LOCALIZATION AND ALTERATION OF MONO-, DI-, AND TRIFUCOSYL α1 → 3 TYPE 2 CHAIN STRUCTURES DURING HUMAN EMBRYOGENESIS AND IN HUMAN CANCER

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Development and differentiation could be mediated by continuous changes of cell surface glycoconjugates through which cell-cell interaction may take place (1). Rapid, dramatic changes of specific carbohydrate structures defined by various antibodies during the development of the preimplantation (2-11) as well as the postimplantation (12) mouse embryo have suggested that these structures are signals for cell recognition. Despite the fact that a great deal of information has accumulated on cell surface carbohydrate changes during mouse embryogenesis, our knowledge of the cell surface glycoconjugates in human embryos is extremely meager. Only one series of studies by Szulman (13) indicates the occurrence of an orderly appearance and disappearance of blood group A, H, and Lewis antigens during human embryogenesis. A systematic knowledge of the changes of cell surface carbohydrates during organogenesis is important for understanding the oncofetal expression of tumor-associated antigens expressed in many human cancers. A series of glycolipids bearing the X determinant have been found to be accumulated in various types of human adenocarcinoma, and their structures have been identified as shown in Table I. A number of monoclonal antibodies produced by various investigators, originally assigned as being directed to “tumor-specific antigens”, have been identified as being directed to the same structure as an embryonic antigen, SSEA-1 (14-16). The glycolipid lactofucopentaosyl(III)ceramide, designated as X hapten (17), was found to be greatly accumulated in various types of human cancer (18) and has the same terminal structure as SSEA-1. However, the antigen defined by the SSEA-1 antibody is widely distributed in normal human tissues, such as gastric epithelia and kidney tubules, as well as in a large variety of tumors (19). More recently, glycolipids with di- or trimeric X determinant, difucosyllactonorhexaosylceramide (II1V3Fuc2nLc6) and trifucosyllactonooctaosylceramide (II1V3, VII1Fuc3nLc8), have been found to accumulate in colonic and liver cancers (20). Two monoclonal antibodies have been established; one antibody, FH4, is directed...
Reactivity of FH3 and FH4 to Fucosylated Type 2 Chain Structures

| Antibodies | Ig class | Reactivities with: |
|------------|----------|--------------------|
| FH3        | IgG3     | $^1$V$^3$FucnLc$_6$ $^*|$ $^3$V$^3$FucnLc$_6$ $^*$ $^3$V$^3$V$^3$VII$^3$FucnLc$_6$ $^4$ |
| FH4        | IgG3     | -                  | $^+$ $^+$ $^+$ $^+$ $^+$ |

$^*$ Gal$^\beta$1 $\rightarrow$ 4GlcNAc$^\beta$1 $\rightarrow$ 3Gal$^\beta$1 $\rightarrow$ 4Glc$^\beta$1 $\rightarrow$ 1Cer

$^2$ Gal$^\beta$1 $\rightarrow$ 4GlcNAc$^\beta$1 $\rightarrow$ 3Gal$^\beta$1 $\rightarrow$ 4GlcNAc$^\beta$1 $\rightarrow$ 3Gal$^\beta$1 $\rightarrow$ 4Glc$^\beta$1 $\rightarrow$ 1Cer

$^3$ Gal$^\beta$1 $\rightarrow$ 4GlcNAc$^\beta$1 $\rightarrow$ 3Gal$^\beta$1 $\rightarrow$ 4GlcNAc$^\beta$1 $\rightarrow$ 3Gal$^\beta$1 $\rightarrow$ 4Glc$^\beta$1 $\rightarrow$ 1Cer

Materials and Methods

Tissues. 27 human embryos and fetuses$^1$ were collected at the Division of Human Embryology and Teratology, Department of Pediatrics, University of Washington. The ages of the embryos and fetuses were between 38 and 127 d, and were organized as described by Shepard et al. (22). Two newborn tissue samples were donated from the Department of Pathology, Children’s Orthopedic Hospital, Seattle. Various normal adult tissues, including colon, stomach, ovary, lung, skin, breast, liver, spleen, and skeletal muscle, were obtained from specimens associated with the surgical removal of tumors. In total, 65 cases of various cancer tissues were obtained from the Department of Surgery, Swedish Hospital, Seattle, and from Japan Immunoresearch Laboratories, Takasaki, Japan. Formalin-fixed, paraffin-embedded sections of normal bladder, urethra, and testis were obtained from the Department of Urology, Tohoku University School of Medicine, Sendai, Japan.

