Institute of Experimental Morphology, Pathology and Anthropology with Museum
Bulgarian Anatomical Society

Acta morphologica et anthropologica, 20
Sofia • 2014

Expression of carbohydrate-binding proteins in culture medium from MCF-7 cells treated with metal complexes of the cholic acid

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Abstract: Levels of N-Ac-ß-D-mannosamine-binding proteins were higher in the culture medium from MCF-7 (human adenocarcinoma cells) treated with $Co(Chol)_2.2H_2O$ and $La(Chol)_3\ 2H_2O$ as compared their levels in the culture medium from non-treated cells. We also found higher levels of D-galactosamine- mannose- and galactose-binding proteins in culture media from tumor cells treated with $Cu(Chol)_2.4H_2O$, $Co(Chol)_2.2H_2O$ and $La(Chol)_3\ 2H_2O$, as compared to their levels in the culture media from non-treated cells. D-glucosamine-binding proteins were down-regulated after treatment with all of the metal complexes. Treatment of MCF-7 with $La(Chol)_3\ 2H_2O$ led to higher levels of N-Ac-D-glucosamine- and D-mannosamine-binding proteins in the culture medium, compared to non-treated cell. D-fucose-binding proteins were up-regulated in cell culture treated with $Co(Chol)_2.2H_2O$ and $La(Chol)_3\ 2H_2O$, as compared to their expression in non-treated cells.

Key words: carbohydrate-binding proteins, MCF-7 adenocarcinoma, metal complexes.

Introduction

Carbohydrate-binding proteins (CBPs) play important role in the processes of malignant transformation and metastasis in a variety of tumor cells. Qualitative and quantitative changes in the expression of intracellular and cell surface galectins have been correlated with transformation and metastasis of tumor cells [7]. Carbohydrate-binding proteins with specificities other than galactose are expressed in many tumor cells. However their role in tumor cell biology is not as clear as the role of galectins. Fucose-binding proteins are expressed in rhabdomyosarcomas [2] and human epithelial tumor [3]. Liver metastases of three other types of primary tumors showed a tendency towards preferential expression of additional fucose-binding proteins [6]. Secretion of these carbohydrate-binding proteins was not followed up, but such secreted receptor could participate in

cell adhesion phenomena through binding to terminal fucose residues on the complex type N-linked glycans. Mannose–binding proteins were found in human teratocarcinoma cells [4]. Specific anti-carbohydrate immunotherapy of Guerin tumor cells correlate strongly with their proliferation index [1]. Metastatic lesions to lung from three different types of primary tumors revealed tumor-associated mannan-binding proteins [6]. Spontaneous strongly metastatic variants (ESb) of a murine lymphoma contained additional sugar receptors for N-acetylglucosamine. In another model system derived from the murine mastocytoma cell line P815×2A, biochemical analysis of the liver-metastasizing variant P815×2B revealed additional characteristic acetylgalactosamine- and maltose-specific binding proteins [5].

Materials and Methods

Synthesis of metal complexes of cholic acid: A solutions of 10 ml containing 0.5 mM of $Cu(CH_3COO)_2\,H_2O$, $Co(NO_3)\,6H_2O$, $La(NO_3)\,6H_2O$ were added to 10 ml 1 mM solution of sodium cholate ($C_{24}H_{39}O_5Na$). The resulting mixture was stirred and heated for 1 hour. Formed precipitates were filtered, washed with water and dried over P_4O_{10}.

Culturing and treatment of MCF-7 cells: MCF-7 (human breast adenocarcinoma) cells were routinely grown as monolayer cultures in a combination of E-199 and Iscove's modified Dulbecco's medium (IMDM) supplemented with 10% fetal calf serum, penicillin (100 U/ml) and streptomycin (100 mg/ml). The culture was maintained at 37 °C, 5% CO_2 in a humidified atmosphere. At the 24th h cells from monolayers were washed and covered with media modified with 100 mg/ml of the compound examined.

Haemagglutination experiments: Agglutination assays were done in microtitter U plates using serial two-fold dilutions of cell culture samples. For sugar inhibition studies, 1 M of the corresponding sugars were added in place of the 0.15 M NaCl and preincubated with the lectin source for 30 min at room temperature.

Results

N-Ac-β–D-mannosamine-binding proteins: Levels of N-Ac-β–D-mannosamine-binding protein were of the same order in MCF-7 cells treated $Co(Col)_2.2H_2O$ and non-treated cells. The highest levels of expression were found in the culture medium from MCF-7 cells treated with $Cu(Chol)_2.4H_2O$, whereas tumor cells treated with $La(Chol)_3\,2H_2O$ complex expressed an intermediate levels of N-Ac-β–D-mannosamine-binding proteins, see Fig 1.

