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# The evidence of potential tumor suppressor properties of *TAGLN in vitro*

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**Aim.** To prove potential tumor-suppressor properties of *TAGLN* in baculovirus expression system *in vitro*. **Methods.** *TAGLN* cloning into baculovirus expression system. Cell line transduction of recombinant baculoviruses (PC3, PNT2, LNCaP, HEK293). Cell viability and fluorescent microscopy detection to investigate the qualitative and quantitative cell's characteristics. Relative gene expression of *TAGLN, MKI67* and *CASP3* investigation by a quantitative RT-PCR. **Results.** Transduction of AcGFP-TAGLN resulted in increased cell death on 24 and 48 hours after transduction in all cell lines compared to the virus-free groups. Fluorescence microscopy confirmed massive cell death of transduced cell lines with *TAGLN*. The study of *TAGLN* relative gene expression after AcGFP-TAGLN transduction has shown a significant increase in *TAGLN* expression in the PC3 and PNT2 cell lines. Increased *CASP3* expression in PNT2 cells upon AcGFP-TAGLN transduction was detected. **Conclusions.** The effect of *TAGLN* administration on cell survival of both normal and cancer cell lines was shown. This confirms the evidence of the potential tumor suppressor function of *TAGLN* by a direct action on the tumor cells. Increased expression of *CASP3* in PNT2 cells and the absence of this effect in PC3 cells upon *TAGLN* transduction indicate different mechanisms of cel ldeath. These facts require further research.

**K e y w o r d s:** tumor suppressor genes, *TAGLN*, cell lines, recombinant baculoviruses, relative gene expression

## Introduction

The tumor suppressor genes (TSG) are a very important group among the tumor-associated genes. They play a pivotal role at all stages of carcinogenesis according to the modern conceptions of the oncological diseases development [1, 2]. TSGs have been found in different functional groups of genes. They are usually inactivated in cancer tissues and cancer cell lines [3, 4]. TSGs loss of function is one of the properties, which helps to find new genes in this group [5]. TSGs are very important targets for the cancer gene therapy [6, 7]. Their functional restoration could help to overcome the cancer drug resistance [8, 9]. Therefore, TSGs

cloning and their subsequent expression in cancer cells will help to reveal the functional properties of these genes in carcinogenesis and to develop new methods of therapy.

Our previous studies on the search for the potential prostate cancer suppressor genes showed a decreased expression of the *TAGLN* gene, which coded Transgelin (SM22), in the cancer cell lines compared to the normal prostate cell line [10, 11]. In the prostate tumor tissues, the *TAGLN* gene revealed a high level of the expression heterogeneity [12]. It is known that this gene has potential TSG properties in particular in colorectal and prostate cancer [13–15]. But in some other tumor types, on the contrary, it has the properties that stimulate the cancer growth, in particular, in the lung tumor cells and some types of breast cancer cell lines [16–18]. The TAGLN protein functioning is associated with the cancer cell migration, apoptosis and small molecule biochemistry [18]. Our previous investigations have shown the efficient gene delivery to the mammalian cells by the Baculovirus expression system [19, 20]. This expression system was chosen for the *TAGLN* cloning.

Considering these data, the aim of our investigation was to clone the *TAGLN* gene in the Baculovirus system and transduce it to cancerous and normal cell lines in order to determine the effect of the *TAGLN* gene expression on cell survival and marker genes expression.

## Material and methods

**Construction of recombinant baculoviruses.** Recombinant baculoviruses were obtained on the basis of Autographa californica multiple nuclear polyhedrosis virus (AcMNPV) in the Bac-to-Bac Baculovirus Expression System (Thermo Fisher Scientific, USA) according to the manufacturer's protocol.

Plasmid pFastBac was used as a donor plasmid. According to the protocols, recombinant baculoviruses AcGFP were obtained under the CMV virus gene promoter for expression in mammalian cells with the Green fluorescent protein (GFP) gene embedded in the cassette under the CV40 promoter [20, 21]. Recombinant baculovirus AcGFP-TAGLN contained both the *TAGLN* and the *GFP* gene in the cassette.

