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Mouse embryonic fibroblasts expressing IFNβ or IL-21 inhibit proliferation of melanoma cells *in vitro*

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Aim. To study an influence of mouse embryonic fibroblasts (C57Fb) transduced with baculovirus vectors (BVs), encoding genes for murine *Ifn-β* or human *IL21*, on survival and proliferation of the malignant mouse melanoma cells (B16) *in vitro*. **Methods.** Transduction of cells, construction of BVs, RNA isolation, quantitative PCR. **Results.** We have shown that the normal C57Fb and B16 tumor cells are sensitive to the anti-proliferative effect of IFNβ. Rapidly proliferating B16 cells were the most sensitive. BV ensured a stable expression of *Ifnβ* mRNA for 5 days in C57Fb/IFNβ cells. The growth of B16 cells was suppressed upon co-cultivation with C57Fb/IFNβ or C57Fb/IL21 cells. **Conclusions.** IFNβ and IL-21 synthesized by the mouse embryonic fibroblasts transduced with BVs carrying the *Ifnβ* or *IL-21*genes inhibited proliferation of B16 melanoma cells *in vitro*.

Keywords: mouse embryonic fibroblasts, melanoma cells, interferon β (IFNβ), interleukin-21 (IL-21), baculovirus vector, cell transduction.

Introduction

One of the most promising trends in anti-tumor therapy is the use of genetically modified cells. Over the past several years, genetically engineered mesenchymal stem cells (MSCs) have been extensively investigated as a novel vehicle for the cancer gene therapy. They are weakly immunogenic and exhibit a remarkable migratory property towards tumors. The adult human fibroblasts demonstrate similar properties [1–5]. Nowadays a possible application of embryonic and fetal fibroblasts, in particular mouse embryonic fibroblasts (MEFs), in the regenerative cell therapy and in model systems draw more and more attention. It has been shown that the majority of these cells express antigens of the major histocompatibility complex (MHC-I and MHC-II) at the low levels. Embryonic and fetal organs contain, along with differentiated cells, stem and activated (blast) cells, endowed with a high potential of proliferation and secreting a unique set of cytokines and growth factors in a recipient after grafting [6, 7]. MEFs actively proliferated in culture until passage 12; they are widely used as a feeder layer to support the growth of embryonic stem cells and also they are involved in regulating the organ development, wound and burn healing, inflammation and fibrosis [8, 9]. Several reports have conclusively shown that MEFs are similar to MSCs phenotypically, genotypically and functionally [10, 11]. Both MEFs and MSCs are defined as plastic adherent, clonogenic, multipotent fibroblast like the cells that have similar morphological appearance, identical cell surface markers and the ability to undergo mesoderm-type cell differentiation into osteocytes, adipocytes and chondrocytes [11–13]. Additionally, MEFs expressed the markers of embryonic stem cells [11]. These unique features make them an at-

tractive model for the delivery of therapeutic genes directly into the tumor microenvironment.

In the present work we have used interferon β (IFNβ) and interleukin-21 (IL-21) as the anti-tumor agents. It is known that IFNβ promotes apoptosis, exhibits the anti-proliferative effect on tumor cells, suppresses the angiogenesis in tumor tissues, reduces an ability of tumor to metastasize, and shows the immunomodulatory effects and anti-viral activity [14, 15]. However, the clinical application of IFNβ had been limited by its severe toxicity associated with the excess protein distributing freely throughout the body upon systemic administration. Due to its short drug half-life and insufficient delivery to the target tumor cells, a systemic administration of IFNβ has shown a limited response to the initial therapy for most solid tumors [16]. Transduced stem cells can be used as a vehicle to deliver IFNβ or other cytokines into the tumor microenvironment reducing toxicity and increasing the local concentration of these therapeutic products at the tumor sites. Earlier, some model experiments demonstrated the effectiveness of this approach [16–19]. Interleukin-21 (IL-21) is a cytokine with broad pleiotropic actions including the regulation of development, differentiation and function of lymphoid and myeloid cells. The basic mechanism, which is associated with the antitumor activity of IL-21, is the activation of different subsets of killer cells [20, 21]. The studies using mouse tumour models *in vivo* as well as clinical trials in patients with advanced solid tumours have demonstrated that IL-21 can function as a potent antitumour agent [22, 23]. Human and murine IL-21 and IL-21 receptors show approximately 60 % overall amino acid sequence homology with significant conservation in the regions of cytokine–receptor interaction, and to a large extent the function of IL-21 has been shown to be similar in mouse and human, although several discrepancies have also been described [21]. We used baculoviral vectors (BVs), derived from an insect virus, for the gene delivery into MEFs. This insect DNA virus has the ability to enter mammalian cells without replicating or causing toxicity to the transduced cell [24]. Previously we have demonstrated efficacy of the baculoviruses for such task [25].