D-galactosamine-binding proteins: CBPs with specific to D-galactosamine in treated MCF-7 cells were upregulated compared to non-treated cells, see Fig. 1. Among treated cells the ones treated with $La(Chol)_3\,2H_2O$ secreted the highest levels of D-galactosamine CBP in the culture medium.

D-mannose-binding proteins: We found higher levels of D-mannose CBPs in treated cells compared to non-treated ones, see Fig. 1. Tumor cells treated with $La(Chol)_3\,2H_2O$ complex had the highest levels of D-mannose-binding proteins in their culture media when compared to levels of these CBPs in culture medium from tumor cells treated with $Cu(Chol)_2.4H_2O$, $Co(Chol)_2.2H_2O$ complexes.

D-glucosamine-binding proteins: We found that levels of D-glucosamine specific carbohydrate-binding proteins were lower in culture media from MCF-7 cells treated with $Cu(Chol)_2.4H_2O$ and $La(Chol)_3\,2H_2O$, as compared to the levels of these CBP in culture medium from tumor cell treated with the $Co(Chol)_2.2H_2O$ complex, see Fig. 1. Culture media from tumor cells treated with $Co(Chol)_2.2H_2O$ and $La(Chol)_3\,2H_2O$ had lower levels of D-glucosamine-binding proteins, as compared to their levels in non-treated cells.

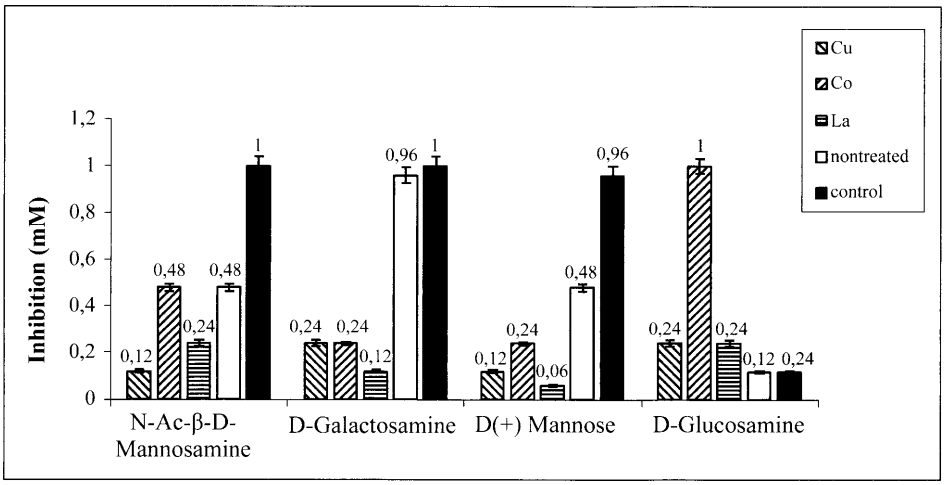

Fig. 1. Inhibition of haemagglutination with N-Ac-β-D-mannosamine, D-galactosamine, mannose and D-glucosamine between rat erythrocytes and culture media from MCF-7 breast adenocarcinoma cells treated with 100 μg/ml $Cu(Chol)_2.4H_2O$ (Cu), $Co(Chol)_2.2H_2O$ (Co) and $La(Chol)_3\,2H_2O$ (La) complexes of the cholic acid. Culture media from non-treated cells (nontreated), only culture media (control).

N-Ac-D-glucosamine-binding proteins: N-Ac-D-glucosamine CBPs were upregulated in treated MCF-7, as compared to their levels in the control, see Fig. 2. Culture media from MCF-7 tumor cells treated with 100 μg/ml $La(Chol)_3\,2H_2O$ had higher levels of this carbohydrate-binding protein, as compared to tumor cells treated with $Cu(Chol)_2.4H_2O$ and $Co(Col)_2.2H_2O$.

Galactose-binding proteins: We found that the levels of carbohydrate-binding proteins (CBPs) with specificity towards galactose were higher in culture media from MCF-7 cells treated with 100 μg/ml $Cu(Chol)_2.4H_2O$, $Co(Chol)_2.2H_2O$ and $La(Chol)_3\,2H_2O$, as compared to their levels in culture medium from cells without treatment, see Fig. 2. The highest levels of expression of this CBP were detected in the tumor cell treated with $Cu(Chol)_2.4H_2O$.

Fucose-binding proteins: D-fucose CBPs were upregulated in treated cells, compared to non-treated, see Fig. 2. MCF-7 cells treated with $La(Chol)_3\,2H_2O$ had the highest secretion of fucose-binding proteins in the culture media.