**Insect and human cell cultures.** A monolayer culture of Sf21 insect cells was grown in TC-100 medium (Sigma) with the addition of 10 % FBS at 28 °C. Transfection of cells by recombinant baculoviruses was carried out according to the standard procedures [21].

Human cells were cultured in DMEM medium (Sigma) with the addition of 10 % fetal calf serum FBS (Sigma) at 37 °C in a CO2 incubator.

Two prostate cancer cell lines (LNCaP, PC3), one conventionally normal prostate cell line (PNT2) and human embryonic kidney cell line HEK293 were used to study the potential tumor suppressor activity of the *TAGLN in vitro*. The transduction of these cell lines by recombinant baculoviruses with the *TAGLN* (AcGFP-TAGLN) and without it (AcGFP) was performed three times in three replicates [20, 21]. All types of cell lines without baculoviruses under transduction procedures were performed as controls. The cells were cultured for 24 and 48 hours after transduction. The cell viability was investigated in different variants of transduction. It was examined by staining with Trypan blue according to a standard protocol [22]. The visual assessment of transduc-

tion and the evaluation of cell quality characteristics were performed using a Leica fluorescence microscope. Additionally, all studied groups were taken to detect the relative gene expression levels in the PC3 and PNT2 cell lines (described below).

**Total RNA isolation and cDNA synthesis.** Cell lines were washed three times with PBS before the RNA isolation. Total RNA was extracted by TRI-reagent (SIGMA), according to the manufacturer's protocol. The total RNA concentration was analyzed by a spectrophotometer (NanoDrop Technologies Inc. USA). cDNA was synthesized from 1 μg of the total RNA, treated with RNase free DNase I (ThermoFisher Scientific, USA), using RevertAid H Minus M-MuLV Reverse Transcriptase (ThermoFisher Scientific, USA), according to the manufacturer's protocol.

**Quantitative PCR (qPCR).** The levels of a relative gene expression (RE) of the *TAGLN, MKI67, CASP3* genes were assessed by qPCR, using a Maxima SYBR Green Mastermix (ThermoFisher Scientific, USA) on a Bio-Rad CFX96 Real-Time PCR Detection System (USA) under the following conditions: 95 °C — 10 min, following 40 cycles of 95 °C — 15 s, 60 °C — 30 s, elongation 72 °C — 30 s. Primers for all genes were selected from a qPrimerDepot (https://primerdepot.nci.nih.gov/) database and confirmed, using the https://www.ncbi.nlm.nih.gov/tools/primer-blast/ algorithm.

The reference gene *TBP* was used for the gene expression normalization [23]. The Model 2-ΔCt described earlier [24] was used for calculation and analysis of the RE levels.

**Statistical analysis.** The Kolmogorov-Smirnov test was applied to assess the normality of distribution. The Kruskal-Wallis test was used to determine differences between the experimental groups. The Dunn-Bonferroni test for multiple comparisons was performed as post hoc test to determine RE differences between groups [23, 24].

## Results

The transduction of cell lines LNCaP, PC3, PNT2 and HEK293 was carried out in three variants. The cell viability studies were performed 24 and 48 hours after transduction (Fig. 1*A–C*): *A* — Transduction of cell lines without baculovirus; *B* — Transduction of cell lines with recombinant AcGFP; *C* — Transduction of cell lines with recombinant AcGFP-TAGLN.

The results of the cell viability study after transduction without baculovirus have shown the insensitivity of LNCaP, PC3, HEK293 cells to the transduction procedure (Fig. 1*A*) whereas there was a tendency of decreasing the survival of PNT2 cells by about 80 % of living cells. However, these changes did not have statistically significant values.

The same tendency of decreased cell survival (up to 80 % of alive cells) of all four cell lines (Fig. 1*B*) was observed after transduction with recombinant baculovirus with green fluorescent protein (AcGFP), especially 24 hours after transduction. However, these changes statistically also did not significantly differ from the non-transduced control cell groups (not shown) and the groups with transduction without baculoviruses.