In the present study we investigated the influence of MEFs (C57Fb) transduced with recombinant baculoviruses, bearing the mouse *Ifnβ* or human *IL-21* genes on the survival and proliferation of mouse melanoma B16 malignant cells *in vitro.*

Materials and Methods

Cell lines

Cells of mouse melanoma B16 were obtained from the Bank of Cell Lines of R. E. Kavetsky Institute of Experimental pathology, oncology and radiobiology, NAS of Ukraine (IEPOR NASU). Mouse embryonic fibroblasts were isolated by enzymatic disaggregation of the soft tissue of 13.5 day old C57BL/6j mouse embryos [26]. Primary cells were passaged 2–3 times. All cells were cultured in DMEM medium (Sigma) supplemented with 10 % fetal bovine serum FBS (Sigma), 100 U/ml penicillin and 100 µg/ml streptomycin at 37 °C in a CO_2 incubator. Monolayer culture of insect cell line Sf21 was grown in TC-100 medium (Sigma) supplemented with 10 % FBS at 28 °C.

Baculovirus vectors (BVs) and MEF transduction

BVs were obtained from the *Autographa californica* Multiple NucleoPolyHedrovirus (AcMNPV), using the Bac-to-Bac expression system (Invitrogen). The following BVs were created: Ac-M-IFN, containing the murine *Ifnβ* gene under regulation of promoter of chicken β-actine in the CAG cassette; Ac-IFN-GFP, containing two genes: reporter *GFP* under the CMV promoter and the murine *Ifnβ* gene in the CAG cassette; and also Ac-CMV-IL21 containing the human interleukin *IL-21* gene under the CMV promoter. As controls, the following BVs were used: Ac-M-GFP; Ac-M-S18 carrying a gene, encoding the mitochondrial ribosomal protein S18-2; and AcFastMam that was designed on the base pFastBac plasmid with the CAG cassette [27]. The virus was concentrated by

centrifugation at 100000 g. The titer of viruses preparations was 2–4×10^8 pfu/ml. Transduction was performed as desribed previously [28]. C57Fb cells were incubated with recombinant BVs at a concentration of 200 moi (multiplicity of infection = pfu per cell) for 4 hours at 28 °C in phosphate buffered saline (PBS), followed by addition of 1.5 ml DMEM medium and culturing for 16 hours at 37 °C. At the end of the incubation period, the virus solution was discarded, cells were washed with PBS and 2 ml DMEM medium containing 10 % FBS was added.

Analysis of cell proliferation

B16 melanoma cells were seeded on 6-well plates at a concentration of 2×10^5 cells/well and cultured together with C57Fb cells transduced with BVs carrying the murine *Ifnβ* or human *IL-21* gene at a concentration of 2×10^4 cells/well. In the control experiments, cells were seeded in the same amounts and ratios (1:10). As a control, we used the following variants of co-culturing cells: C57Fb: B16; C57Fb/GFP: B16; C57Fb/S18: B16; C57Fb/FastMam: B16. Also cells C57Fb and B16 were transduced with the above BVs cultured alone (2×10^5 cells/well). And "pure" control – cells B16 and C57Fb cultured alone (not transduced) at a concentration of 2×10^5 cells/well.

