A COMPARATIVE STUDY OF LAMPs AND YKL-40 TISSUE EXPRESSION
IN GLIAL TUMORS

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ABSTRACT

INTRODUCTION: YKL-40 is a glycoprotein believed potentially to be a marker of various pathological processes. High levels of YKL-40 have been found in cancer and chronic inflammatory diseases. The function of the glycoprotein is not completely known yet. A possible involvement in angiogenesis and tumor aggressiveness is supposed. Lysosome-associated membrane glycoproteins (LAMP) 1 and 2 are highly conserved proteins with still undefined biological functions. There is evidence that they are implicated in autophagy, angiogenesis and tissue remodeling. AIM: The aim of the present study was to investigate the potential relationship between the tissue expression of YKL-40, LAMP-1 and LAMP-2 in glial tumors. MATERIAL AND METHODS: LAMPS were found on cell membranes of glial and endothelial cells, while YKL-40 was detected in the cytoplasm of these cells. Intensive immunohistochemical reaction was present in tumor cells. LAMP-2 showed a more intensive staining compared to LAMP-1. RESULTS: LAMPS were found on cell membranes of glial and endothelial cells, while YKL-40 was detected in the cytoplasm of these cells. Intensive immunohistochemical reaction was present in tumor cells. LAMP-2 showed a more intensive staining compared to LAMP-1. CONCLUSION: We present the first comparative study of YKL-40 and LAMPS in astroglial tumors. The relationship between the expression of the three glycoconjugates indicates a possible participation in the processes of angiogenesis and tissue remodeling during tumor development.

Key words: YKL-40, LAMPS, glial tumors

REZIOME

ВВЕДЕНИЕ: YKL-40 представляет собой гликопротеин, который считается потенциальным маркером при патологических процессах. Высокие уровни YKL-40 установлены при ряде опухолевых и хронических заболеваний. Несмотря на то, что конкретные функции гликопротеина все еще неизвестны, предполагается его участие в ангиогенезе и в опухолевой агрессивности. Лизосомно-ассоциированные мембранные протеины (LAMPS) 1 и 2 представляют высокую консервативные белки с дискутабельными функциями. Имеется доказательства об их участии в процессах как аутофагия, ангиогенез и тканевое ремоделирование. ЦЕЛЬ: Исследовать потенциальную связь между тканевой экспрессией YKL-40, LAMP-1 и LAMP-2 при глиальных опухолях. МАТЕРИАЛ И МЕТОДЫ: Экспрессия LAMPS и YKL-40 детектирована через иммуногистохимический анализ в 36 глиальных опухолях. Проведены морфометрическое исследование и анализ тканевой экспрессии YKL-40 с помощью компьютерной системы Quick-patho Micro 2.3. Вычислены площадь (мм2), периметр (мм) и экспрессия (%) трех гликопротеинов. РЕЗУЛЬТАТЫ: Лизосомные белки позитивируются на клеточную мембрану глиальных и эндотелиальных клеток, что в то время как YKL-40 локализован в их цитоплазме. Интенсивная иммуногистохимическая реактивность наблюдается в опухолевых клетках. При LAMP-2 отмечается более демонстративная экспрессия по сравнению с LAMP-1. ЗАКЛЮЧЕНИЕ: Впервые представлены данные о сравнительном иммуногистохимическом исследовании YKL-40, LAMP-1 и LAMP-2 при глиальных опухолях. Сходство между экспрессией этих белков предполагает их участие в процессах ангиогенеза и в процессах тканевого ремоделирования во время опухолевого развития.

Ключевые слова: YKL-40, LAMPS, глиальные опухоли

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Folia Medica 2014; 56(3): 194-198
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doi: 10.2478/folmed-2014-0028
INTRODUCTION

Gliomas are highly malignant and aggressive tumors that extend far from the primary tumor site. They are characterized by rapid expansion, invasion of adjacent central nervous system tissues and abnormal vascularization.1

It is extremely important to find sensitive and specific markers that can provide valuable information for early diagnosis and identification of poorly responsive tumors.

YKL-40 is a novel potential biomarker of tumor and inflammatory processes, which is expressed and secreted by activated macrophages and neutrophils.2

Genetic analysis showed that YKL-40 gene is over-expressed in high-grade gliomas.3 The corresponding protein belongs to the family of chitinase-like proteins but due to a mutation of glutamic acid to leucine, YKL-40 lacks chitinase activity.4 Although the function of YKL-40 is not well characterized, several studies have demonstrated that this glycoprotein participates in extracellular tissue remodeling, migration, angiogenesis and inflammation.5

Lysosomal-membrane-associated proteins (LAMPs) 1 and 2 are highly conserved in sequence across species.6 Despite their similar structure and localization, LAMP-1 and LAMP-2 have distinctly separate functions.7 The biological role of these proteins is not entirely studied. Some authors demonstrated their involvement in processes associated with apoptosis, tissue remodeling and vascularization.8,9

This is the first study aiming to search for a correlation between YKL-40 and LAMPs expression in the tumor site of malignant gliomas.

