples from the first case were taken from the spleen, from a small splenic hilar lymph node, from the bone marrow and the liver. In the second case tissue samples were taken from the primary colon tumor and lymph node biopsies and at autopsy from the bilateral ovarian tumor (Krukenberg metastasis). After fixation (in 10% formaldehyde in 0.1 M sodium phosphate, pH 7.2 at room temperature), dehydration in ethanol at increasing concentration (70% - 100%) and clearing in xylene, tissue blocks were embedded in paraffin wax and 5 - 6 micron-thick sections were cut using rotatory microtome. After deparaffinization and rehydration by routine protocol, sections were incubated for 15 min in 0.3% H_2O_2 in methanol to block endogeneous peroxidase. Then the slides were incubated with monoclonal mouse antibodies (all diluted 1:100 in 0.05 Tris buffer, pH 7.6) against breast carcinoma antigen (BioGenex), cytokeratins, epithelial membrane antigen (EMA), complement membrane attack complex (C5b-9), A, B and O/H blood group antigens all from (DAKO), for 1 h at room temperature. After washing sections in Tris buffer, slides were incubated at first with biotinylated anti-mouse immunoglobulins, after then with peroxidase-labelled streptavidin (both diluted 1100 for 10 min), the chromogen reagent solution freshly prepared with 1.5% diaminobenzidine (Sigma) in TBS buffer with 0.01% H_2O_2. The sections were counterstained with hematoxylin. Sections were labelled for the binding of biotinylated lectins that recognize blood group antigen determinants, as detailed in **Figure 1**. The applied lectins all were biotinylated and purchased from Vector Laboratories. The slides were incubated with biotinylated lectins at a concentration of 10 microg in TBS (pH 7.4 - 7.5), for 1 h

at room temperature. After washing in TBS, slides were covered with streptavidin-horseradish peroxidase at a concentration of 50 ng/ml for 1 h. The chromogen solution was prepared as described above.

In early (6 - 48 h) myocardial infarctions there is a strong local terminal complement cascade (C5b-9) activation around the necrotic muscle fibers [50]. For this reason, sections of human infarcted heart tissue were employed as positive controls for the binding of anti C5b-9. Positive controls for blood group antigens were normal tissues from individuals with known ABH blood groups. The reaction of the patient's own endothelial cells and erythrocytes served as endogenous controls.

SRCA—(Specific Red Cell Adherence) test. 5 - 6 micron thin frozen tissue sections (Tissue Tek II) were made and air-dried, then they were incubated with anti-A and anti-B sera (Human, Budapest) in a humidified chamber and washed with phosphate buffered saline (PBS, pH 7.4). Subsequently the cryostat sections were covered with erythrocytes (blood group A and B/1%/ suspended in saline) and incubated. The non-adherent red blood cells were removed by washing with saline. The reaction was fixed in 2% glutaraldehyde solution and the benzidin-peroxydase reaction was used to enhance the contrast of erythrocytes.

The evaluation of the intensity of immunoreaction was calculated using the following scoring sytem: + scattered reacting cells; ++ one third of the tumor cells are weakly positive; +++ two thirds of the tumor cells are weakly positive or one-third show strongly positive reactivity and ++++ all tumor cells show positivity but variable in intensity +++++ all tumor cells label intensely and homogenously.

3. Case Reports

Knowing Levine's case, we searched for similarly behaving, peculiarly, unusually metastasizing carcinoma cases, comparing the primary tumor and metastases with blood group-specific mAb-s and lectins.

Case 1. During laparotomy for mechanical ileus, in a case of a 10-years-old girl, a tumor had been found in the distal colon with metastases in a regional lymph node. Histology confirmed signet ring cell carcinoma. She went into complete remission after chemotherapy for 4 years. Subsequently, she was diagnosed with severe acute myeloid leukemia (FAB M1). She died during induction chemotherapy with symptoms of endotoxin shock. At her autopsy, performed at the Institute of Pathology in Pécs, necrotizing pseudo-membranous enterocolitis, hemorrhagic diathesis, and septic organs were found [51]. In addition, bilateral apple-sized mucinous ovarian (Krukenberg) metastases were also found. During re-examination of the primary colon tumor biopsy samples, the metastatic lymph nodes and the Krukenberg tumor cells of the patient with blood group A, those reacted intensively with Griffonia (Banderiaea) simplicifolia I lectin specific for blood group B antigen. Consequently, the tumor cells of the bilateral ovarian metastasis contained incompatible blood group B antigens. No

tumor was found elsewhere at the autopsy. The signet ring cells of Krukenberg's carcinoma carried tumor-specific blood group B antigen incompatible with the patient's blood group (A) [Griffonia (Banderiaea) simplicifolia I lectin] immunoperoxidase reaction (Figure 6.).

