ONCOLOGY

Cell Localization of Mucin-Type Receptors Assayed with Novel GalNac/Gal-Specific Lectin from Sea Mussel *Crenomytilus grayanus* in Human Colon Tumors

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Cell localization of mucin type receptors in low-differentiated human colon adenocarcinoma was studied. Several types of receptor localization were identified. It was shown that lectin CGL most intensively binds to intracellular membranes, cytoplasm, and secrete of mucin-producing tumor cells.

Key Words: *Crenomytilus grayanus sea mussel lectin; mucin-type glycoprotein; colon cancer*

Lectins are proteins, which specifically bind certain carbohydrates. Lectins specific for galactose (Gal) and N-acetyl-D-galactosamine (GalNac) are used for studying the glycoproteins localization in human colon adenocarcinoma cells. Histological staining revealed receptors to *Helix pomata* snail lectin (HPA) on cell surface and in the cytoplasm of tumor cells, but not in the nucleus and mucoid secret [4]. *Dolichos biflorus* lectin (DBA) binds to the cytoplasmic components and mucins in normal, but not tumor cells [5]. Unlike these lectins, sea mussel *Crenomytilus grayanus* lectin (CGL) used in our experiments primarily binds mucin-type glycoproteins with a great number of side carbohydrate chains with terminal Gal and GalNac [3]. The intensity of interaction depends on saturation of glycoprotein molecule with these groups. Mucin-type glycoproteins consist of protein core and O-glycoside oligosaccharide side chains. In mucins, carbohydrate chains constitute about 80% of the total molecular weight (400-1000 kD). The behavior and the role of mucin-type glycoproteins in tumor transformation remained obscure [2,7].

Studying the distribution of CGL receptors in normal and transformed cells one can reveal the role of these molecules in various cell rearrangements. Here we studied the localization of CGL receptors in human colon adenocarcinoma cells.

MATERIALS AND METHODS

Colon tumors and adjacent epithelium autopsy specimens were obtained from 12 patients with low-differentiated colon adenocarcinoma (Regional Hospital, Vladivostok). The specimens were fixed in 5% formalin and embedded in paraffin. After deparaffinization, 5-μ sections were treated with 1% H_2O_2 in methanol (blockade of endogenous peroxidase). Horseradish peroxidase-conjugated CGL were prepared as described previously [1]. The conjugate (0.02 mg/ml) was incubated for 1 h in 0.1 M phosphate buffer (pH 7.4) containing 2.5% BSA. Peroxidase labeling was visualized with diaminobenzidine tetrachloride (0.05 mg/ml in 0.1 M Tris buffer, pH 7.4) and 0.1% H_2O_2 for 15 min.

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The specificity of peroxidase reaction was verified by incubating some specimens with Gal or GalNac (4 mg/ml in 0.1 M phosphate buffer, pH 7.4) for 1 h. The specimens were poststained with Karachi hematoxylin and/or alcian blue.

RESULTS

In normal epithelial cells of human colon, CGL binding is seen in the perinuclear and apical zones of the cytoplasm (Fig. 1, *a*). The localization of CGL receptors is similar in all cells within the crypt, which indicates synchronization of their secretory apparatus. Lectin probably detects the initial and intermediary membrane-dependent stages of mucin glycosylation. At the same time, the dye is present in small vesicles dispersed throughout the cytoplasm volume, which probably reflects a short-term stage of vesicle accumulation, which then assemble into large Golgi cisterns. As the diameter of these vesicles increases, they concentrate in the apical cytoplasm over the nucleus, while their content becomes CGL-negative. CGL only slightly interacts with goblet cell secret. The intensity of CGL-positive staining reflects the state of cell secretory apparatus, which is different in normal and transformed cells.