Antibodies. IgG3 monoclonal antibodies FH3 and FH4 (21) were purified from culture supernatant or from ascites fluid by affinity chromatography on a protein A-Sepharose column.

According to the definition of Moore (26), the time from 1 to 8 wk after fertilization is called the embryonic period, and 9-38 wk is called the fetal period. The terms embryo and fetus are approximately distinguished according to this definition.
column. Antibody adsorbed on protein A-Sepharose was eluted with 0.1 M citrate buffer, pH 4.2, dialyzed against phosphate-buffered saline (PBS), and stabilized by addition of 0.1% bovine serum albumin. The final concentration of antibody was adjusted to 100 \( \mu \text{g/ml} \), which was approximately equivalent to a sixfold dilution of ascites. The secondary antibody (rabbit IgG directed to mouse IgG) conjugated with horseradish peroxidase was purchased from Accurate Chemicals Co., Westbury, NY.

**Preparation of Tissue Sections.** Tissues were embedded in OCT compound (Tissue-Tek II Division, Miles Laboratories, Inc., Naperville, IL), frozen in dry ice-acetone, and stored in a Revco freezer at \(-80^\circ\text{C}\) until use. Frozen sections (4–6 \( \mu \text{m} \) thick) were prepared on a cryostat. Each section was dried on objective glass for 30 min at room temperature, fixed in acetone at \(\text{4}^\circ\text{C}\) for 10 min, and washed with PBS at \(\text{4}^\circ\text{C}\). Tissue sections (6–8 \( \mu \text{m} \) thick) were also prepared from formalin-fixed, paraffin-embedded specimens according to established procedures. Sections were deparaffinized in xylene for 5 min at \(\text{4}^\circ\text{C}\), dehydrated in ethanol, and washed with PBS. Before antibody labeling, frozen sections and paraffin-embedded sections were blocked by incubation with 15% normal rabbit serum in PBS for 1 h at room temperature.

**Immunostaining Procedure.** After blocking with normal rabbit serum, sections were incubated at room temperature with the primary antibody solution (FH3 and FH4 as prepared above) for 18 h in a moist chamber. Sections were washed three times with PBS at \(\text{4}^\circ\text{C}\) (5 min per washing). Sections were then incubated with the peroxidase-conjugated secondary antibody (diluted 1:30) for 1 h at room temperature in a moist chamber and washed three times in PBS at \(\text{4}^\circ\text{C}\) as above. Bound antibodies were detected by incubating tissue sections in 0.05 M Tris/HCl buffer, pH 7.6, containing 0.03% 3,3′-diaminobenzidine (Sigma Chemical Co., St. Louis, MO) and 0.008% hydrogen peroxide. After 10 min, the sections were washed with distilled water, counter-stained with hematoxylin, dehydrated in ethanol, washed with xylene, and mounted. Two controls were performed for each staining experiment: sections treated without the primary antibody and sections treated with normal mouse serum. Specific tissue labeling was not observed after either of the above treatments.

**Comparison of Immunostaining of Frozen Sections and Paraffin-embedded Sections.** In view of a possible deletion of glycolipid antigens during preparation of sections from paraffin-embedded specimens, staining of the antigens in these sections was carefully compared with cryostat sections from frozen samples. There were no significant differences in immunoreactivity between frozen sections and paraffin sections for both fetal and newborn specimens.