Once in the circulation, the carcinoma cells with incompatible blood group antigens could undergo complement and antibody-mediated immunocytolysis but due to the weakness of the specific local microenvironmental immune surveillance in the ovaries, the signet ring cells were able to survive and proliferate.

Similarity can be suggested with the isolated, tumor-like testicular recurrence during remission in boys with acute lymphoid leukemia. Leukemic cells are able to settle in an immunologically inert, protected place in the testes.

Case 2. In another case an autopsy was performed on a 78-year-old female patient, (who happened to spend only 4 hours before her death in the hospital) at the Pathology Department of Kanizsai Dorottya Hospital in Nagykanizsa [52] [53]. The clinical diagnosis was circulatory failure. Regarding her history, severe anemia and thrombocytopenia had been found in the laboratory examination performed before a cataract surgery. The patient refused the proposed hematological workup. At the autopsy, the only positive finding was the moderately enlarged (420 g) very congested, board-hard to the touch spleen, with a blurred structure and a blackberry-reddish color. On the sections, it mostly gave the impression of a ham spleen with primary amyloidosis (Figure 7). The visceral organs were pale and anemic. The spine was severely kyphyscoliotic with consequent chronic cor pulmonale. Samples were taken for histological examination, according to the primary amyloidosis protocol (spleen, liver, splenic hilar lymph node, bone marrow).

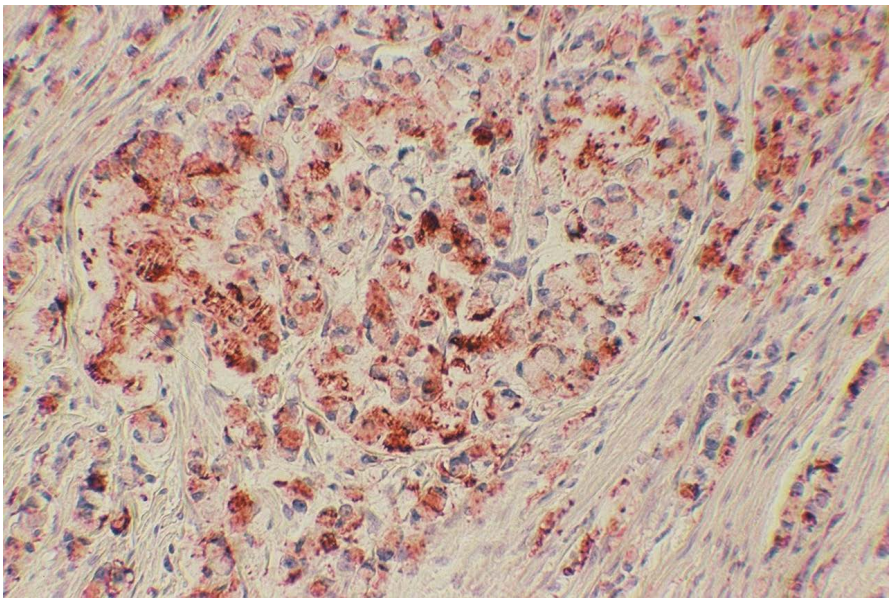

Figure 6. Krukenberg metastasis signet ring cells carried an incompatible blood group B antigen that did not match the patient's blood group (A) Griffonia (Banderiaea) simplicifolia I lectin-immunoperoxidase reaction.