PATIENTS AND METHODS

PATIENTS

The study included 36 patients newly diagnosed preoperatively with high-grade gliomas. The median age for anaplastic astrocytoma patients was 60.4 ± 6.42 yrs and for glioblastoma multiforme (GBM) patients - 57.93 ± 19.02 yrs. Symptoms of the disease preceded hospitalization by an average of 3.8 weeks.

The patients had surgeries to remove the tumors at the Department of Neurosurgery of St George University Hospital. No prior chemotherapy or radiotherapy had been performed. A microsurgical resection of the tumor lesion was carried out. In 91% of cases total or subtotal lesionectomy was performed. Histologically, 14 patients were diagnosed with astrocytoma grade IV (glioblastoma multiforme, GBM) and 22 patients - with astrocytoma grade III (anaplastic astrocytomas) according to WHO grading.

Brain tissue was collected from all patients during surgical removal of the tumor. Tumor tissue was fixed for 6-12 hrs in neutral formalin and then embedded in paraffin. Paraffin sections were used for routine histopathological examination and for immunohistochemical analysis.

The study was approved by the University Ethics Committee (protocol №3/ 25.11.2009) in accordance with the Helsinki Declaration. Informed consent was signed by all patients.

METHODS

IMMUNOHISTOCHEMISTRY

Immunohistochemistry was performed on formalin-fixed, paraffin-embedded tissue samples after antigen retrieval. Goat anti-human chitinase 3-like antibody (R&D Systems, Minneapolis) and monoclonal antibodies to human LAMPs (H4A3 and H4A4; Developmental Studies Hybridoma Bank, University of Iowa) were used as primary antibodies.

The Avidin-Biotin system was applied (Vectorstain Elite ABC Kit, Vector Laboratories, Burlingame, CA). The final reaction was demonstrated by freshly prepared DAB as a chromogen. Nuclei were counterstained with Mayer’s hematoxylin.

MORPHOMETRIC ANALYSIS

Morphometric analysis of the intensity of tissue expression was performed using the Quick-photo Micro 2.3 system. Area (μm), perimeter (μm), and expression level (%) of the three glycoconjugates were calculated. Cells with strong granular cytoplasmic YKL-40 and diffuse membrane LAMPs staining were scored. Only tumor tissues without necrotic areas were examined. Ten consecutive fields were studied for each sample by two independent observers.

STATISTICAL ANALYSIS

Pearson correlation coefficient was calculated to evaluate correlation between studied variables. Statistical analysis was carried out with the SPSS v 17.0 statistical software. All P-values were two-tailed.

RESULTS

The three glycoproteins studied showed similar intensity of the immunohistochemical reaction but different cellular localization. LAMPs were found on cell plasma membranes, while YKL-40 was detected in the cytoplasm.
YKL-40 Expression
YKL-40 was found in endothelial cells with positive reactivity in places characterized by neoangiogenesis. The most intensive immunohistochemical reaction was detected in tumor cells. Large tumor zones were stained for YKL-40. A diffuse cytoplasmic immunoreactivity was determined. The intensity of YKL-40 staining (Fig. 1A) and expression level (%) (Fig. 2) is presented.

LAMPs Expression
LAMPs were found in endothelial cells with high positivity in zones with intensive angiogenesis. Large areas of positive malignant cells were labeled in the sites where the most prominent immunohistochemical reaction was detected. LAMP-2 showed a higher staining intensity compared to LAMP-1 (Fig. 1). The data from the morphometric analysis confirmed the microscopic examination (Fig. 2).

(A) Expression of YKL-40 protein in endothelial cells (arrow) and intensive positive immunoperoxidase reaction in large areas of glial cells was observed; (B) LAMP-1 expression in endothelial cells (arrow) of high-grade glioma; Numerous malignant cells were labeled with intensive immunoreactivity; (C) LAMP-2 expression in the endothelial cells (arrow) and positive detection in glial tumor cells.

Figure 1. Immunohistochemical staining of YKL-40 and LAMPs in endothelial and tumor cells in gliomas. Biotin-streptavidin peroxidase reaction.

DISCUSSION
The short survival of patients with high-grade gliomas is a consequence of the limited effects of chemotherapy and radiation therapy combined with a very aggressive behaviour of these tumors. In the present study the immunohistochemical expression and distribution of LAMPs and YKL-40 in malignant glioma is investigated.

The lysosomal cell death pathway is a novel concept in which lysosomal membrane permeabilization participates in the induction of cell death, using death pathways still functional in cancer cells. Some researchers reported that LAMP-1 was found on the cell surface of highly metastatic tumor cells. Metastatic colon cancer cells showed high expression of LAMP-1 compared to cancer cells with low metastatic potential, which is an indication for a role of LAMP-1 in cell adhesion and migration.

