ONCOLOGY

Expression of Type IV Collagen by Lewis Pulmonary Carcinoma Cells

G.P. Gaenko, S. V. Khaidukov, and Yu. G. Molotkovskii

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Study of the expression of type IV collagen by Lewis pulmonary carcinoma cells (PLMG-T, PLMG-M1, PLMG-M2) showed that these cells produce type IV collagen. The expression of type IV collagen in PLMG-T and PLMG-M2 cells approximately 2-fold surpassed that in PLMG-M1 cells. The number of active cells (synthesizing type IV collagen) in PLMG-M1 culture was 1.6 and 2.2 times lower than in PLMG-M2 and PLMG-T cultures, respectively. It was found that cell culture medium modulated expression of type IV collagen and this effect was mediated via cell receptors.

New Words: *basal membrane; type IV collagen; glycoconjugates; extracellular matrix; cellular receptors*

Type IV collagen is the main structural protein of the extracellular matrix. Previous studies demonstrated the role of extracellular matrix in the regulation of tumor growth and metastases. Presumably, type IV collagen plays an important role in tumor invasion, because the level of this protein affects the metastatic potential of transformed and malignant cells [4,6]. Phenotypical characteristics of primary tumor cell pool, including receptor status, determine the interactions of tumor cells and extracellular matrix and, eventually, clinical picture of the disease. It was found that human breast tumor cells expressing surface estrogen receptors form more encapsulated and less metastasizing tumors compared to estrogen receptor-negative cells [3].

We previously showed that three cell strains derived from primary tumor tissue and metastasis of mouse Lewis pulmonary carcinoma differed by physiological activity after subcutaneous injection in a syngeneic system and by their ligand-receptor characteristics *in vitro* [2].

M. M. Shemyakin and Yu. A. Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow

Here we investigated the effect of microenvironment on the expression of type IV collagen by Lewis pulmonary carcinoma cells.

MATERIALS AND METHODS

PLMG-T, PLMG-M1, and PLMG-M2 cell strains were derived from mouse Lewis carcinoma tumor and metastatic tissue cells [2]. The cells were cultured under standard conditions in RPMI-1640 with 7% embryonic calf serum.

Glycoconjugates on polyacrylamide matrix 6-HSO_3LacNAc (disaccharide ligand) and SiaLe^x (Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ; tetrasaccharide ligand) [1] (Syntesome GmbH, Munich) and fibronectin (80 μg/ml) and antibodies (Imtek) were used in the study.

Glycoconjugate (0.3 mg/ml normal saline) and fibronectin were added to the culture medium (1/10 of the total volume).

Type IV collagen in the intracellular matrix was detected by immunohistochemical staining. To this end, the cells were cultured on a fibronectin feeder. Sterile fibronectin solution was applied onto degreased

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sterile slide and dried for 24 h at 18-20°C. The glass was washed in nutrient medium, placed into a Petri dish (*d*=3 cm), after which cell suspension in culture medium (2 ml) was added and incubated until the needed amount of cells was obtained. The slide with cells was washed from the medium with a buffer, fixed with acetone for 5 min, and washed in distilled water. Rabbit antibodies to type IV collagen (RIH C44, 0.1 mg/ml normal saline, Imtek) diluted 100-fold were layered onto fixed cells and incubated for 1 h at 18-20°C, after which the slides were washed tree times in phosphate buffer and dried on air at 18-20°C. Then the cells were incubated with goat antibodies to rabbit immunoglobulins (Amersham, No. 2034) labeled with Texas Red, washed in distilled water, dried, and examined under a microscope (Amplival).

Immunochemical detection of type IV collagen on cell surface was carried out as described previously [7]. The cells were harvested with 0.2% EDTA, washed twice in 0.2% BSA in phosphate buffer. Aliquots of cell suspensions were incubated with antibodies (RIH C44) in a concentration of 20 μg/ml (30 min at 18-20°C), washed twice in BSA solution, and incubated with FITC-labeled goat antibodies to rabbit immunoglobulins (f-GARI, Imtek) for 30 min at 18-20°C, after which they were washed twice in BSA solution, suspended in 0.5 ml phosphate buffer, and the fluorescence was measured on an EPICS ELITE flow cytofluorometer (Coulter Electronics). The mean fluorescence intensity (at least 5000 cells) was evaluated in arbitrary units (Multy Graph, IMMUNO, Coulter Electronics).

RESULTS

Cells of Lewis pulmonary carcinoma and metastasis expressed type IV collagen (Fig. 1), but the intensity of this synthesis in PLMG-M2 and PLMG-T cells almost 2-fold surpassed that in PLMG-M1 cells. The number of cells with type IV collagen on the surface