The opposite situation was observed when all cell lines were transduced by recombinant baculovirus with Transgelin (AcGFP-TAGLN) (Fig. 1*C*). The amount of dead cells increased

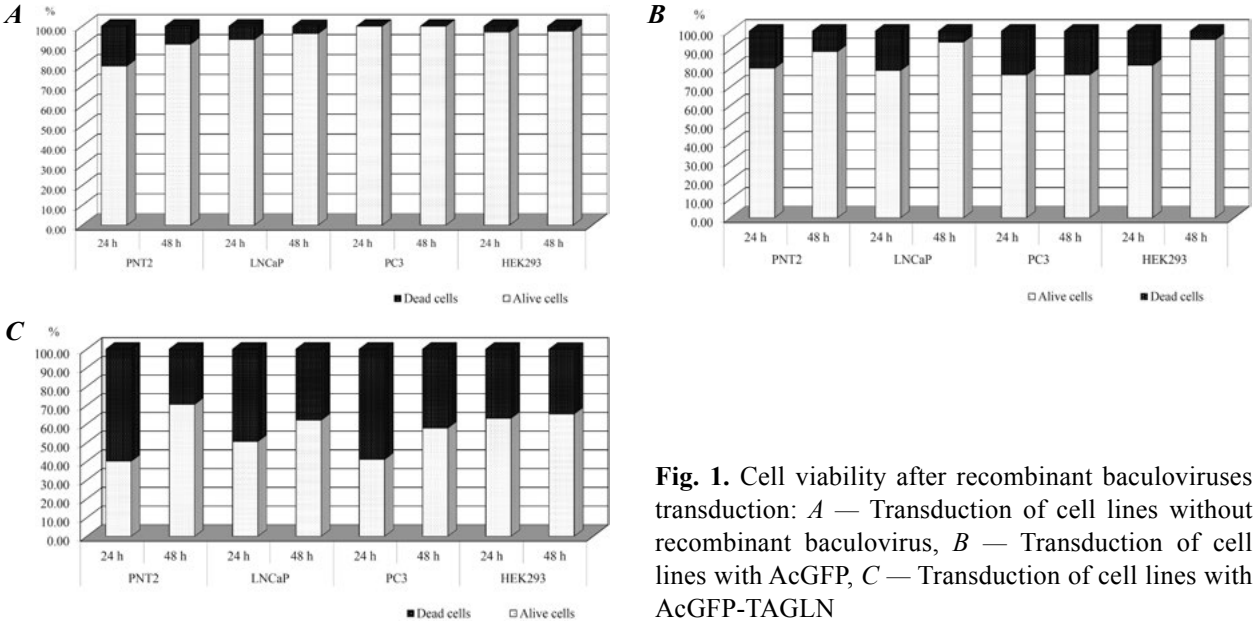


**Fig. 1.** Cell viability after recombinant baculoviruses transduction: *A* — Transduction of cell lines without recombinant baculovirus, *B* — Transduction of cell lines with AcGFP, *C* — Transduction of cell lines with AcGFP-TAGLN

by about 2–3 times after 24-hour transduction, so it was significantly higher ($p < 0.05$) than in the corresponding groups of the cells transduced without baculovirus and with AcGFP. The maximal effect on cell survival (almost 60 % dead cells) was observed with the expression of TAGLN in the PC3 cell line after 24 hours of transduction with AcGFP-TAGLN.

The PC3 cell viability after 48 hours of AcGFP-TAGLN transduction was slightly increased compared to that after 24 hours, but remained significantly lower than of the PC3 cells transduced without baculovirus ($p < 0.05$).

The study of morphological characteristics of cells using a fluorescent microscope was carried out after the end of transduction (0 hours), after 24 and 48 hours. Figure 2 shows the micrographs of the PC3 cell line after transduction with AcGFP and AcGFP-TAGLN recombinant baculoviruses. The results confirm the successful cell lines transduction with AcGFP and AcGFP-TAGLN. The cells have a green color, regular shapes with clear contours, nuclei and nucleolus. Accumulation of the green fluorescent protein was found in the cytoplasm of cells. The cells in different phases of division were observed.