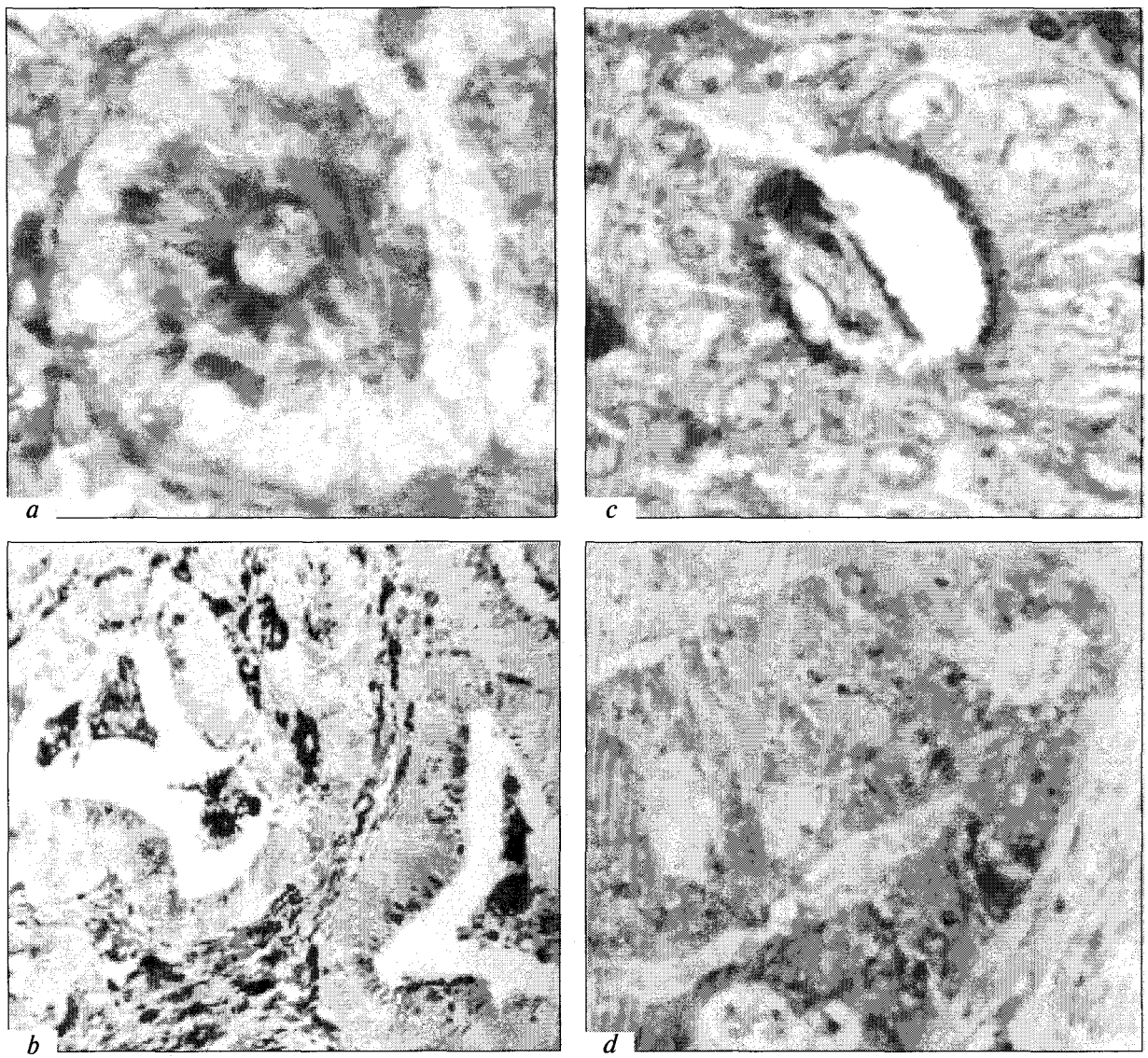

Fig. 1. Distribution of CGL receptors visualized with CGL in normal and tumor cells of human colon. *a*) basement cross-section of a Lieberkunh crypt in colonic epithelium. Diaminobenzidine tetrachloride spots in the medium (above nucleus) and apical zones, ×1000, slight hematoxylin poststaining; *b*) cross-section of glandular tubules of colon adenocarcinoma. Diaminobenzidine tetrachloride staining looks like irregular border around gland lumen, ×200, hematoxylin poststaining; *c*) cross-section of a tubular gland of colon adenocarcinoma. Diaminobenzidine tetrachloride staining looks like fine border around gland lumen, ×1000, hematoxylin poststaining; *d*) parenchyma of adenocarcinoma tissue with villous transformation. Diaminobenzidine tetrachloride fills up the cytoplasm, ×250, hematoxylin poststaining.

CHL visualizes specific apical vesicle complexes bordering the adenocarcinoma lumen (Fig. 1, *b*). These complexes usually consist of oval vesicles with CGL-positive secret in the gland lumen. This probably reflects a blockade of mucin synthesis at a late stage. The apical complex most probably passively dissociates from the cell and appeared in the adenocarcinoma lumen. Membrane vesicles can be preserved. Sometimes the apical vesicular complex looks like fine border along the apical cell edge (Fig. 1, *c*). In tissues with signs of villous transformation, sites with intensively stained cell cytoplasm an secret are seen (Fig. 1, *d*). Morphologically, these glands are characterized by higher parenchyma/stroma ratio. In poorly differentiated adenocarcinomas practically no CGL-positive staining are detected.

All types of mucin-type receptors can be classified as follows: 1) apical vesicular, 2) diffuse vesicular, 3) cytoplasmic, and 4) perinuclear. Perinuclear localization of glycoprotein responsible for CGL binding is typical of normal cell and is absent in tumor cells. Diffuse vesicular localization of CGL receptors is present in both normal and tumor cells, while apical vesicular and cytoplasmic localization is specific for villous adenocarcinoma (hyperplasia). Another characteristic feature of this tumor is aberrant mucin synthesis [6]. We assume that CGL-positive staining of tumor cells on histological sections reflects this phenomenon, since this lectin specifically interacts with secretory vesicles and their content. In normal colon epithelium CGL does not visualize goblet cells secret, while in colon adenocarcinoma it binds to the secret in tumor cells and adenocarcinoma lumen. Alcian blue intensively stains acid mucins in goblet cells but does not interacts with the content of these vesicles. This confirms our assumption on late disturbances of the processing (blockade of sialylation and glycosylation) of CGL-binding tumor mucin.

Compartments of secretory apparatus associated with mucin-type glycoproteins in adenocarcinoma cells differ from those in normal cells. Secretory granules do not form a goblet, but remained in the apical part of the cytoplasm and are gradually displaced by CGL-negative structures of secretory apparatus. This process is associated with cell dedifferentiation, since less differentiated adenocarcinomas are CGL-negative. These observation confirm our conclusion that aberrant glycoprotein glycosylation accompanies neoplastic cell transformation [2].

It can be concluded that expression of CGL receptors is not a strict sign of neoplastic transformation. The observed patchy pattern of CGL-binding sites reflects a balance between differentiation and dedifferentiation of mucin-secreting apparatus in human colon adenocarcinoma. The highest expression of CGL receptors is characteristic of mucin-producing colon adenocarcinomas.

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