**Results**

**General Developmental Changes of Antigens Defined by FH3 and FH4 Antibodies**

Strong staining of embryonic and fetal tissue by both FH3 and FH4 antibodies was limited to gastrointestinal and urogenital epithelia, and the patterns of antibody reactivity showed dramatic changes depending on the stage of the embryo. The X determinant defined by the FH3 antibody showed a wider distribution than the multimeric X antigen defined by the FH4 antibody. Both antigens were absent or present in relatively low concentration during early embryonic development (up to 40–50 d), showed maximum expression at a specific stage of development (mostly 50–70 d), and regressed upon further differentiation and development. The antigen defined by FH3 appeared about

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2 Abbreviations used in this paper: PBS, phosphate-buffered saline (10 mM sodium phosphate buffer, pH 7.2, containing 0.9% NaCl).

3 Since the staining behavior and its change in urogenital epithelia associated with development from pronephros through metanephros are highly complex, and since there was a great deal of variation in the staining of a variety of kidney tumors, this subject will be described elsewhere.
TABLE II
Reactivities of Digestive Organs of Human Embryo with FH3 and FH4

| Antibody | Organ       | 38 | 40 | 42 | 52 | 53 | 54 | 57 | 58 | 59 | 67 | 69 | 84 | 110 | 127 | Newborn |
|----------|-------------|----|----|----|----|----|----|----|----|----|----|----|----|-----|-----|--------|
|          |             | F* | F* | P  | F  | P  | F  | P  | F  | P  | F  | P  | F  | F  | P  | P  |
| FH3      | Esophagus   | +  | -  | +  | -  | +  | -  | +  | -  | +  | -  | +  | -  | +  | -  | +  |
|          | Stomach     | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ |
|          | Small intestine | +  | -  | +  | +  | +  | -  | +  | -  | +  | -  | +  | -  | +  | -  | +  |
|          | Colon       | ±  | -  | ±  | -  | ±  | -  | ±  | -  | ±  | -  | ±  | -  | ±  | -  | ±  |
|          | Liver       | ±  | -  | ±  | -  | ±  | -  | ±  | -  | ±  | -  | ±  | -  | ±  | -  | ±  |
|          | Gall bladder | ±  | -  | ±  | -  | ±  | -  | ±  | -  | ±  | -  | ±  | -  | ±  | -  | ±  |
|          | Pancreas    | ±  | -  | ±  | -  | ±  | -  | ±  | -  | ±  | -  | ±  | -  | ±  | -  | ±  |
| FH4      | Esophagus   | -  | -  | +  | ±  | -  | -  | +  | ±  | -  | -  | +  | ±  | -  | -  | -  |
|          | Stomach     | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ |
|          | Small intestine | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  |
|          | Colon       | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  |
|          | Liver       | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  |
|          | Gall bladder | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  |
|          | Pancreas    | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  |

* Paraffin section.
† Frozen section.
‡ +, Deeply folded portion; -, superficial portion.
§ +, Chief cells; +, surface mucous cells; ++, parietal cells and other epithelial cells.
¶ Some unidentified cells are strongly positive.
** ++, Paneth's cell, basal granular cells; -, other epithelial cells.
†† ±, Cells of islets of Langerhans; -, other cells.
TABLE I
Reactivities of Circulatory, Respiratory Organs, Adrenal, Skin, and Nervous System of Human Embryo with FH3 and FH4 Antibodies

| Antibody | Organ | Stage of embryo (in gestation days) | Newborn |
|----------|-------|------------------------------------|---------|
|          |       | 58 50 52 53 54 58 60 62 64 66 69 72 | 84 100 127 |
|          | P     | F | F | P | F | P | F | F | F | P | P | F |
| FH3      | Heart, artery and vein | - | - | - | - | - | - | - | - | - | - | - |
|          | Lung airway | + | + | - | - | - | - | - | - | + | + | - |
|          | Trachea | - | - | - | - | - | - | - | - | - | - | - |
|          | Cerebrum, cerebellum and pons | - | - | - | - | - | - | - | - | - | - | - |
|          | Spinal cord | - | - | - | - | - | - | - | - | - | - | - |
|          | Adrenal | - | - | - | - | - | - | - | - | - | - | - |
|          | Spleen | + | + | + | + | + | + | + | + | + | + | + |
|          | Skin | - | - | - | - | - | - | - | - | - | - | - |
|          | Bone and Muscle | + | + | + | + | + | + | + | + | + | + | + |
| FH4      | Heart, artery and vein | - | - | - | - | - | - | - | - | - | - | - |
|          | Lung airway | - | - | - | - | - | - | - | - | - | - | - |
|          | Trachea | - | - | - | - | - | - | - | - | - | - | - |
|          | Cerebrum, cerebellum and pons | - | - | - | - | - | - | - | - | - | - | - |
|          | Spinal cord | - | - | - | - | - | - | - | - | - | - | - |
|          | Adrenal | - | - | - | - | - | - | - | - | - | - | - |
|          | Spleen | + | + | + | + | + | + | + | + | + | + | + |
|          | Skin | - | - | - | - | - | - | - | - | - | - | - |
|          | Bone and Muscle | + | + | + | + | + | + | + | + | + | + | + |