The divergent morphological patterns of the PC3 cell line are detected 24 hours after the AcGFP and AcGFP-TAGLN transduction. Thus, after cell line transduction with AcGFP it was observed cell growth and proliferation, accumulation of green fluorescent protein, whereas after transduction with AcGFP-TAGLN the massive death of transduced cells was found. This is evidenced by the loss of clear cell outlines and cell nuclei. Shapeless clouds of the green fluorescent protein were observed instead of live cells. Similar changes were detected for other cell lines upon the

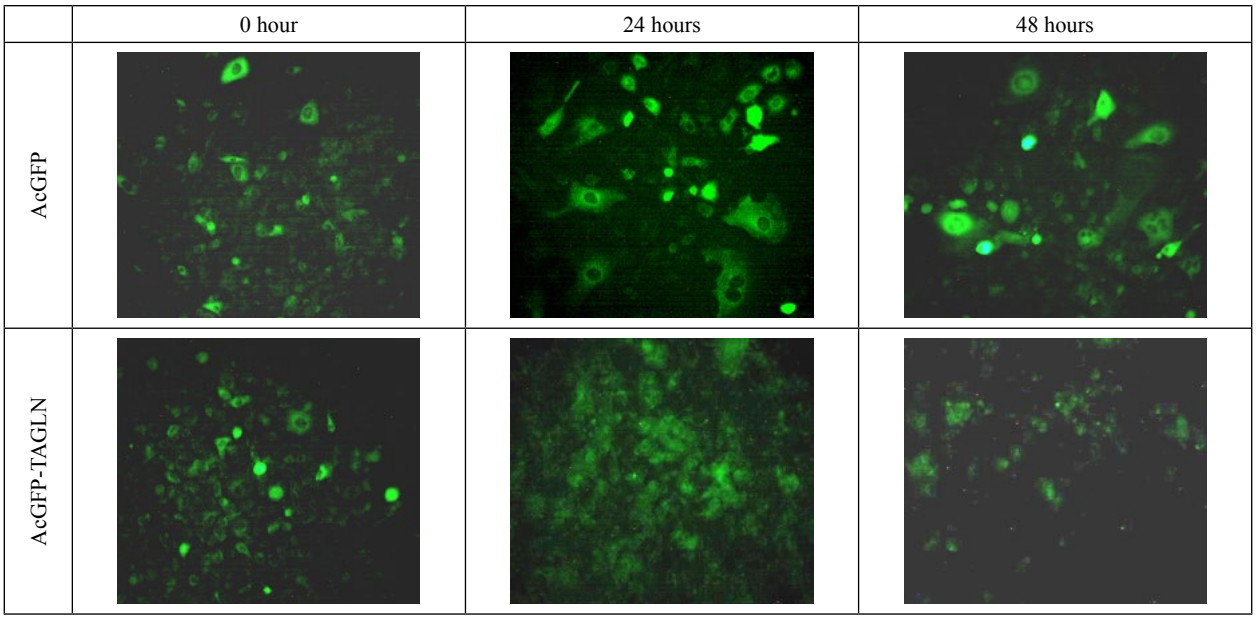


**Fig. 2.** Transduction of the PC3 cell line with recombinant AcGFP and AcGFP-TAGLN.

transduction with the recombinant AcGFP-TAGLN.

The continued cell growth and accumulation of the fluorescent protein were observed in the PC3 cells after 48 hours transduction with AcGFP. At the same time, after transduction with AcGFP-TAGLN no living green cells were detected and the clouds of the green fluorescent protein were less significant. Noteworthy, the live cells are observed under light microscopy in these samples, but most likely these cells did not have transduced recombinant baculovirus.

Similar changes in the cell morphology were also observed for other studied cell lines (LNCaP, PNT2, HEK239) after transduction with AcGFP and AcGFP-TAGLN.

