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EXPRESSION OF CYTOSKELETAL PROTEINS IN GLIOBLASTOMA CELL LINE

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EXPRESSION OF CYTOSKELETAL PROTEINS IN GLIOBLASTOMA CELL LINE (Abstract): The overexpression of vimentin is linked to tumors with low differentiation, metastatic diseases, and reduced overall survival. The precise function of transgelin, an actin-binding protein, in the development of cancer is still debatable. Nevertheless, its participation in the mobility, differentiation and programmed cell death of malignant cells has been well-established. **Material and methods**: To investigate transgelin and transgelin expression, we used glioblastoma U87 cell line 2D culture. We conducted immunofluorescence staining to examine cultured cells. **Results**: the studied proteins were co-expressed in the studied cell line. **Conclusions**: How transgelin is expressed in cancerous cells is similar to the expression of vimentin. This similarity could suggest that transgelin may serve as an indicator of the level of aggression in cancer. **Keywords**: VIMENTIN, TRANSGELIN, CANCER, CELL CULTURE.

Glioblastoma is the most common brain tumor, with a poor prognosis and survival rate. Despite the complex approach, including surgery, radiotherapy and adjuvant chemotherapy, most of the patients develop relapse of the disease in the first ten months after initial treatment (1, 2, 3).

Vimentin is a type III filament characteristic of mesenchymal cells coded at the level of chromosome 10p13 (4). In the adult body, it is found exclusively in specific cell types, namely fibroblasts, glial cells, endothelial cells and lymphocytes. Its roles include participation in the maintenance of focal adhesions, potentiation of lamellipodia formation through CARMIL2, and migration through interaction with actin (5, 6). Although they do not seem to be directly involved in cell motility, numerous studies have demonstrated that some intermediate filaments of the cytoskeleton, such as vimentin, affects the epithelial to mesenchymal transition (EMT), as well as on neoplastic cell invasion and migration (7- 9).

In neoplastic cells, vimentin seems to potentiate their migratory character by influencing cytoskeleton dynamic in EMT; its overexpression is correlated to poorly differentiated tumors metastatic disease and shorter overall survival (10 -12).

Originally named SM22alpha, trans-

gelin belongs to the calponin family, being an actin binding protein with multiple roles in cell dynamic and cell cycle. In the embryonic stage, the functions of transgelin are podosome formation and smooth muscle differentiation. In the adult, transgelin is found in the cytoplasm of fibroblasts and some epithelial cells, having a role in the regulation of smooth muscle fiber contraction, cell migration in response to inflammation, the formation of podosomes in smooth muscle cells and the reorganization of the cytoskeleton during cell differentiation (13, 14).

In the context of carcinogenesis, the role of transgelin is controversial, although its participation in the processes of migration, differentiation and apoptosis of neoplastic cells is known(15). Data from previous research cannot establish a pattern of transgelin's expression in cancer; studies performed on tissue samples and cell lines exert contradictory results, as transgelin being overexpressed in colorectal (16), lung (17) and hepatocellular cancer (18).

In other ways, there are some evidence of transgelin being under expressed in prostate(19), esophageal (20) and cervical cancer (21) .

The role of transgelin as a tumor marker cannot be discussed as long as it may found in significant amounts in normal tissue. To fully comprehend the role of transgelin in the spread of cancer, its function must be studied thoroughly. This will allow for an evaluation of its potential role in determining the prognosis of the disease. The exact mechanism through which transgelin operates on cancer cells remains unclear, but it is believed that it results from a modification in the expression of genes that regulate cell mobility (22).

MATERIAL AND METHODS

We investigate vimentin and transgelin co-expression in glioblastoma U87 cell line.

Cell cultures

To investigate transgelin and transgelin expression, we used a glioblastoma U87 cell line cultured in 2D system. The cells were provided by the University of Bergen, Norway with the support of Mr. James Lorens.

Cell line was cultured in DMEM/F12 medium supplemented with 10 μg/mL of insulin 5% horse serum, 20 ng/mL of EGF, 0.5 μg/mL of hydrocortisone, 100 ng/mL cholera toxin, 100 U/mL of penicillin and 100 μg/mL of streptomycin (Sigma-Aldrich, St. Louis, MO, USA). We used 96-well culture plates which were incubated at 37°C for seven days.

Immunofluorescence

We performed fixation of the plates with 4% paraformaldehyde for 1 h then we washed them with PBS at room temperature for 5 minutes. A solution containing 0.3% saponin, 0.3% Triton X 100 and 0.3% digitonin was used for permeabilization.

The process of permeabilization was exclusively conducted on cytoplasmic targets, namely vimentin and transgelin. Primary antibodies, vimentin (Monoclonal Mouse anti-vimentin Clone V9) (1:25) and transgelin (rabbit SM22 Alpha (1:25) were diluted with Dako antibody diluent and left to incubate for 72 hours at a temperature of 4°C. Afterward, cells that were labeled for vimentin and transgelin underwent two washes with PBS, each lasting for five minutes. Following this, they were subjected to a 24-hour incubation with Alexa Fluor 647 goat anti-mouse (1:200) and FITC anti-rabbit (1:200) before being observed. To protect the dyes, they were incubated

overnight at a temperature of 4°Celsius with Prolong (R) Gold antifade with DAPI.