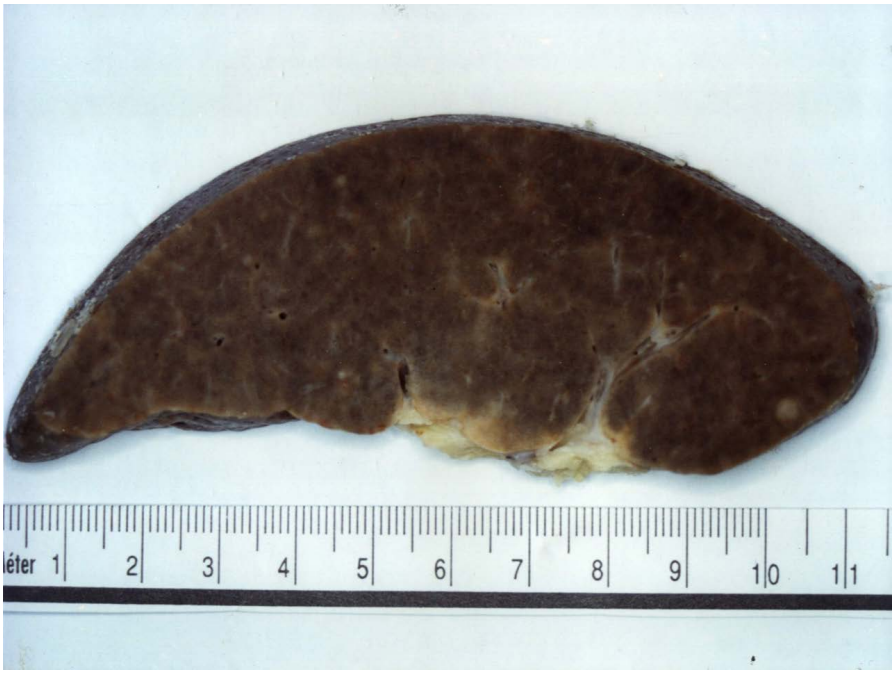

Figure 7. On gross examination, the spleen was mildly enlarged (440 g), with a homogeneous congested cut surface. There was no sign of a tumor.

Macroscopically, no signs of tumorous process were found in the organs. Surprisingly, the histological examination of the spleen revealed carcinoma. The pattern of the adenocarcinoma raised the suspicion of breast adenocarcinoma metastasis. However, the breasts, chest, and pleura were tumor-free. The metastatic cells of the spleen and bone marrow reacted with the antibody against Breast Carcinoma Antigen (BioGenex) on the sections of the spleen, lymph node, and bone marrow. The patient had blood type O. The metastatic cells of the spleen, splenic hilar lymph node and bone marrow were intensively marked by blood group B specific Banderiaeasimplicifolia agglutinin I and anti-B mAb. In the sinusoids of the splenic hilar lymph node, the metastatic cell groups displaying a syncytial arrangement showed intense positivity with Banderiaeasimplicifolia agglutinin I lectin (**Figure 8**.). Thus, in addition to blood group O, tumor-specific blood group B antigens were expressed.

We did not get a reaction with blood group A antigen specific mAb or blood group A specific lectin (Dolichos biflorus). O/H blood group specific antibody and Ulex europeaus I lectin showed weak intensity positivity with the precursor antigen on the metastatic cells.

4. Results and Discussion

Based on the cases the following was hypothetised: in both cases all the carcinomatous cells underwent lysis in the circulation—most probably by antibody and complement mediated immune reaction—but were able to survive and multiply in the oophoron, spleen and the bone marrow. It seems that by examining tumor tissue blood group antigens, we are facing a promising opportunity for tumor