We detected that the own *TAGLN* expression is present in the PNT2 cells at a sufficiently high level (Fig. 3. columns 1, 2), whereas in the cancer PC3 cell line the RE of TAGLN expression is very low (Fig. 3. columns 7, 8). These results confirm our previous studies [10, 11]. Transduction of the PNT2 cells with AcGFP-TAGLN increases the *TAGLN* gene expression almost by 2.5 times, which is significantly higher than in the control groups (Fig. 3 columns 1–4) ($p < 0.05$). The relative expression levels of *TAGLN* are more than 10-fold higher in the PC3 cells after 24 and 48 hours of the transduction with AcGFP-TAGLN compared to the PC3 cells without baculovirus and with AcGFP (Fig. 3 columns 7–10) ($p < 0.05$). However, the *TAGLN* expression levels in these PC3 cell groups do not differ significantly from their own expression levels in PNT2 (Fig. 3 columns 1–4).

Analysis of the *MKI67* relative expression levels after 24 hours of transduction revealed no significant differences between experimen-

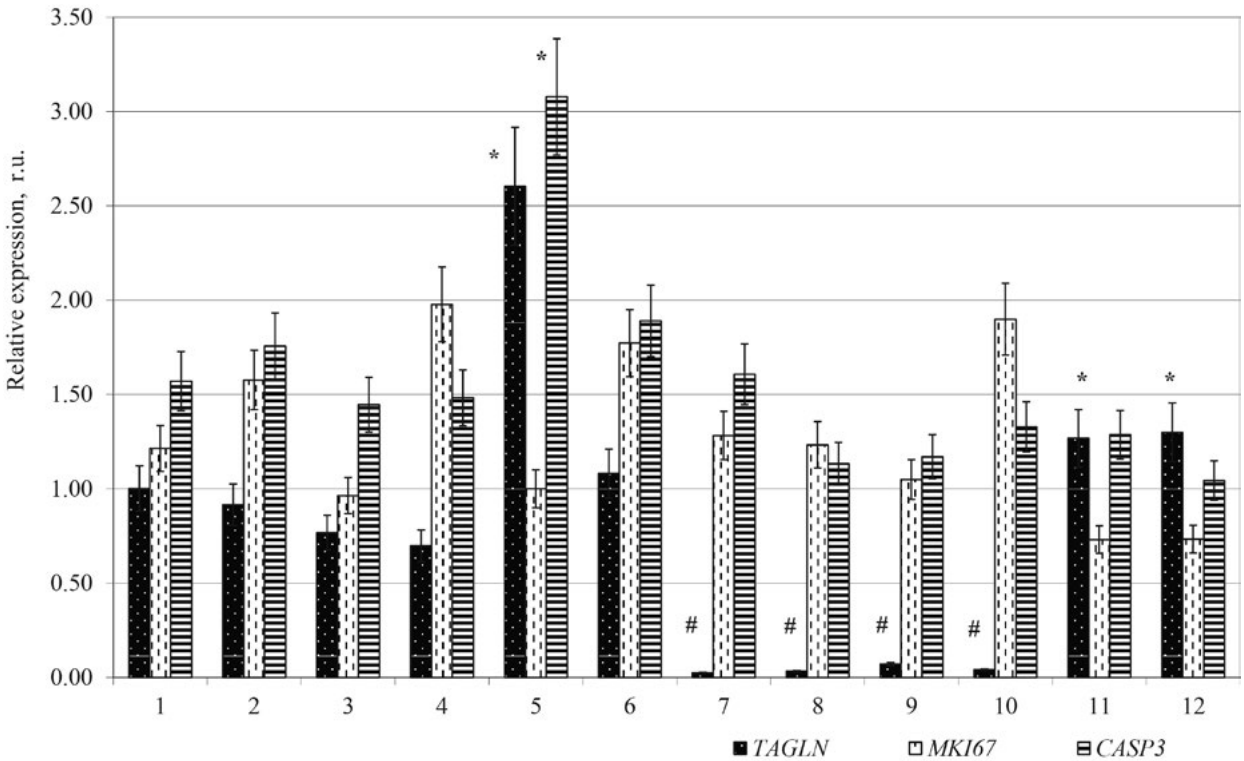


**Fig. 3.** Relative gene expression levels in the PC3 and PNT2 cell lines after 24 and 48 hours transduction with recombinant baculoviruses: *1* — PNT2 transduction without baculovirus — 24 hours; *2* — PNT2 transduction without baculovirus — 48 hours; *3* — PNT2 transduced with AcGFP — 24 hours; *4* — PNT2 transduced with AcGFP — 48 hours; *5* — PNT2 transduced with AcGFP-TAGLN — 24 hours; *6* — PNT2 transduced with AcGFP-TAGLN — 48 hours; *7* — PC3 transduction without baculovirus — 24 hours; *8* — PC3 transduction without baculovirus — 48 hours; *9* — PC3 transduced with AcGFP — 24 hours; *10* — PC3 transduced with AcGFP — 48 hours; *11* — PC3 transduced with AcGFP-TAGLN — 24 hours; *12* — PC3 transduced with AcGFP-TAGLN — 48 hour; * — $p < 0.05$ between the studied group and the "transduction without baculovirus" group; # — $p < 0.05$ between the PC3 and PNT2 cell lines corresponding groups

tal groups. However, there was a certain tendency to a decrease in the *MKI67* expression levels in the groups after 24 hours of AcGFP-TAGLN transduction, both in the PNT2 cell line and in the PC3 cell line (Fig. 3, columns 5, 11). Additionally, a significant decrease in the *MKI67* expression was noted in the PC3 group after 48 hours of AcGFP-TAGLN transduction (Fig. 3, column 12) in comparison with the same time groups of the PNT2 cell line in all transduction variants. Such a significant decrease of expression level was also detected in the PC3 group after 48 hours of the AcGFP-TAGLN transduction in comparison with the PC3 group after 48 hours of the AcGFP transduction.

Investigation of the relative expression levels of the *CASP3* gene showed a single sig-