In all experiments, the cells were cultured for 5 days at the standard conditions without replacement of the culture medium. Cells were removed from the culture plates by the trypsin EDTA solution and re-suspended in PBS supplemented with serum (10 % FBS). The total cell number was then calculated. All experiments were performed in triplicate. The results are presented as the relative number of cells (in % of the cell number in controls, see [16] for description).

Assessment of the Ifnβ gene expression

To monitor the kinetics of *Ifnβ* gene expression in transduced MEFs, the samples were collected at 0, 24, 48, 72, 96, and 120 hours after transduction. As the controls the C57Fb intact cells and C57Fb cells transduced with AcFastMam at 0 and 24 hours were used.

RNA isolation and cDNA synthesis

RNA was isolated, using the RNA isolation kit (Fermentas/Thermo Fisher Scientific), according to the manufacturer's protocol. RNA concentration was measured, using a Nanodrop spectrophotometer (Thermo Fisher Scientific). The RNA was treated with DNase (Fermentas/ Thermo Fisher Scientific), and 1 μg of RNA was applied in cDNA synthesis, using oligo-dT primer and cDNA synthesis kit (Fermentas). 2 μl of the synthesized cDNA was used for q-PCR.

Real-Time q-PCR

The relative expression level of the gene *Ifnβ* was assessed by q-PCR, using SYBR Green (Maxima SYBR qPCR Master Mix/Thermo Fisher Scientific) on a Real-Time thermocycler (CFX96 Real-time System C1000 Thermal Cycler / BIO-RAD). The *TBP* was used as the reference gene. The following primers were used: TbpF1 5'-CCCCACAACTCTT CCATTCT-3'; TbpR1 5'-GCAGGAGTGATAGGG GTGAT-3'; Ifn-bF 5'-CCCTATGGAGATGACGG AGA-3'; Ifn-bR5'-CAGTCTGGAGAAATTGT-3'. Amplification parameters were: 50 °C – 20 min, 95 °C – 10 min, (94 °C – 25 s, 60 °C – 25 s, 72 °C – 25 s) for 40 cycles, and 72 °C – 2 min.

Statistical analysis

Statistical analysis was performed using the Student's t-test.

Results and Discussion

Effect of MEF (C57Fb) and tumor B16 cells transduced with BVs, on the proliferation of melanoma cells B16 in vitro

To examine a possible anti-proliferative effect of the C57Fb cells transduced with the BVs carrying the *Ifnβ* or *IL-21* genes, these cells were co-cultured with the malignant B16 cells *in vitro*. We have found that proliferation of the B16 cells was inhibited by the C57Fb transduced BVs with gene *Ifnβ* (Fig. 1, columns 6, 7) in comparison with the controls (columns 1–5). The amount of B16 melanoma cells in

the control on 5th day of culturing – $6x10^6$ cells/well was taken for 100 %. The relative number of the B16 cells co-cultured with C57Fb/IFNβ or C57Fb/IFNβ-GFP cells (4.5±2.7 % and 2.8±1.2 %, respectively) was decreased significantly in comparison with all the controls, and these differences were statistically significant ($p < 0.001$). The number of B16 cells cultured together with C57Fb/FastMam cells (Fig. 1, column 5) was significantly lower compared to the number of B16 cell cultured alone ($p < 0.01$). Moreover, the significant reduction in the number of these cells was observed ($p < 0.05$) when compared with the control mixtures of cells (Fig. 1, columns 2–4). It was shown earlier that baculovirus transduction could activate the TLR3 (toll-like receptor) pathway in MSCs and triggered secretion of various cytokines, including IFNα and IFNβ, although a level of their secretion was low [29, 30]. Nevertheless, the properties of MEFs and their production of endogenous IFN in response to transduction of BVs were not investigated earlier. We also found that in all control mixtures of B16 cells with C57Fb the number of B16 cells was statistically significantly reduced ($p < 0.05$) compared with the growth of B16 cells alone. Therefore, modeling *in vitro* interaction of heterologous cells in tumor – melanoma cells (B16) and fibroblasts (MEFs), we have shown that IFNβ, synthesized by C57Fb/IFNβ or C57Fb/IFNβ-GFP cells, inhibits the proliferation of tumor B16 cells. The presented results are consistent with the earlier reports [16, 31, 32]. Notably, in the referred here studies MSCs used showed a stimulating effect on the tumor cells (including melanoma cells) growth when co-cultured *in vitro*. Recently, there are increasing evidences that MSCs exhibit the promotion or inhibition of the tumor growth and the invasion through direct or indirect interaction with the tumor cells [33]. As discussed above, the C57Fb cells exerted an inhibitory effect on the B16 tumor cell growth. This fact also indicates the perspective of using MEFs as a vector in the experimental mouse models of melanoma *in vivo*.