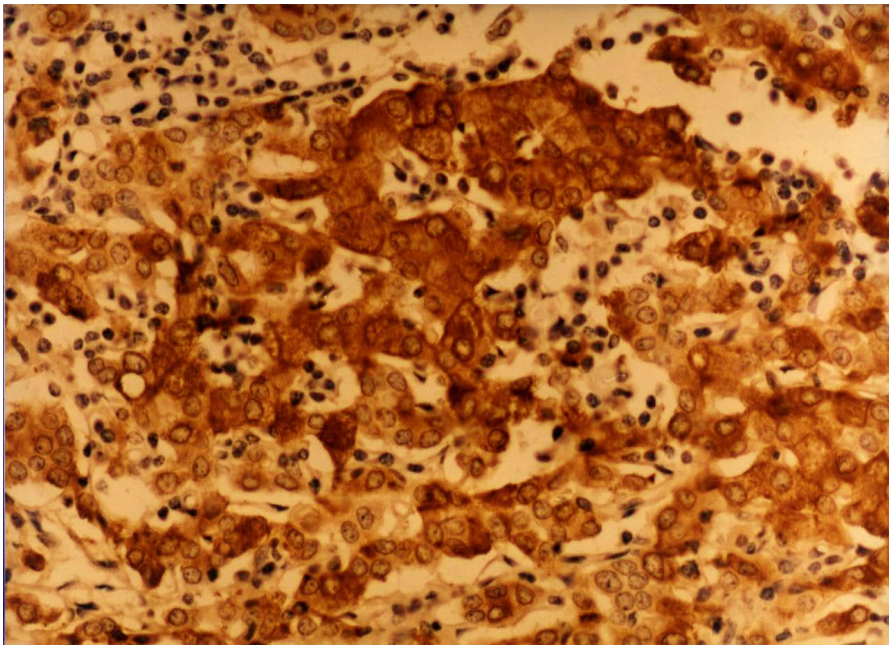

Figure 8. All the metastatic cell groups with syntitial pattern of the sinusoids of the spleen show intense positivity for binding of BSA-I lectin. All the metastatic cell groups with syntitial pattern of the sinusoids of the spleen show intense positivity for binding of BSA-I lectin.

research and tumor immunotherapy. Further studies are required in order to proceed with comparative lectin/mAb blood group antigen studies on comprehensive (primary tumor, metastasis) tissue blood group antigens. It is likely that due to the genetic instability that appears during carcinogenesis, a disturbance—a very fine-tuned mechanism—occurs in the functioning of glycosyltransferases (**Figure 3**).

Blood group glycosyltansferases were used to modify HeLa cells of H specificity (O group) into cells of A and B specificity [54]. In the author's opinion this method can be applied to tumor cells in general and constitutes an attempt to stimulate the immunocompetent system. The presence of tumor-specific blood group antigenic determinants may be suspected in cases of carcinomas that give rise to late solitary metastasis (over 10 years). People with O/H blood group already have anti-B antibodies in their serum (produced against intestinal bacteria's capsular antigens), whose affinity and avidity can increase against incompatible blood group antigenic determinants. The patient's humoral immune system could be stimulated by vaccination with the specific blood group antigen of the incompatible tumor.

Cummings and Mazur [55] reported 2 cases of breast carcinoma verily similar to the one described here, in which idiopathic thrombocytopenic purpura (ITP) occurred after radio- and chemotherapy. However, they were in remission for 5 and 13 years. After splenectomy, ITP disappeared. Similar to our case, diffuse metastases were found in both removed spleens, microscopically only. The occurrence of splenic metastasis of epithelial tumors is generally believed to be

rare. However, some autopsy examinations found the involvement of the spleen in 5% - 13% of cancer cases [56]. In our case number 2 patient, severe anemia and thrombocytopenia were confirmed before her cataract surgery, however, the patient did not agree to the suggested workup. Metoki *et al.* detected tumor-specific incompatible A antigen expression in two cases of 10 patients with blood group B, and in two cases of 9 patients with blood group O, from 53 ovarian carcinomas [57]. Clausen *et al.* [58] observed the presence of A-blood group antigen on the tumor cells in two patients with O blood group out of 15 colon carcinoma cases. Hattori *et al.* [59] found an incompatible A blood group active glycolipid in the tumor tissue sample of a gastric cancer patient with blood group O, which was absent from the tumor-free mucosa, so only the tumor cells contained the incompatible A antigen. Hakkinen [60] found A-like blood group antigens on tumor cells in O and B blood group gastric cancer patients. Itzkowitz *et al.* detected carcinoma-associated blood group antigens in 33% of pancreatic carcinoma cases. The incompatible tumor tissue antigens A and B are certainly recognized by the body's immune system in the early stages of the development of carcinoma. The incidence of the incompatible A-antigen in B and O blood group tumors can be higher than 10% - 15% [61].

It may be correct to conclude that vaccination with tumor-specific antigen A (N-acetyl-galactosamine) can be effective in the prevention and treatment of carcinoma in B and O blood group individuals. In the case of the girl with the Krukenberg tumor, in addition to blood group A, the naturally present anti-B antibodies eliminated the carcinoma cells with their anti-tumor specific cytotoxicity and only tumor cells that were exempt from immune surveillance were able to persist. In the case of the O-blood type patient with occult breast cancer, the carcinoma (metastasis) was essentially limited to the spleen, as her immune system had destroyed the B-incompatible antigen containing tumor cells by means of immune recognition and immunocytolysis, thanks to the natural anti-B antibody presence in her body.