nificant (more than twofold) increase in the expression level in the PNT2 cell line after 24 hours of AcGFP-TAGLN transduction ($p < 0.05$), whereas no increase in the *CASP3* gene expression was detected in the PC3 cell line.

## Discussion

The *TAGLN* gene encodes the protein Transgelin (SM22), which belongs to the calponin family. It is expressed in various types of cells, in particular, smooth muscle, epithelial, fibroblast, endothelial cells [13, 25]. The encoded actin-binding protein takes part in a number of physiological and pathological processes, including the development of asthma [26], suppression or progression of various types of cancer [14, 26–29], vascular and cardiac inflammation [30, 31].

For prostate cancer, the *TAGLN* gene is a potential tumor-suppressor gene. Thus, a decrease in its expression in the prostate cancer cell lines was demonstrated in our previous studies [10, 11] and by other researchers [15]. In this study, we confirmed a decrease in the *TAGLN* gene expression in the PC3 cell line compared to the conditionally normal prostate epithelial cell line PNT2 (Fig. 3).

The *TAGLN* gene has an increased level of expression in lung tumors, oral squamous cell carcinoma, pancreas cancer, some types of breast cancer that is a marker of poor prognosis [16, 28, 29, 32] whereas in colorectal cancer tumors it has reduced expression and is a potential tumor suppressor gene [14, 15]. High variance is found among the *TAGLN* expression levels in prostate tumor samples [12]. This may indicate the expression of this gene in different cell types, in particular, prostate cancer cells, and tumor stromal elements, for example in fibroblasts, endothelial cells *etc.* [33].

The results of these studies on the cell lines with the expression of the *TAGLN* gene in the baculovirus expression system showed an increase in the number of dead cells in the conventionally normal prostate PNT2 cell line, embryonic kidney HEK293 cell line and in the prostate cancer LNCaP and PC3 cell lines. Moreover, it was detected a decrease in the expression levels of the proliferation marker *MKI67* after 48 hours of AcGFP-TAGLN transduction in both PNT2 and PC3 cell lines. This indirectly suggests the death of transduced cells under the influence of TAGLN expression. The *TAGLN* expression levels after 24-h transduction with AcGFP-TAGLN have shown significant overexpression in the PNT2 cells and re-expression levels in the PC3 cell line compared to the normal PN2 cell line without baculovirus transduction (Fig. 3). In this case, we are talking about different levels of the *TAGLN* expression leading to the death in normal and tumor cells.