Analysis

In order to acquire images in digital format at 20x, the TissueFAXS 4.2 software (provided by Tissue Gnostic) and the Zeiss Axio Observer Z1 microscope were utilized.

The analysis of the markers under investigation was conducted using Histo Quest 6.0 software, which expresses the average of the "sum intensity" of the fluorescent signal for each cell.

Research ethics. The research was conducted with the authorization the Institutional review board (IRB) approval from Regional Institute of Oncology (No. 108/ 29.03.2023).

RESULTS

To evaluate the levels of transgelin and vimentin expression, a glioblastoma cell line was utilized.

We conducted immunofluorescence staining to examine cultured cells.

The stained material was captured through digital imaging fluorescence microscopy. In the examined cell line, both transgelin and vimentin were simultaneously expressed, as shown in first figure.

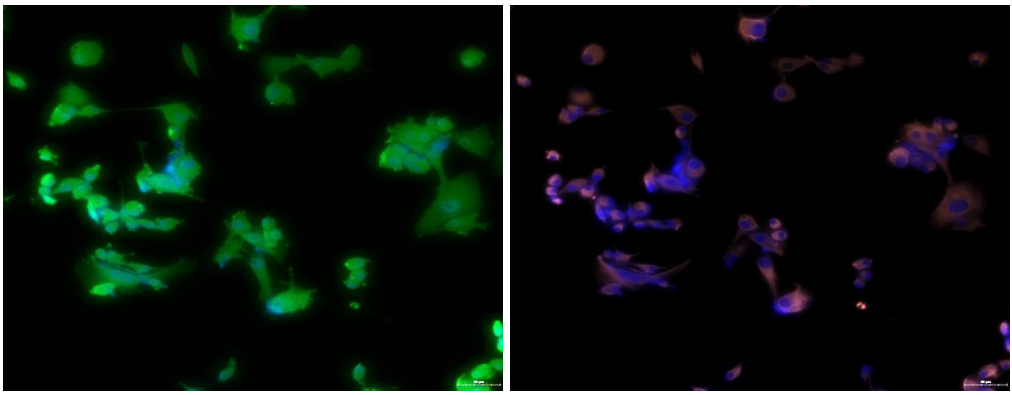

Fig. 1. Transgelin (green) and vimentin (pink) co-expression in U87 glioma cells. Pictures were acquired at 20X.

DISCUSSION

As a component of an extensive research project, this particular section focuses on transgelin and vimentin co-expression in a malignant cell line. It is an essential fragment of the project as a whole, and its findings will contribute significantly to the overall conclusions.

The focus of our research is on the expression of transgelin in an in vitro 2D culture system in relation with the expression levels of vimentin. Our findings reveal that transgelin is co-expressed with vimentin in malignant brain cancer cells.

Studies conducted in the past have demonstrated that inhibiting the function of vimentin in mice leads to the suppression of smooth muscle development and impediments in the process of scarring (6, 23). In recent years, vimentin has been recognized as a marker of EMT, a complex process that is often related to cancer spread. This is of particular importance in the context of cancer because the acquisition of a mobile

and invasive phenotype is associated with the development of metastases, which is the primary cause of death for cancer patients (8).

The involvement of vimentin in cancer cells infiltrating their surroundings is a subject of interest in the field. Understanding the role of this protein in the process of cancer cell invasion could have significant implications for the development of treatments, as its role in chemoresistance is already established (24).

In presented data, the expression of vimentin is explained by the invasive, aggressive character of glioblastoma cells. Nevertheless, a two-dimensional cell culture model would not be able to exhibit the identical progression pattern as a prototype in vivo. The primary outcome deduced from results provided should center on the co-expression of vimentin and transgelin, with both serving as possible indicators of an invasive and aggressive cellular behavior.

The represented results may vary from cell line to cell line and could be also different from in vivo studies; glioblastoma is a very heterogenous pathology, with cellular and molecular diversity not only between tumors but also within the tumor (25). Co-expression of transgelin with a well-known poor prognosis factor as vimentin may indicate a potential similar role in cancer biology. Results may also vary from type of cell line or used tissue samples, as shown before. Immunofluorescence transgelin staining sustain previous research of transgelin being expressed in aggressive cancers (26). The expression of transgelin and also its functional role appear to contrast across different tumor cell types and stromal tissues and may be potentially modified due to various factors such as microenvironmental conditions, gene expression or even cancer progression (27).

CONCLUSIONS

As cancer pathology continues to be a significant global issue, there is a strong conviction that fresh insights into the conduct of cancer with regard to invasion and metastasis are crucially required.

The expression of transgelin in cancer cells appears to resemble that of vimentin, implying that it may function as an indicator of cancer aggressiveness.

Despite conflicting data in the literature, our study of transgelin expands the knowledge base. Specifically, we aim to enhance our comprehension of the part transgelin plays in the invasion and spread of cancer, with potential implications for treatment modalities in clinical settings. Additional research is required to validate the co-expression and correlation of vimentin and transgelin with cancer metastasis.

CONFLICT OF INTEREST AND FUNDING

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STATEMENTS

Conceptualization: Elena Daniela Semen, writing original draft: Elena Daniela Semen; data acquisition and interpretation: G. Luta, manuscript revision: Elena Daniela Semen, Manuela Ciocoiu, I. Prutianu; supervising : Manuela Ciocoiu.

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