In view of the success of active, specific immuno-oncology treatments in recent years, it appears that—if detected earlier—the presented two patients with regressive blood group-associated malignant tumors could have been cured by treating them with an anti-B monoclonal antibody and/or by vaccinating them with the sugar antigen (e.g. beta-galactose) of the blood group B antigen.

In particular, in patients with adenocarcinoma, it could be recommended to determine the tumor tissue blood group antigens in the primary tumor and metastasis, supplemented by testing the titer of antibodies against the blood group antigens in the serum and the activation of the complement system. In some cases, specific immunological and oncotherapeutic treatment of the tumor based on blood group may be considered.

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Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

References

[1] Landsteiner, K. (2001) Agglutination Phenomena of Normal Human Blood. *Wiener klinische Wochenschrift*, **113**, 768-769. (In German)

[2] Franchini, M. and Liumbruno, G.M. (2013) ABO Blood Group: Old Dogma, New Perspectives. *Clinical Chemistry and Laboratory Medicine*, **51**, 1545-1553. https://doi.org/10.1515/cclm-2013-0168

[3] Kenéz, J. (1963) In Memoriam K. Landsteineri, Inventor of Blood Grouping. *Orvosi Hetilap*, **104**, 1232-1234. (In Hungarian)

[4] Kenéz, J. (1968) Milestones in Blood Transfusion Development. *Orvosi Hetilap*, **109**, 2441-2445.(In Hungarian)

[5] Rex-Kiss, B. (1969) Karl Landsteiner. Commemoration of Centenary of His Birth. *Orvosi Hetilap*, **27**, 1567-1570. (In Hungarian)

[6] Rex-Kiss, B. (1980) Brief History of Blood Group Research A vércsoportkutatásrövid története. *Orvosi Hetilap*, **121**, 2335-2339. (In Hungarian)

[7] Boyd, W.C. and Shapleigh, E. (1954) Specific Precipitating Activity of Plant Agglutinins. *Science*, **119**, 419.

[8] Sharon, N. and Lis, H. (1989) Lectins as Recognition Molecules. *Science*, **346**, 227-234. https://doi.org/10.1126/science.2552581

[9] Stillmark, H. (1888) About Ricin, the Gift Ferment from the Seed of *Ricinus communis* L. and Some Other Euphorbiaceen. Inaug.-Diss. Dorpat., Schnakenburg. (In German)

[10] Nowell, P.C. (1960) Phytohaemagglutinin: An Initiator of Mitosis in Cultures of Normal Human Keukocytes. *Cancer Research*, **20**, 462-466.

[11] Aub, J.C., Stanford, B.H. and Cote, M.N. (1965) Studies on Reactivity of Tumor and Normal Cell Reactivity to a Wheat Germ Agglutinin. *Proceedings of the National Academy of Sciences of the United States of America*, **54**, 396-399. https://doi.org/10.1073/pnas.54.2.396

[12] Khan, F., Khan, R.H., Sherwani, A., *et al.* (2002) Lectins as Markers for Blood Grouping. *Medical Science Monitor*, **8**, 293-300.

[13] Prokop, O. and Uhlenbruck, G. (1966) Lehrbuch der menschlichen Blut-und Serumgruppen. Leipzig. (In German)

[14] Fischer, J. (1985) Membrán receptorok oligoszacharid componenseinek szerkezete és funkciói. Fejezetek a korszerű immunologiából. Pécsi Akadémiai Bizottság. 89-103. (In Hungarian)

[15] Bellis, S.L., Reis, C.A., *et al.* (2022) Glycosylation Changes in Cancer. In: Varki, A., Cummings, R.D., Esko, J.D., *et al.*, Eds., *Essentials of Glycobiology*, 4th Edition, Cold Spring Harbor Laboratory Press, New York.

[16] Varki, A., Kannagi, R., Toole, B., Cummings, R.D., Esco, J.D., Stanley, P., *et al.* (2017) Glycosylation Changes in Cancer. In: Varki, A., Cummings, R.D., Esko, J.D., Stanley, P., Hart, G.W., Aebi, M., *et al.*, Eds., *Essentials of Glycobiology*, 3rd Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 2015-2017.