Mannosamine-binding proteins: Mannosamine-specific carbohydrate-binding proteins were found to be down regulated in MCF-7 cells treated with 100 μg/ml $Cu(Chol)_2.4H_2O$, $Co(Chol)_2.2H_2O$, as compared to the levels of these CBPs in non-treated cells, see Fig. 2. $La(Chol)_3\,2H_2O$ treated cells had the highest expression of mannosamine-binding proteins.

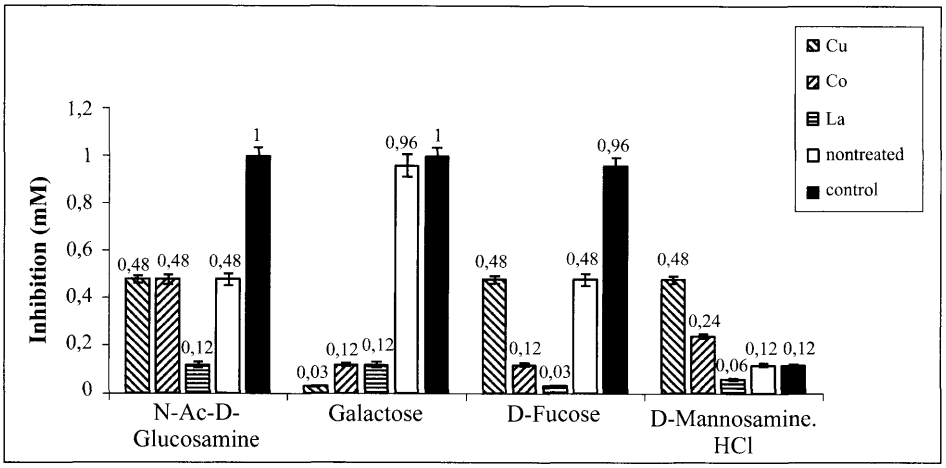

Fig. 2. Inhibition of haemagglutination with N-Ac-D-glucosamine, galactose, D-fucose and D-mannosamine. HCl between rat erythrocytes and culture media from MCF-7 breast adenocarcinoma cells treated with 100 μg/ml $Cu(Chol)_2.4H_2O$ (Cu), $Co(Chol)_2.2H_2O$ (Co) and $La(Chol)_3$ $2H_2O$ (La) complexes of the cholic acid. Culture media from non-treated cells (nontreated), only culture media (control)

Discussion

Carbohydrate-binding proteins (CBPs) are usually up-regulated in many tumor cell lines. CBPs are involved in biological events concerning tumor behavior such as homo- and heterotypic cell adhesions and adhesion to the extracellular matrix (ECM). The aim of our study was to evaluate expression and secretion of carbohydrate-binding proteins in culture media from MCF-7 adenocarcinoma cells treated with metal complexes of the cholic acid. To our knowledge there are no data on the influence of cholic acid and its metal complexes on expression of tumor associated carbohydrate-binding proteins.

We followed up expression of carbohydare-binding proteins with different specificities in Guerin tumor cells treated with metal complexes of the cholic acid. For N-Ac-β–D-mannosamine-binding proteins we found that $Cu(Chol)_2.4H_2O$ complex is most potent effector for upregulation of these proteins. On the other hand D-galactosamine-binding proteins were upregulated after treatment with $La(Chol)_3$ $2H_2O$ complex. Similar to D-galactosamine-binding proteins, D-mannose-binding proteins were also upregulated after treatment with $La(Chol)_3$ $2H_2O$ complex. Levels of expression of D-glucosamine-binding proteins were higher after treatment with $Co(Chol)_2.2H_2O$ complex. Upregulation of N-Ac-D-glucosamine-binding proteins was also observed after treatment of tumor cells with $La(Chol)_3$ $2H_2O$ complex. Expression of Galactose-binding proteins, on the other hand, was affected by all three of the investigated metal complexes. Treatment with $La(Chol)_3$ $2H_2O$ complex of cholic acid led to upregulation of Fucose-binding proteins and Mannosamine-binding proteins.

In conclusion we can say that $La(Chol)_3$ $2H_2O$ complex of cholic acid is the most potent effector leading to upregulation of five of all 8 investigated carbohydrate-binding proteins. Following-up expression of CBPs can be helpful to assess treatment of tumor cells. Combined with measuring of the proliferation index expression of these proteins can be used to follow effectiveness of anti-cancer drugs.

Acknowledgements. This study was partially supported by Grant CC 1402/2004, National Scientific Council, Ministry of Education and Science, Bulgaria.

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