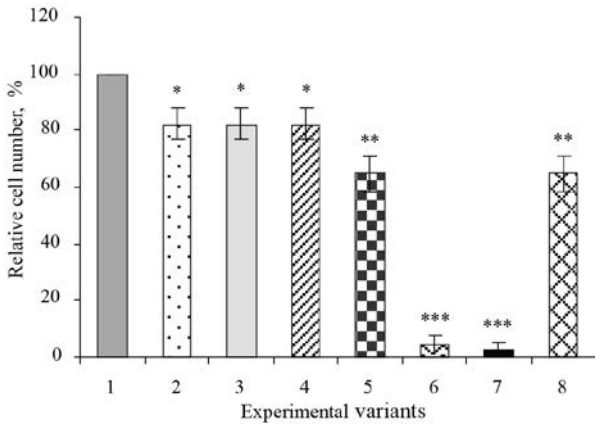

Fig. 1. Effect of co-culturing of embryonic fibroblasts C57Fb expressing IFNβ or IL-21, on the proliferation of B16 cells *in vitro*. 1 – "pure" control B16 cells; control mixtures of cells: 2 – C57Fb: B16; 3 – C57Fb/GFP: B16; 4 – C57Fb/S18: B16; 5 – C57Fb/FastMam: B16; cell mixtures in experiment: 6 - C57Fb/IFNβ: B16; 7 – C57Fb/IFNβ-GFP: B16; 8 – C57Fb/IL21: B16. The differences are significant at *$p < 0,05$; **$p < 0,01$; ***$p < 0,001$ compared to the control.

When B16 cells were co-cultured with C57Fb/IL21 cells (Fig. 1, column 8) a significant decrease in the number of B16 cells was observed, compared with a "pure" control ($p < 0.01$) and the control mixtures of cells ($p < 0.05$). Although IL-21 was originally described as an important regulator of T, B and NK cells, which play key roles in antitumoral immunity, our results have shown that IL21 inhibits proliferation of malignant melanoma cells upon cocultivating with C57Fb/IL21 cells *in vitro*. Further studies are needed to clarify, whether the melanoma cells are a direct target of IL-21 or this cytokine acts indirectly by activating intracellular processes in fibroblasts.

Since the tumor cells interact with each other and with the surrounding normal cells, we considered interesting to model the interaction between homologous cells in tumor, using B16 cells and B16, transduced with BVs carrying the *Ifnβ* or *IL-21* genes. A significant decrease in the number of tumor cells (Fig. 2, columns 5–6) occurred when B16 cells were co-cultured with B16, transduced with BV containing the *Ifnβ* gene, in comparison with the controls (columns 1–4): 21.7 % (B16/IFNβ:B16) and 30.3 % (B16/IFNβ-GFP:B16). The observed difference was statistically significant ($p < 0.001$).