Today, a number of programmed death pathways of normal and tumor cells are known. Among them, apoptosis, necrosis, necroptosis, autophagy occupy the leading places. Moreover, these pathways are different in normal and cancer cells [34]. It is known that the acquisition of aggressive properties by cancer cells is accompanied by the blocking of apoptotic pathways [35].

Massive cell death upon overexpression of *TAGLN* in epithelial cells suggests the activation of an apoptotic program, as shown for normal epithelial cells [14]. This fact can be confirmed by the data we obtained regarding an increase in the *CASP3* gene expression in

the PNT2 cell line upon overexpression of the *TAGLN* gene in the baculovirus system (AcGFP-TAGLN) (Fig. 3, column 5). Activation of caspase pathways is a key feature of apoptosis [36]. However, such increase in the *CASP3* expression in the PC3 cancer cell line was not observed when the *TAGLN* gene was expressed, although significant cell death was detected upon transduction of PC3 with AcGFP-TAGLN. This probably happens through the caspase-independent pathways, possibly including autophagy, necroptosis, *etc.* [35, 37]. This supposition requires further investigations.

## Conclusions

The study demonstrated the potential tumor suppressor properties of the *TAGLN* gene in the prostate cancer cell lines using baculovirus expression system. The transduction of the *TAGLN* gene into normal and cancer cells causes the death of the studied cell lines. This confirms the potential tumor suppressor functions of the *TAGLN* gene by direct action on tumor cells.

An increased expression of the *CASP3* gene in the PNT2 cells and the absence of this effect in the PC3 cells upon transduction with AcGFP-TAGLN indicate different mechanisms of the *TAGLN* influence on the death of different cell types. These facts require further research.

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**Свідчення потенційних супресорних властивостей *TAGLN in vitro***

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**Мета.** Довести потенційні пухлино-супресорні властивості *TAGLN* у бакуловірусній системі експресії *in vitro*. **Методи.** Клонування *TAGLN* в бакуловірусну систему експресії. Трансдукція клітинних ліній (PC3, PNT2, LNCaP, HEK293) рекомбінантними бакуловірусами. Визначення життєздатності клітин, флуоресцентна мікроскопія для дослідження якісних і кількісних характеристик клітин. Дослідження відносної експресії генів *TAGLN, MKI67* і *CASP3* в клітинних лініях за допомогою кількісної RT-PCR. **Результати.** Трансдукція рекомбінантного бакуловіруса з геном *TAGLN* (AcGFP-TAGLN) призводила до підвищення смертності клітин через 24 та 48 годин після трансдукції в усіх досліджуваних клітинних лініях у порівнянні з групами без вірусу. Флюоресцентна мікроскопія підтвердила масову загибель трансдукованих *TAGLN* клітин. Дослідження рівнів відносної експресії *TAGLN* після трансдукції AcGFP-TAGLN показало значне збільшення експресії *TAGLN* у PC3 та PNT2. Детектовано підвищення експресії *CASP3* у PNT2 при трансдукції AcGFP-TAGLN. **Висновки.** Було показано вплив введення *TAGLN* на виживаність клітин як умовно-нормальних, так і пухлинних клітинних ліній. Це підтверджує потенційні пухлино-супресорні функції *TAGLN* прямою дією на пухлинні клітини. Підвищення експресії *CASP3* у PNT2 та відсутність цього ефекту у PC3 при трансдукції *TAGLN* свідчить про різні механізми впливу гена на загибель клітин. Ці факти потребують подальших досліджень.

**Ключові слова:** гени супресори росту пухлин, *TAGLN*, клітинні лінії, рекомбінантні бакуловіруси, відносна експресія генів

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