[17] Feizi, T. (1981) Carbohydrate Differentiation Antigens. *Trends in Biochemical Sciences*, **6**, 333-335. https://doi.org/10.1016/0968-0004(81)90119-5

[18] Hakomori, S.-I. and Strycharz, G.B. (1968) Cellular Blood-Group Substances. I. Isolation and Chemical Composition of Blood-Group ABH and Leb Isoantigens of Sphingoglycolipid Nature. *Biochemistery*, **7**, 1279-1286. https://doi.org/10.1021/bi00844a005

[19] Feizi, T., Kabat, E.A. Vicary, G. anderson, B. and Marsh, W.L. (1971) Immunochemical Studies on Blood Groups: XLVII. The I Antigen Complex—Precursors in the A, B, H, LE^a, and LE^b Blood Group Sytem—Hemagglutination-Inhibition Studies. *Journal of Experimental Medicine*, **133**, 39-52. https://doi.org/10.1084/jem.133.1.39

[20] Grollmann, E.F., Kobata, A. and Ginsburg, V. (1969) An Enzymatic Basis of Lewis Blood Type in Man. *The Journal of Clinical Investigation*, **48**, 1489-1493. https://doi.org/10.1172/JCI106115

[21] Feizi, T. (1985) Demonstration by Monoclonal Antibodies That Carbohydrate Structures of Glycoproteins and Glycolipidsare Omcodevelopmental Antigens. *Nature*, **314**, 53-57. https://doi.org/10.1038/314053a0

[22] Wiener, A.S. and Herman, M. (1939) The Second Stage of the Agglutinative Reaction. *The Journal of Immunology*, **36**, 255-259. https://doi.org/10.4049/jimmunol.36.4.255

[23] Coombs, R.R.A., Bedford, D. and Rouillard, I.M. (1956) A and B Blood Group Antigens on Human Epidermal Cells Demonstrated by Mixed Agglutination Reaction. *The Lancet*, **1**, 461-463. https://doi.org/10.1016/S0140-6736(56)90528-1

[24] Davidsohn, I. (1972) Early Immunologic Diagnosis and Prognosis of Carcinoma. *American Journal of Clinical Pathology*, **57**, 715-730. https://doi.org/10.1093/ajcp/57.6.715

[25] Köhler, G. and Milstein, C. (1975) Continuous Cultures of Fused Cell Secreting Antibody of Predefined Specificity. *Nature*, **256**, 495-497. https://doi.org/10.1038/256495a0

[26] Okada, Y., Arima, T., Togawa, K., Nagashima, H., Jinno, K., Moriwaki, S., Kunitomo, T., Thurin, J. and Koprowski, H. (1987) Neoexpression of ABH and Lewis Blood Group Antigens in Human Hepatocellular Carcinomas. *Journal of the National Cancer Institute*, **78**, 19-28. https://doi.org/10.1093/jnci/78.1.19

[27] Szulman, A.E. (1962) The Histological Distribution of the Blood Group Substances in Man, as Disclosed by Immunofluorescence. *Journal of Experimental Medicine*, **115**, 977-1005. https://doi.org/10.1084/jem.115.5.977

[28] Vowden, P., Lowe, A.D., Lennox, E.S., Bleehen, N.M., *et al.* (1986) Thyroid Blood Group Expression: A Paralell with ABH Isoantigen Expression in the Distal Colon. *British Journal of Cancer*, **53**, 721-725. https://doi.org/10.1038/bjc.1986.124

[29] Baranyay, F., Knels, R. and Somogyi, L. (1992) The Distribution of ABO(H) Isoantigens in Urinary Bladder Tumours. *Acta Medica Hungarica*, **49**, 129-136.

[30] Coon, J.S. and Weinstein, R.S. (1986) Blood Group Related Antigens as Markers of Malignant Potential and Heterogeneity in Human Carcinomas. *Human Pathology*, **17**, 1089-1106. https://doi.org/10.1016/S0046-8177(86)80413-0

[31] Orntoft, T.F., Wolf, H., Clausen, H., Dabelsteen, E. and Hakomori, S. (1989) Blood

Group ABH-Related Antigens in Normal and Malignant Bladder Urothelium: Possible Structural Basis for the Deletion of Type-2 Chain ABH Antigens in Invasive Carcinomas. *International Journal of Cancer*, **43**, 774-780. https://doi.org/10.1002/ijc.2910430505

[32] Weinstein, R.S., Coon, J. and Alroy, J. (1981) Tissue-Associated Blood Group Antigens in Human Tumors. In: DeLellis, R.A., Ed., *Diagnostic Immunochemistry*, Masson Publishing, New York, 239-261.