* Adrenal cortex, -; medulla, ±.
† Adrenal cortex, -; medulla, +.
‡ Entire layer of skin, +.
§ Duct of eccrine sweat gland, +; epidermis, +; corium, -.
¶ Entire layer, ±.

2 wk earlier than the antigen defined by FH4 and regressed later than that defined by FH4 or was continuously expressed after birth. Thus, the FH4 antigen was often limited to a specific type of cell in certain epithelial tissues (see below). The reactivities of the FH3 and FH4 antibodies with fetal, newborn, and adult tissues are summarized in Tables II, III, and IV.

Weak staining was also observed in the pulmobronchial epithelia, adrenal medulla, and entire layers of the epidermis by both FH3 and FH4 in embryonic stages. The reactivities of FH4 regressed completely in newborn tissues. A weak staining with FH3 remained, however, in the adrenal medulla as well as in sebaceous and sweat glands of the epidermis (Table III).

The antigens recognized by FH3 and FH4 were not found in connective tissues, nervous tissues (brain and spinal cord), tissues of the circulatory system (heart, arteries, and veins), skeletal tissue (bones and muscles), or other parenchymatous organs, such as liver and spleen, at any stage of development. Distribution of the antigen defined by FH4 in tissues of newborns and adults was limited to specific types of cells in gastric and intestinal epithelia and the antigen was completely absent in colonic epithelia.
TABLE IV
Reactivities of Human Adult Tissues with FH4 Antibody

| Tissue                 | Reactivity (number of cases) |
|------------------------|-----------------------------|
|                        | Paraffin section | Frozen section |
| Stomach                |                |                |
| Surface mucous cells   | –,±*            | ±              |
| Chief cells            | –               | –              |
| Parietal cells         | ++ (4)          | + (3)          |
| Pyrolic gland          | ++              | +              |
| Other cells            | –,+†            | ±              |
| Small intestine        |                |                |
| Paneth’s cells         | ++              | ++             |
| Basal granular cell    | ++              | ++             |
| Cuticular or brush border cells | – (2) | – (1) |
| Globlet cell           | –               | –              |
| Colon                  |                |                |
| Crypt cells            | –               | –,+†           |
| Cuticular cell         | – (4)           | – (7)          |
| Globlet cells          | –               | –              |
| Mammary gland          |                |                |
| Parenchyma and duct cells | – (3) | – (4) |
| Lung                   | – (5)           | – (4)          |
| Skin                   | – (2)           | – (1)          |
| Testis                 | – (3)           |                |
| Prostate               | – (4)           |                |
| Muscle                 | –               | – (2)          |

A single grading indicates that all cases examined showed the same degree of reactivity.
* –, Three of four cases; ±, one of four cases.
† –, Three of four cases; +, one of four cases.
‡ –, Six of seven cases; +, one of seven cases.

Distribution of Antigens Defined by FH3 and FH4 Antibodies in Gastrointestinal Tissues of the Human Embryo

The strongest expression of the antigens defined by FH3 and FH4 antibodies was found in stomach epithelia. The reactivity was intense in all cell populations of stomach epithelia at 35 d of development (Fig. 1A and B, arrow a). This intense reactivity continued up to 90 d. After 100 d, the reactivity of many stomach cell populations regressed, became limited to a deep layer at later stages (Fig. 1C, arrow a), and was finally limited to the parietal cells (Fig. 1D) and pyloric glands (data not shown) of newborn and adult gastric epithelia. The chief cells and other epithelial cells in stomach epithelia became completely negative in newborn and adult epithelia (Table IV, Fig. 1D).