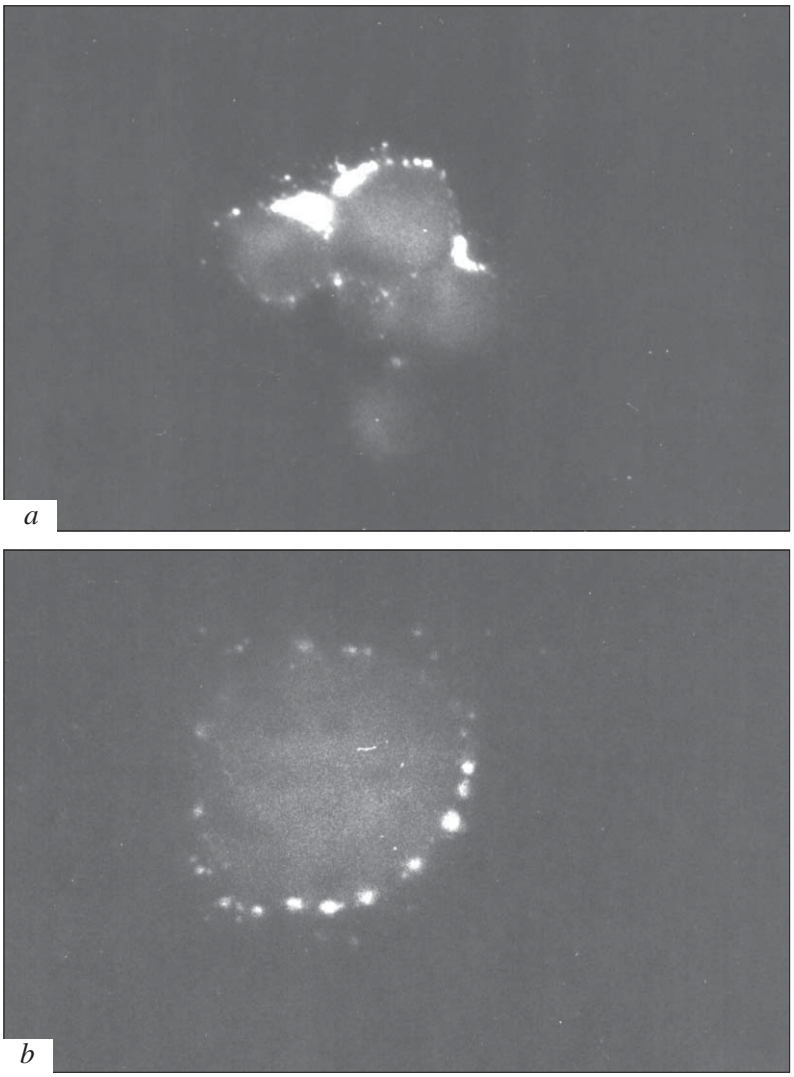

Fig. 1. Type IV collagen on the surface of Lewis pulmonary carcinoma cells; Texas Red staining, ×500 (*a*); ×1250 (*b*).

TABLE 1. Expression of Type IV Collagen by Lewis Pulmonary Carcinoma Cells under Different Conditions of Incubation

Cell strain	RPMI without additives	Added		
		fibronectin	6-HSO_3LacNAc	$SiaLe^x$
PLMG-T	2.0/42.0	1.9/8.5	2.3/24.5	4.0/60.0
PLMG-M1	0.9/19.0	0.9/44.0	2.4/80.0	0.9/44.0
PLMG-M2	1.8/31.0	1.8/49.0	2.2/30.0	1.8/30.0

Note. Numerator: fluorescence (in arb. units); denominator: percentage of active cells.

in PLMG-M2 and PLMG-T cultures 1.6- and 2.2-fold surpassed that in PLMG-M1 culture (Table 1).

Cell receptors mediate the effects of microenvironment on the expression of genes encoding protein synthesis [3]. For example, the growth of human mammary carcinoma cells in collagen matrix is paralleled by the formation of invasions, in contrast to growth in agarose matrix. The growth of these cells in agarose-collagen matrix was also invasive. The formation of invasion is determined by interactions of adhesion receptors with matrix structures and induction of protease expression [3].

The effect of culture medium on the synthesis of extracellular matrix and the role of cell receptors in this process were studied using glycoconjugate with 6-HSO_3LacNAc determinant exhibiting affinity for PLMG-T cell lectins and $SiaLe^x$ determinant exhibiting affinity for receptors of PLMG-M1 and PLMG-M2 cells [2] and fibronectin as a basal membrane structural protein.

Addition of fibronectin, 6-HSO_3LacNAc, and $SiaLe^x$ to PLMG-M1 cells increased the number of active cells in the culture: fibronectin and $SiaLe^x$ increased number of active cells by almost 2-fold, while 6-HSO_3LacNAc by more than 4-fold. Addition of 6-HSO_3LacNAc into the medium increased collagen synthesis by 167%, while addition of $SiaLe^x$ and fibronectin did not change the mean level of fluorescence on the cell surface. Addition of conjugate with carbohydrate determinant $SiaLe^x$ to PLMG-T culture 1.4-fold increased the number of active cells and stimulated production of type IV collagen (by 100%). In the presence of fibronectin the number of PLMG-T cells producing type IV collagen decreased almost 5-fold and addition of 6-HSO_3LacNAc decreased it 1.7-fold, though the mean level of type IV collagen expression did not change in comparison with cells cultured under standard conditions. Presumably, the decrease in the number of cells carrying type IV collagen on their surface is a result of modulated expression of genes encoding synthesis of proteases destroying collagen in the extracellular matrix [3]. Modification of PLMG-M2 cell culture medium by fibronectin, 6-HSO_3LacNAc, or $SiaLe^x$ virtually did not modulate the synthesis of type IV collagen and the number of active cells, though a slight (58%) increase in the number of active cells in the medium was observed after addition of fibronectin.

These findings suggest that culture medium components acting as ligands for cell lectins can modulate expression of type IV collagen. The regulation of the synthesis of extracellular matrix implies the possibility of purposeful suppression of malignant growth. M. Nakajima *et al.* stated that collagenase-suppressed rat mammary adenocarcinoma cells were encapsulated in the basal membrane and lost the capacity to metastasize. Resumption of collagenase synthesis and degradation of the extracellular matrix restored the initial metastatic potential of these cells [6].

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