[33] Dabelsteen, E., Mandel, U. and Clausen, H. (1988) Blood Group Antigens as Differentiation and Tumor Associated Narkers in Oral Epithelium. *Proceedings of the Finnish Dental Society*, **84**, 19-29.

[34] Feizi, T. (1985) Demonstration by Monoclonal Antibodies That Carbohydrate Structures of Glycopropteins and Glycolipids Are Oncodevelopmental Antigens. *Nature*, **314**, 53-57. https://doi.org/10.1038/314053a0

[35] Kannagi, R., Miyake, M., Zenita, K.M., Itai, S., Hiraiwa, N., Shigeta, K., *et al.* (1988) Cancer-Associated Carbohydrate Group Substances and Onco-Developmental Antigens on Tumor Cells. *Gann Monograph on Cancer Research*, **34**, 15-28.

[36] Hakomori, S.-I. (1981) Glycosphingolipids in Cellular Interaction, Differentiation, and Oncogenesis. *Annual Review of Biochemistry*, **50**, 733-764. https://doi.org/10.1146/annurev.bi.50.070181.003505

[37] Fox, N., Damjanov, J., Knowles, B.B. and Solter, D. (1983) Immunohistochemical Localization of the Mouse Stage Specific Enbryonic Antigen 1 in Human Tissues and Tumors. *Cancer Research*, **43**, 669-674.

[38] Hakomori, S.-I. (1991) Possible Functions of Tumor-Associated Carbohydrate Antigens. *Current Opinion in Immunology*, **3**, 646-653. https://doi.org/10.1016/0952-7915(91)90091-E

[39] Fujimoto, J., Hata, S.I., Ishii, S.Y., Tanaka, S., Kannagi, R., Ueyaey, Y. and Tamaoki, N. (1987) Differentiation Antigens Defined by Mouse Monoclonal Antibodies against Human Germ Cell Tumors. *Laboratory Investigation*, **57**, 350-358.

[40] Hsu, S.M., Ho, Y.S., Li, P.-J., Monheit, J., Ree, H.J., Sheibani, K. and Winberg, C.D. (1986) L-H Variants of Reed-Sternberg Cells Express Sialylated Leu M1 Antigens. *The American Journal of Pathology*, **22**, 199-203.

[41] Limas, C. and Lange, P.H. (1985) Lewis Antigens in Normal and Neoplastic Urothelium. *The American Journal of Pathology*, **121**, 176-183.

[42] Orntoft, T.F., Holmes, E.H., *et al.* (1991) Differential Tissue Expression of the Lewis Blood Group Antigens: Enzymatic, Immunohistologic and Immunochemical Evidence for Lewis a and Lewis b Antigen Expression in Le(a-b-) Individuals. *Blood*, **77**, 1387-1396. https://doi.org/10.1182/blood.V77.6.1389.1389

[43] Watkins, W.M. (1980) Biochemistry and Genetics of the ABO, Lewis and P Blood Group Systems. In: Harris, H. and Hirschhorn, K., Eds., *Advances in Human Genetics*, 10th Edition, Plenum Press, New York, 1-136. https://doi.org/10.1007/978-1-4615-8288-5_1

[44] Hakomori, S.-I. and Kannagi, R. (1983) Glycosphyngolipids as Tumor Associated and Differentiation Markers. *Journal of the National Cancer Institute*, **71**, 231-251.

[45] Sato, M. and Muramatsum, I. (1986) Oncodevelopmental Carbohydrate Antigens: Distribution of ECMA2 and 3 Antigens in Embryonic and Adult Tissues of the Mouse and in Teratocarcinomas. *Journal of Reproductive Immunology*, **2**, 123-135. https://doi.org/10.1016/0165-0378(86)90006-9

[46] Sindrevicz, P., Lian, L.Y. and Yu, L.G. (2016) Interaction of the Oncofetal

Thomsen-Friedenreich Antigen with Galectins in Cancer Progression and Metastasis. *Frontiers in Oncology*, **6**, Article No. 79. https://doi.org/10.3389/fonc.2016.00079

[47] Brooks, S.A., Carter, T.M., Royle, L., *et al.* (2008) Altered Glycosylation of Protein Sin Cancer: What Is the Potential for New Antitumor Strategies. *Anti-Cancer Agents in Nedical Chemistry*, **8**, 2-21. https://doi.org/10.2174/187152008783330860