Small intestinal epithelial cells were not stained by FH4 at 40 d of development (Fig. 1A, arrow b), but were strongly stained by FH3 (Fig. 1B, arrow b). At later stages, the majority of small intestine epithelial cells became negative; however, a strong reactivity with both FH3 and FH4 was observed in some unidentified cells of fetal tissue (Fig. 1E). The antigens defined by both FH3 and FH4 in the majority of cell populations regressed subsequently, becoming restricted only to
Paneth's cells and basal granular cells in the cryptic region of the newborn and adult small intestine (Fig. 1 F, arrow a; Table IV).

The reactivity of FH3 and FH4 with colonic epithelial cells showed a similar pattern. These cells were weakly positive in the early embryo, became positive and maximally expressed between 50 and 60 d (Fig. 1 H, arrow a), and subsequently regressed. At later stages, FH3 antigen expression was limited to crypt cells in the newborn and adult (Fig. 1 I, arrow a). Crypt cells in newborn and adult tissues were not stained by FH4 (data not shown). The antigen defined by FH3 was present in esophageal epithelia in the early fetus (Fig. 1 G) as well as in the newborn and adult (data not shown), but the antigen defined by FH4 was absent in esophageal epithelia throughout all stages of embryonic development examined. A similar distribution was found in the fetal pancreas; the ducal epithelium was weakly stained by FH3, but not by FH4, and became completely negative in the newborn. It is interesting to note that the antigen defined by FH3 was present in cells of Langerhans' islets, but that defined by FH4 was absent (data not shown). Neither antigen was detected in the various stages of liver development (data not shown).

**Respiratory Organs and Other Tissues**

The epithelial cells of trachea and secretory glands of bronchus were stained by FH3, but not by FH4 in newborns. No fetal tissue of bronchus was available. The epithelial cells of the lung airway found in fetal lung bud were positive with FH3, but were negative with FH4 (data not shown). The entire layer of epidermal tissue of fetal age was positive with both FH3 and FH4, but the reactivity with FH4 became negative in adult tissue. A weakly positive reaction was observed with FH3 in sebaceous glands and in eccrine sweat glands; no reactivity was observed with FH4 (data not shown). Heart, aorta, arteries, veins, cerebrum, cerebellum, pons, spinal cord, muscles, and bones were all negative with both FH3 and FH4 throughout all developmental stages (data not shown).
Distribution of the Antigen Defined by FH4 in Human Cancer in Comparison with Adult Normal Tissues

Gastric cancer. 8 out of 11 cases of paraffin-embedded sections and four out of five frozen sections of gastric cancers were strongly or clearly positive with FH4 (Table V). All the positive cases were tubular or papillary adenocarcinoma, while all the negative cases were undifferentiated adenocarcinoma (Table V). A typical positive example is shown in Fig. 1L. In normal adult gastric epithelia, only parietal cells and pyloric gland cells were consistently positive in both paraffin and frozen sections (Table IV).

Colonic cancer. Three out of three cases of paraffin-embedded sections and seven out of eight cases of frozen sections were strongly or clearly positive with FH4 (Table V). A typical section from colonic cancer is shown in Fig. 1J. Only one case was negative, which was not correlated with the histological characteristics of the case. Normal parts of colonic epithelia were all negative, including crypt cells in both paraffin and frozen sections, except for one case that showed a positive reaction in the crypt cells.