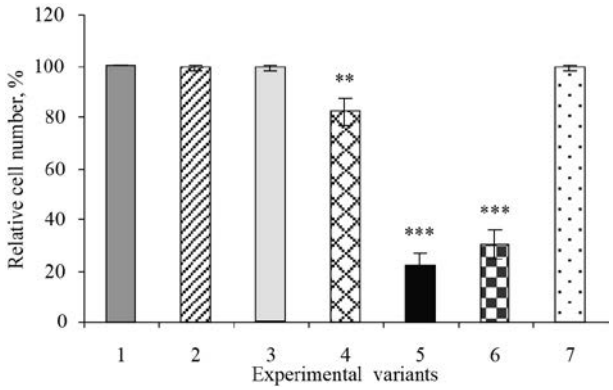

Fig. 2. Effect of co-culture of melanoma B16 cells transduced with BVs and non transduced B16 cells *in vitro*. 1. – B16 control cells; 2. – B16/GFP: B16; 3. – B16/S18: B16; 4. – B16/FastMam: B16; 5. – B16/IFNβ: B16; 6. – B16/IFNβ-GFP: B16; 7. – B16/IL21: B16. The differences are significant at **$p<0.01$; ***$p< 0.001$ compared to the control.

Thus, when co-cultured the homologous cells (B16/IFNβ:B16, B16/IFNβ-GFP:B16) or the heterologous (C57Fb/IFNβ:B16, C57Fb/IFNβ-GFP:B16) cells as well, IFNβ synthesized by B16 or C57Fb cells inhibits the proliferation of tumor B16 cells (Fig. 3). However, the observed effect is more pronounced when the heterologous cells were co-cultured. Thus, at the 5th day of co-cultivation of B16 cells with C57Fb/IFNβ or C57Fb/IFNβ-GFP the number of B16 cells decreased 5 and 11 fold, in comparison with co-culturing the homologous cells – B16/IFNβ:B16 and B16/IFNβ-GFP:B16.

The number of B16 cells cultured together with B16/FastMam cells was lower, compared to the number of B16 cells in a "pure" control and the control mixtures (Fig. 2, columns 2, 3) with statistically significant differences ($p<0.01$).

Notably, the control cell mixtures containing MEFs (Fig. 1) showed a higher inhibiting capacity compared with the similar mixtures that contained the homologous B16 cells (Fig. 2), and compared with the transduced cell B16 cultured alone (Fig. 4). Similar results on suppression of the tumor cell proliferation by fibroblasts were also reported earlier [34–36]. It was shown that mouse and human fibroblasts can inhibit the proliferation of tumor cells, when co-cultured *in vitro*. Yet, their inhibitory ability varies, depending on the origin of the tumor cells and fibroblasts. The effective inhibition was observed when fibroblasts formed a monolayer, and inhibition was due to direct cell-to-cell contacts. Tumor cells interact with other cells and extracellular matrix through a wide range of adhesion molecules localized on the surface of tumor cells and fibroblasts. It was also noted that soluble factors (different proteins, including cytokines) secreted by fibroblasts contribute to the inhibition of proliferation and motility of tumor cells. In our study, despite the fact that the ratio of MEFs and tumor cells was 1:10, the growth of B16 tumor cells was inhibited significantly on the fifth day of co-cultivation. When B16 cells were co-cultured with B16, transduced with