[48] Ragupathi, G. (1996) Carbohydrate Antigens as Targets for Active Specific Immunotherapy. *Cancer Immunology, Immunotherapy*, **43**, 152-157. https://doi.org/10.1007/s002620050316

[49] Levine, P. (1976) Illegitimate Blood Group Antigen P1, A, and MN(T) in Malignancy. A Possible Therapeutic Approach with Anti-Tja, Anti-A and Anti-T. *Annals of the New York Academy of Sciences*, **277**, 428-436. https://doi.org/10.1111/j.1749-6632.1976.tb41719.x

[50] Yasojima, K., Schwab, C., McGeer, E.G. and McGeer, P.L. (1988) Human Heart Generates Complement Proteins That Are Upregulated and Activated after Myocardial Infarction. *Circulation Research*, **83**, 860-869. https://doi.org/10.1161/01.RES.83.8.860

[51] Baranyay, F., Szélig, L. and Kajtár, P. (1993) Comparative ABH Specific Lectin/mAb Immunohistochemical Analysis of Signet Ring Cell Colon Cancer with Krukenberg Metastasis. 15*th International Lectin Meeting*, Szeged, 22-28 August 1993, 8.

[52] Baranyay, F. (2009) Diffuse Splenic Metastasis of Occult Breast Cancer with Incompatible Blood Group Antigenic Determinants. *Acta Histochemica*, **111**, 344-349. https://doi.org/10.1016/j.acthis.2008.11.015

[53] Baranyay, F. (2021) Regarding Tumor-Specific ABO Histo-Blood Group Antigens. *Orvosi Hetilap*, **162**, 1422-1427. (In Hungarian) https://doi.org/10.1556/650.2021.HO2679

[54] Mousseron-Canet, M., Magoni, R., Lecou, C. and Levallois, C. (1975) Enzymatic Modification of the Transplant Antigens (ABO System) on Human Tumor Cells. *Cancer*, **36**, 1309-1314. https://doi.org/10.1002/1097-0142(197510)36:4<1309::AID-CNCR2820360418>3.0.CO;2-L

[55] Cummings, O.W. and Mazur, M.T. (1992) Breast Carcinoma Diffusely Metastatic to the Spleen. *American Journal of Clinical Pathology*, **97**, 487-489. https://doi.org/10.1093/ajcp/97.4.484

[56] Berege, T. (1974) Splenic Metastases. Frequences and Patterns. *Acta Pathologica Microbiologica Scandinavica Series A*, **82**, 499-506. https://doi.org/10.1111/j.1699-0463.1974.tb00379.x

[57] Metoki, R., Kakudo, K., Tsuji, Y., Teng, N., Clausen, H. and Hakomori, S. (1989) Deletion of Histo-Blood Group A and B Antigens and Expression Incompatible A in Ovarian Cancer. *Journal of the National Cancer Institute*, **81**, 1151-1157. https://doi.org/10.1093/jnci/81.15.1151

[58] Clausen, H., Hakomori, S.-I., Graem, N. and Dabelsteen, E. (1986) Incompatible A Antigen Expressed of Blood Group O Individuals: Immunochemical, Immunohistologic, and Enzymatic Characterization. *The Journal of Immunology*, **136**, 326-331. https://doi.org/10.4049/jimmunol.136.1.326

[59] Hattori, H., Uemira, K.I., Ogata, H., Katsuyama, T., Takatomi, T. and Kanfer, J.N. (1987) Characterisation of Glicolipids from the Gastric Cancer of a Patient of p,O,Le (1-,b+) Blood Type: Presence of Incompatible Blood Group Antigens in Tumor Tissues. *Cancer Research*, **47**, 1968-1972.

[60] Hakkinen, I. (1970) A-Like Blood Group Antigen in Gastric Cancer Cells of Patients in Blood Group O or B. *Journal of the National Cancer Institute*, **44**, 1183-1193.

[61] Itzkowitz, S.H., Yuan, M., Ferrell, L.D., Ratclifte, R.M., Chung, Y.S., Satake, K., *et al.* (1987) Cancer Associated Alterations of Blood Group Antigens Expression in Human Pancreas. *Journal of the National Cancer Institute*, **79**, 425-434.