Breast cancer. Three out of four cases of both paraffin-embedded and frozen

| Tumor tissue         | Reactivity (number of cases) |
|----------------------|-----------------------------|
|                      | Paraffin section | Frozen section |
| Stomach cancer       | Adenocarcinoma       | ++ (5/11)      | ++ (2/5)       |
|                      |                   | + (5/11)       | + (2/5)       |
|                      |                   | - (4/11)       | - (1/5)       |
| Colon cancer         | Adenocarcinoma       | ++ (1/3)       | ++ (5/8)      |
|                      |                   | + (2/3)        | + (2/8)      |
|                      |                   | - (0/3)        | -            |
| Ovary                | Clear cell carcinoma | - (1/1)        |              |
|                      | Serous cystoadenocarcinoma | - (1/1)        |              |
|                      | Mucinous cystoadenocarcinoma | - (1/1)       |              |
| Testis               | Seminoma           | - (3/3)        |              |
| Breast               | Infiltrating ductal carcinoma | ++ (3/4)    | ++ (3/4)   |
|                      |                   | - (1/4)        | - (1/4)    |
|                      | Lymph node metastasis | ++ (2/2)       | ++ (2/2)   |
| Lung                 | Squamous cell carcinoma | - (2/2)       | - (4/4) |
| Gall bladder         | Adenocarcinoma       | + (1/1)        |              |
| Prostate             | Adenocarcinoma       | - (3/3)        |              |
|                      | Benign adenoma       | - (5/3)        | - (1/1)    |
| Skin                 | Malignant melanoma   | - (1/1)        | - (1/1)    |
| Muscle               | Leiomysarcoma        | - (1/1)        | - (1/1)    |
sections of infiltrating ductal carcinoma were positive. A typical case is shown in Fig. 1 K. Metastatic lesions in lymph nodes were also strongly positive (Table V).

Kidney cancer. A great deal of variation in the staining of kidney tumors was observed, which will be described elsewhere. These results may appropriately reflect the normal variation in the reactivity of FH3 and FH4 with urogenital epithelia during their development from pronephros to mesonephros to metanephros (manuscript in preparation).

Other cancers. Various types of ovarian carcinoma, seminoma, lung squamous cell carcinoma, prostate adenocarcinoma, malignant melanoma, and leiomyosarcoma were all negative.

Discussion

The reactivity of FH3 and FH4 with the developing human embryo and fetus can be summarized as follows: (a) The antigens detected by both FH3 and FH4 are most strongly expressed in the epithelial cells of the gastrointestinal and urogenital organs at specific stages of development. Expression of these antigens, particularly FH4, regressed upon further development with functional differentiation, and disappeared from most of the epithelial cell populations of those tissues with the exception of a few specific types of cells in normal adult tissue. (b) The antigen defined by FH3 appeared at an earlier stage of fetal development than the antigen defined by FH4; however, the antigen defined by FH4 regressed rapidly and completely at later stages of development and its expression became highly limited in adult epithelial tissues. The antigen defined by FH3 remained in a wider variety of cells than the antigen defined by FH4 in developed tissues. (c) In adult epithelial tissue, the antigen defined by FH4 was found to be limited to parietal cells and pyloric glands of stomach epithelia, Paneth's cells and basal granular cells of the intestine, and proximal convoluted tubules of the kidney. The antigen was not detected by FH4 in the entire colonic epithelia, including crypt cells. The crypt cells in colonic epithelia were positive with FH3. (d) A clear differential reactivity was found between the FH3 and FH4 antibodies in sebaceous and sweat glands of the epidermis, Langerhans' islet of the pancreas, adrenal medulla, esophageal epithelia, bronchial epithelia, airway of lung buds, and vaginal epithelia. Cells in these tissues were clearly or strongly stained by FH3, but were not reactive with FH4 throughout fetal development as well as in newborn and adult tissues.

The antigens defined by FH3 and FH4 may not be expressed or may be expressed weakly in preimplantation human embryos, in contrast to mouse embryos. This possibility is suggested by the absence of SSEA-1 in undifferentiated human teratocarcinoma and its appearance on differentiation (7), in contrast to a strong expression of SSEA-1 in undifferentiated mouse teratocarcinoma and its decline on differentiation (8, 12). There were no cases in which tissues were negative at fetal stages, followed by increasing expression in newborn or adult tissues.