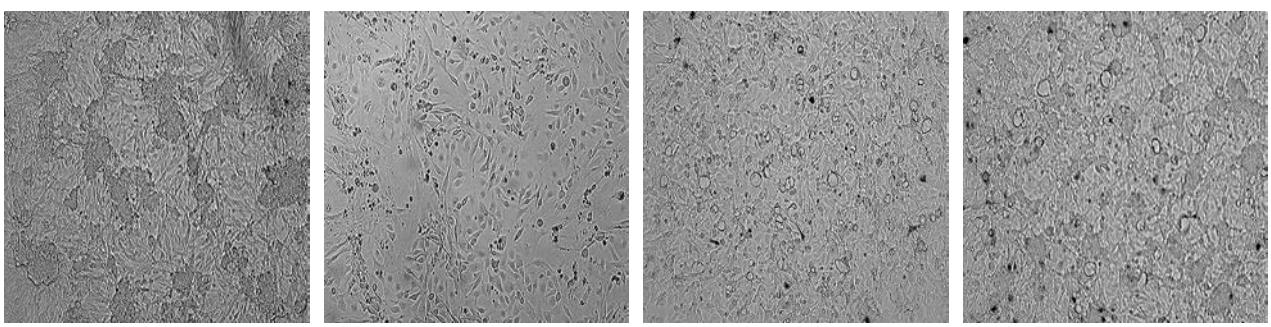

Fig. 3. Antiproliferative influence of IFNβ, synthesized by C57Fb or by tumor B16 cells, transduced with BVs, on melanoma cells (magnification × 10).

BV carrying *IL-21* no difference in the growth of tumor cells was observed (Fig. 2, column 7).

Analysis of proliferation of B16 and C57Fb cells (cultured alone), transduced with BV carrying *Ifnβ*, revealed inhibition of the cell growth on the fifth day in comparison with all the control variants (Fig. 4, 5): 19.0 % B16/IFNβ and 22.4 % B16/IFNβ-GFP; 49.8% C57Fb/IFNβ and 33.3 % C57Fb/IFNβ-GFP. The number of C57Fb cells in a "pure" control on the fifth day of culturing – $8.6x10^5$ cells/well was taken for 100 %.The observed difference was statistically significant ($p < 0.001$).

The growth of B16/FastMam cells was also significantly inhibited ($p < 0.05$) in comparison with the growth of B16 cells in control (Fig. 4, column 4). The number of B16/IL21 cells does not differ from this index in the control variants. We also observed a significant reduction in the number of C57Fb/GFP, C57Fb/S18 and C57Fb/FastMam cells in comparison with a "pure" control ($p < 0.05$, $p < 0.01$) (Fig. 5). Thus, C57Fb cells showed increased responsiveness to the transduction with BVs as compared with B16 cells. As previously reported, this effect is probably associated with the induction of endogenous interferon or other cytokines in response to the baculovirus transduction of these cells and also depends on the expression of introduced transgenes. The number of C57Fb/IL21 cells was statistically significantly reduced ($p < 0.05$) compared with the growth of C57Fb cells alone. This is in line with previous findings, showing that IL-21 inhibits proliferation of C57Fb as B16 cells which were co-cultured with C57Fb/IL21 (Fig. 1).

Noteworthy, C57Fb cells were more resistant to the anti-proliferative effect of IFNβ produced by these cells. It is known that the secreted IFNβ binds to specific receptors on the membrane and functions as a regulator of the cell proliferation through autocrine (affects the secreting cell itself), and paracrine (acts on neighboring cells and tissue) pathways [37]. Thus, IFNβ produced by the transduced C57Fb/IFNβ, C57Fb/IFNβ-GFP, B16/IFNβ, B16/IFNβ-GFP cells inhibited their proliferation, and when co-cultured with B16 cells it suppressed the melanoma cells proliferation.

Analysis of Ifnβ expression in C57Fb cells, transduced with BV carrying Ac-M-IFNβ

To assess the kinetics of *Ifnβ* expression in C57Fb cells, transduced with BV, q-PCR analysis was per-

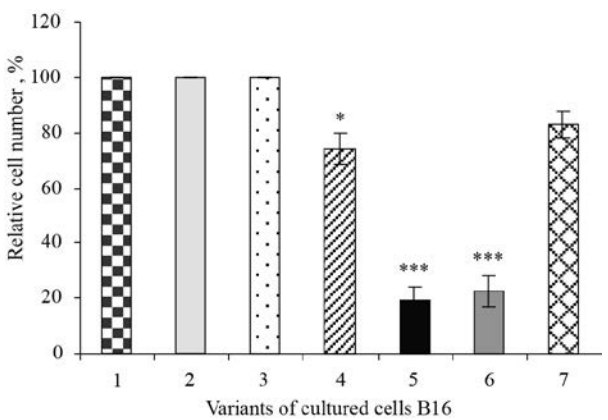

Fig. 4. The results of cell culture of melanoma B16, transduced with various BVs: 1 – control cells B16; 2 – B16/GFP; 3 – B16/S18; 4 – B16/FastMam; 5 – B16/IFNβ; 6 – B16/IFNβ-GFP; 7 – B16/IL21 The differences are significant at *$p < 0.05$; ***$p < 0.001$ compared to the control.