Killing of glioma cells surpassing apoptotic pathways has previously been described in a survey using parovirus H-1. We detected that LAMPs were expressed with high positivity in endothelial cells in the sites with active neoangiogenesis. Our results confirmed the idea that LAMPs could be involved in tumor progression by facilitating neoangiogenesis. This statement is in accordance with data that the lysosomal proteases – cathepsins are associated with angiogenesis in tumor development. It was revealed that these extracellularly released enzymes correlated with worse prognosis in glioblastomas. It has been suggested that the...
cathepsins might play a dual role being involved in both tumor progression and in the lysosomal cell death pathway.\textsuperscript{11}

Recent studies determined elevated LAMP-1 protein expression in glioblastomas compared to diffuse and anaplastic astrocytomas. It was supposed that the high amount of lysosomes in glioblastomas made these tumors very sensitive to drugs targeting lysosomes.\textsuperscript{14}

On the other hand, YKL-40 is a promising marker in breast, ovary and colon cancer. Increased concentrations are associated with disease progression, histopathological type and survival time.\textsuperscript{15-18} YKL-40 expression has been demonstrated by some researchers to have no prognostic value in breast cancer.\textsuperscript{19} The exact cellular source of YKL-40 in tumor lesions has not yet been identified. Some authors have found that YKL-40 is produced by peritumoral macrophages in small-cell lung cancer and in glioblastomas.\textsuperscript{20,21} In inflammatory diseases, the glycoprotein is generated by activated macrophages and neutrophils.\textsuperscript{22} In contrast, Horbinski et al. showed that YKL-40 was directly produced by neoplastic glial cells.\textsuperscript{23}

YKL-40 could facilitate migration of endothelial cells and tubule formation. In some research YKL-40 angiogenic capability on endothelial cells was found to be was identical to that of VEGF.\textsuperscript{24} This is confirmed by the fact that the levels of neovascularization under the influence of YKL-40 in some tumors are greater than those in control tumors, suggesting that YKL-40 promotes vessel formation and tumor growth.\textsuperscript{24} Such angiogenic properties of the glycoprotein have also been demonstrated in glioblastomas.\textsuperscript{5}

In the present study, we examined the immunohistochemical expression of YKL-40 in parallel with LAMPs in order to provide a local and particular evaluation of its expression in gliomas. We found that the three glycoproteins were expressed by endothelial cells in tumor samples indicating potential involvement in vascularization of tumors.

Our results indicate that YKL-40 protein expression in the tumor site parallels with the malignancy grade of tumors, suggesting a potential role of the protein in neoplastic development. Our data are in accordance with other investigations concerning the role of YKL-40 in breast and gastric cancers.\textsuperscript{24,25} Recent studies reported that the high expression of the glycoprotein promoted proliferation, angiogenesis and malignancy via the phosphorylated-Akt signaling pathway.\textsuperscript{26} This pathway is involved in high-grade malignancy.\textsuperscript{5}

It was found that YKL-40 modulated glioma cell invasion through actin cytoskeleton rearrangement and MMP-2 expression. Ku et al. suggested that the glycoprotein was required for malignant transformation and local invasiveness in gliomas.\textsuperscript{27} Farbish et al. demonstrated that a monoclonal antibody against YKL-40 inhibited \textit{in vitro} and \textit{in vivo} tumor growth and angiogenesis of brain tumors. It could be speculated that YKL-40 might be a potential therapeutic molecular target for gliomas.\textsuperscript{28}

Some researchers reported that anaplastic oligodendrogliaomas and glioblastomas could be distinguished through immunohistochemical detection of YKL-40.\textsuperscript{29} They argued in their study that YKL-40 staining provided a better histologic diagnosis. Our investigation supposed that the immunohistochemical expression of the glycoprotein could support the diagnostic procedure.

Glioblastomas are characterized with intensive hypoxia which is supported by a higher amount of lysosomes in the tumor zones. An increased pattern of LAMP-1 expression was found in glioblastoma tumor stem cell containing spheroids in hypoxia compared to normoxia.\textsuperscript{15} A significant increase of YKL-40 levels in glioblastoma cell line U87 in condition of hypoxia and ionizing radiation was measured.\textsuperscript{2}

We determined the relationship between the expressions of YKL-40 and LAMPs in glial tumors indicating a possible participation in the processes of angiogenesis and tissue remodeling during tumor development.

CONCLUSIONS

We present the first parallel investigation on YKL-40 and LAMPs expression at the cellular level in human high-grade gliomas. The overexpression of the three glycoproteins might be an important feature of malignant glioma suggesting crosslinking of pathogenetic routes involved in the development of these tumors.

ACKNOWLEDGEMENTS

The study is supported by grant DP – 08/2012 from Medical University - Plovdiv and in part by grant DUNK-01-2/2009 from the Bulgarian Ministry of Education and Science.

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