Maximum expression of the antigens defined by FH3 and FH4 was found in the epithelia of tissues at a specific developmental stage, mostly 40–80 d. This may indicate that these structures are essential signals for cell adhesion and recognition, which could be an essential step for further differentiation of fetal
epithelial cells into a variety of functionally differentiated adult epithelial cells. Despite our lack of knowledge of a functional role of these structures, such a
dramatic change, with maximum expression at a defined stage followed by
orderly disappearance, suggests a vital function for these structures in “chemical
conversation” (23) between embryonic cells during epitheliogenesis.

Since a majority of human cancers are derived from gastrointestinal, urogen-
tal, and pulmobronchial epithelia, in which X or oligomeric X antigens are
strongly expressed at embryonic to fetal age, an intense reexpression of these
antigens in a large variety of human cancers strongly suggests that these struc-
tures are essentially oncofetal antigens. Only those cancer cells derived from
epithelial tissues that express a high level of these antigens during a certain stage
of development showed a strong reactivity with FH3 and FH4 antibodies.
Interestingly, differentiated papillary adenocarcinoma of stomach expresses the
antigen defined by FH4, whereas undifferentiated adenocarcinoma does not
express this antigen. Thus, reexpression of the structure defined by FH4 in
tumors could be associated with retrogenesis of tumor cells to a certain stage of
organogenesis rather than to a stage of the very early embryo. If retrogenesis of
cells occurs to a very early, undifferentiated stage of embryonic tissue, tumor
cells may not express the FH4 antigen (Fig. 2).

Immunostaining of both X antigen and multimeric X antigen in paraffin-
embedded sections gave similar or identical results to those from immunostaining
of frozen cryostat sections. This may indicate that (a) glycolipid antigens are not
diminished during preparation of paraffin sections, or (b) the antigens may be
carried by glycoproteins that are not diminished by preparation of paraffin
sections. Since many lacto-series carbohydrates are carried by both glycolipids
and glycoproteins (24), it is reasonable to assume that the antigens detected by
immunostaining of fetal tissue sections are, in fact, glycoproteins with properties
similar to "embryoglycan" or lactosaminoglycans (25).

Oncofetal expression of both FH3 and FH4 should be based on a common
mechanism for activation of α1 → 3 fucosyltransferase in fetal epithelial tissue
and in certain types of human cancer. The fucosyltransferase that makes FH4
antigen can be distinguished from that for synthesis of FH3 antigen, and the
 genetic regulation of these enzymes is a crucial mechanism controlling embry-
ogenesis as well as oncogenesis.

Summary

Distribution patterns of specific fucose-containing antigens having X deter-
minant (Galβ1 → 4[Fucα1 → 3]GlcNAc) as well as the di- or trimeric X
determinants (Galβ1 → 4[Fucα1 → 3]GlcNAcβ1 → 3Galβ1 → 4[Fucα1 → 3]-
GlcNAc) in the developing human embryo and fetus and in human cancer have
been examined using immunohistological techniques. Tissue sections were
stained with monoclonal antibody FH3, which defines X determinant, and with
monoclonal antibody FH4, which defines di- or trimeric X determinant. The
following general trends in the expression of the antigens defined by FH3 and
FH4 have been observed: (a) A well-organized, orderly appearance and disap-
pearance of the antigens was observed during the histogenesis of various epithelia
of gastrointestinal and other organs. The developmental stage exhibiting the
maximum antigen expression is different for each organ. (b) The X determinant defined by FH3 was expressed ~2 wk earlier than the di- or trimeric X determinant defined by FH4, and the antigen defined by FH4 regressed more rapidly and more completely than the X determinant defined by FH3 on further development of epithelial tissue. Thus, expression of the FH4 antigen is highly limited to specific types of cells in newborn and adult epithelial tissues. (c) The antigen defined by FH4 was strongly expressed in the majority of tubular and papillary adenocarcinoma of stomach, adenocarcinoma of colon, and infiltrating ductal carcinoma of breast and its metastatic lesions. No antigen was found in poorly differentiated stomach adenocarcinoma, squamous lung carcinoma, and many other types of tumors from ovary, testis, prostate, skin, and muscle. The
presence of the antigen defined by FH4 is therefore limited to carcinoma of the stomach, colon, and breast and can be regarded as a retrograde expression of the antigen to a certain stage of fetal development in which expression of this antigen was maximal.

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