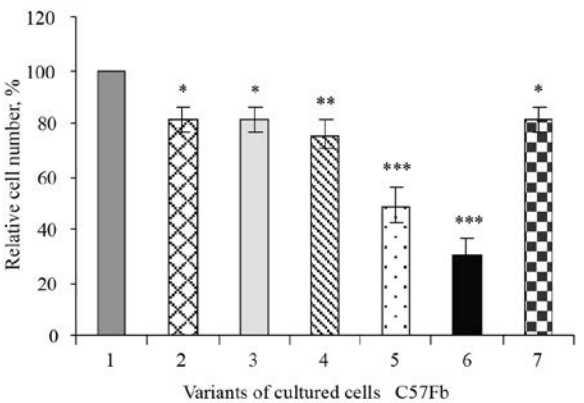

Fig. 5. The results of culturing mouse embryonic fibroblasts C57Fb transduced with various BVs: 1 – control cells C57Fb; 2 – C57Fb/GFP; 3 – C57Fb/S18; 4 – C57Fb/FastMam; 5 – C57Fb/IFNβ; 6 – C57Fb/IFNβ-GFP; 7 – C57Fb/IL21. The differences are significant at *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ compared to the control.

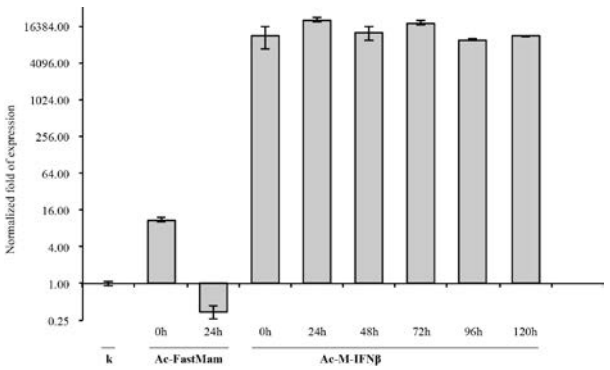

Fig. 6. Dynamics of the *Ifnβ* gene expression in embryonic fibroblasts C57Fb, transduced with BVs. x – axis: time after transduction (h); k – control cells C57Fb; Ac-FastMam – C57Fb cells transduced with control BV; Ac-M-IFNβ – C57Fb cells transduced with BV containing the *Ifnβ* gene.

formed. It is known that BVs provide transient expression of transgenes in non-permissive mammalian cells in the absence of viral replication and a gradual loss of viral DNA upon cell division. As was shown earlier, the expression of GFP reporter protein may last for 30 days gradually decreasing, but stored in some single cells [28, 30]. Using q-PCR, we showed that *Ifnβ* was expressed at a high level for 120 hours, substantially equal to that observed immediately after transduction (Fig. 6). Maximum value in our system was observed on the twenty-fourth hour post transduction. The transduction of C57Fb cells with the control baculovirus (Ac-FastMam) led to a minor increase in the synthesis of endogenous IFNβ that within 24 hours falls to a lower level than in cells without transduction (Fig. 6). These data are consistent with the previously reported and are associated with a response to introduction of BV by secreting cytokines including interferon [30]. Consequently, when B16 cells were co-cultured with C57Fb/IFNβ cells, IFNβ produced by MEF, provides a permanent inhibitory effect on the tumor cells for 120 hours.

Conclusions

We showed that IFNβ, synthesized by C57Fb MEFs or by tumor B16 cells transduced with BVs, carrying the *Ifnβ* gene, inhibited the proliferation of malignant mouse B16 melanoma cells *in vitro*. The effectiveness of inhibition of the B16 cells proliferation was higher when the heterologous cells were co-cultured. Hence, the interactions between MEFs and tumor cells contribute mostly to the inhibition of tumor cell proliferation.

We found that *Ifnβ* was stably expressed in C57Fb cells transduced with BV Ac-M-IFNβ for 120 hours. Noteworthy, C57Fb/IFNβ cells were more resistant to the anti-proliferative effect of IFNβ, than B16/IFNβ cells. The growth of B16 cells was inhibited upon co-cultivating with C57Fb cells transduced with BV carrying the *IL-21* gene. Overall, we have demonstrated that MEFs can be used as potential anti-tumor vehicles delivering IFNβ or IL-21 to malignant melanoma. The preliminary findings of the present study support the further investigation of MEF-based gene therapy as a strategy for the treatment of melanoma B16 or other malignant tumors *in vivo*.

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Ембріональні фібробласти миші, які експресують IFNβ або IL-21, інгібують проліферацію клітин меланоми *in vitro*

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Мета. Вивчення впливу ембріональних фібробластів миші (C57Fb), трансдукованих бакуловірусними векторами (БВ), що містять гени мишачого *Ifnβ* або *IL-21* людини, на виживаність і проліферацію злоякісних клітин меланоми миші (B16) *in vitro*. **Методи.** Трансдукція клітин, конструювання БВ, виділення РНК, кількісна ПЛР. **Результати.** Показано, що до антипроліферативного ефекту IFNβ виявилися чутливими всі досліджувані клітини як нормальні C57Fb, так і пухлинні B16; максимальну чутливість мали клітини B16, які швидко діляться. БВ забезпечували стабільну експресію гена *Ifnβ* на рівні мРНК протягом 5 діб в клітинах C57Fb/IFNβ. Проліферація клітин B16 пригнічувалася при сумісному культивуванні з C57Fb/IFNβ або C57Fb/IL21 клітинами. **Висновки.** IFNβ і IL-21, синтезовані ембріональними фібробластами C57Fb, трансдукованими БВ з генами *Ifnβ* або *IL-21* інгібували проліферацію клітин B16 при їх сумісному культивуванні *in vitro*.

Ключові слова: ембріональні фібробласти, клітини меланоми, інтерферон β (IFNβ), інтерлейкін-21 (IL-21), бакуловірусні вектори, трансдукція.

Эмбриональные фибробласты мыши, экспрессирующие IFNβ или *IL-21*, ингибируют пролиферацию клеток меланомы *in vitro*

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Цель. Изучение влияния эмбриональных фибробластов мыши (C57Fb), трансдуцированных бакуловирусными векторами (БВ), содержащими гены мышиного *Ifnβ* или *IL-21* человека, на выживаемость и пролиферацию злокачественных клеток меланомы мыши (B16) in vitro. **Методы.** Трансдукция клеток, конструирование БВ, выделение РНК, количественная ПЦР. **Результаты.** Показано, что к антипролиферативному эффекту IFNβ оказались чувствительными все исследуемые клетки как нормальные C57Fb, так и опухолевые B16; максимальной чувствительностью обладали быстро делящиеся клетки B16. БВ обеспечивали стабильную экспрессию гена *Ifnβ* на уровне мРНК в течение 5 суток в клетках C57Fb/IFNβ. Пролиферация клеток B16 подавлялась при со-культивировании с C57Fb/IFNβ или C57Fb/IL21 клетками. **Выводы.** IFNβ и IL-21, синтезируемые мышиными эмбриональными фибробластами, трансдуцированными БВ с генами *Ifnβ* или *IL-21* ингибировали пролиферацию клеток меланомы B16 *in vitro*.

Ключевые слова: эмбриональные фибробласты, клетки меланомы, интерферон β (IFNβ), интерлейкин-21 (IL-21), бакуловирусные векторы, трансдукция.

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