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APPLICATION OF NANOPARTICLES BASED ON RARE EARTH ORTHOVANADATES TO INACTIVATE EHRLICH CARCINOMA GROWTH

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The research of the peculiarities of Ehrlich carcinoma growth *in vivo* after incubation with nanoparticles based on rare-earth orthovanadates of spherical, spindle-like and rod-like shapes under different concentrations was the aim of this study. By immune fluorescence method there were quantitatively assessed the tumor precursors of various differentiation rate on the presence of phenotype markers CD44, CD24, CD 117 and Sca-1. The inhibition of tumor process after pre-treatment of Ehrlich carcinoma cells with nanoparticles of all the shapes and concentrations has been demonstrated. Nano-spindles of 0.875 g/l concentration were in a greater extent capable of tumor growth inhibiting that stipulates a maximal survival of tumor-bearing mice. There has been shown a significant re-distribution in growing tumor of the content of the precursors with the mentioned above phenotype markers after pre-treatment of inoculated Ehrlich carcinoma cells with nanoparticles of all the shapes and concentrations. Predictive value of the coefficient of $CD44^{hi}$ to $CD117^{+}$ cells' ratio when assessing the anti-tumor therapy was found.

Key words: nanoparticles, cancer stem cells, Ehrlich carcinoma.

Nowadays one of the most developing research area of oncology is "cancer nanotechnology", combining the novel scientific achievements in the fields of nanotechnology, engineering and medicine with the power of visualization, molecular diagnostics and targeted therapy of cancer [1].

The most important tools for identifying the tumors are non-invasive means of imaging, including computed and positron emission tomography, as well as nuclear magnetic resonance. Use of the contrasts based on nanoparticles (NPs) with the selective localization in tumor tissues can greatly increase the sensitivity and specificity of current methods of tumor imaging *in vivo* [2]. Nanoluminophores based on orthovanadates doped with europium $ReVO_4$: Eu^{3+} (Re = Y, Gd, La), have a significant Stokes shift of their luminescence, enabling the separating of the cell autofluorescence from a probe fluorescence [3]. Therefore, their application as the means of imaging of biological objects is very promising. Goltsev et al. [4] have previously shown that the NPs of rare-earth orthovanadates $GdYVO_4$: Eu^{3+}, developed by the Institute of Scintillation Materials of the National Academy of Sciences of Ukraine (Kharkiv) [5] were able of visualization of almost all the cells with $CD44^{+}$ phenotype, the functional activity of those determines the tumor intensity. It is of interest to evaluate the ability of these hybrid nano-complexes of such a composition to change the functional activity of the EC cells *in vivo*.

Klochkov et al. [3] reported about the relationship between the shape of NPs based on orthovanadates of rare-earth elements

with their possible transmembrane transport and accumulation in intranuclear structures. When studying the toxicity, accumulation and excretion dynamics of the NPs based on orthovanadates it has been found that their peroral and intraperitoneal injection to rats did not lead to the death of animals and the change in the mass coefficients of internal organs, that allowed the considering of the synthesized samples of nano-vanadates as virtually non-toxic compounds (V toxicity class) [6]. Complete elimination of NPs out of a body occurred 30 days later the injection. However, the elucidation of the ability of NPs of the orthovanadates based on rare-earth elements to change the proliferative and metabolic activity of targeted cells has remained an urgent task, especially when the case is referred to tumor cells [7].

Now it is taken for granted that tumors are heterogeneous in their compositions, and each of their cell sub-population has its own value in its development [8, 9]. Initiation and maintenance of tumor growth are due to the existence of a small population of cancer stem cells (CSCs), which are capable of unlimited proliferation [10]. As well as most regional stem cell the CSCs are in G_0 phase of a cell cycle, that is largely due to their resistance to chemo- and radiotherapy, resulting in frequent relapses of the disease [11]. In this regard, one of the tasks of current oncology is the search for the drugs targeted to recognize and inactivate CSCs.

CSCs and their progenitors are shown to express a certain spectrum of phenotypic markers. Thus, for CSCs of breast cancer (BC), the presence of CD44, CD133, ESA (epithelial surface antigen) markers on their surface and the absence/presence of a low level of CD24 expression is characteristic [10, 12]. It is shown that all the signs of CSCs in breast cancer (capability for self-renewal, high invasiveness and chemoresistance) are inherent to the cells with high expression rate of CD44 marker and absence of CD24 expression, which are identified as $CD44^{hi}CD24^{low}$ [13, 14].

CSCs expansion is implemented under their microenvironment conditions, i.e. due to accessory-regulatory cells, which directly controlling their functional potential [15, 16]. Regulatory and accessory cells make about 90% of total cells of the breast, stomach, pancreas carcinomas [8]. Not surprisingly, that in tumor site there were identified those cells which have been for a long time referred to the cells of hematopoietic elements ($Sca\text{-}1^+$, $CD117^+$). However, their contribution to the regulation of tumor growth has not been completely discovered.

The use of phenotypic identification of progenitor cells of various differentiation levels in tumor focus makes it possible not only to assess the developmental stage and dynamics of invasive tumors, but also to determine its sensitivity to perform therapy, including the effect of different forms of nanocomposites. As Evangelou et al. [17] demonstrated, the vanadium compounds had an anti-tumor effect due to the inhibition of cell phosphatase and activation of tyrosine phosphorylases. This leads to activation of signal transduction pathways triggering apoptosis and/or activation of tumor-suppressor genes. It has been found that the vanadium compounds with malonic acid in certain concentrations inhibited the growth of cultures of various cancer cell lines without causing a cytotoxic effect on normal human skin fibroblasts [18]. According to the authors' data, the antitumor effect was concentration dependent. Thus, after treatment of mouse fibrosarcoma cells (L929) with vanadium compounds with malonic acid at concentrations of 0.5–1 μg/ml an inhibitory effect on cell proliferative activity was not detected, but an increase in the concentration of vanadate up to 1.5 μg/ml contributed to the inhibition of culture growth by 60%, and in a dose of 6 μg/ml by 74% [18].

A convenient model for experimental studies to evaluate the antitumor activity of various therapeutic agents is a transplantable tumor cell line of Ehrlich carcinoma (EC), derived from a spontaneous mouse breast cancer (BC) [19]. As it has been shown above, in a heterogeneous pool of breast cancer cells there were identified the CSCs by a number of phenotypic markers. However, for the EC ascitic form which is cultivated in peritoneal cavity of mice the subpopulation composition and concentration relationship of progenitor cells of various levels of differentiation have not been clearly established yet. In a few studies there have been shown that low-frequency orthovanadates doped with europium, could be selectively accumulated in the $CD44^+$ fraction of EC cells having an increased tumorigenic potential [5]. Taking into account the ability of vanadium salts to manifest an antitumor effect, the goal was to evaluate the ability of NPs of orthovanadates of different shapes and concentrations to inactivate the functional potential of the tumor cells.

Materials and Methods

The experiments were performed in 8-month-old female BALB/c mice weighing 16–18 g in accordance with the "General Principles of Experiments in Animals" approved by the 5th National Congress in Bioethics of Ukraine (Kyiv, 2013) and consistent with the statements of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986).

Stabilization of EC cells after cryopreservation. As primary cultures there were used the cryopreserved by the method of Goltsev et al. [20] EC cells were stored at the low-temperature bank of the Institute for Problems of Cryobiology and Cryomedicine (Kharkiv). Thawed and washed-out with saline the EC cells were thrice inoculated *in vivo* to achieve the morphofunctional characteristics of EC native culture [21]. For this aim the EC cells were intraperitoneally injected in a dose of 3×10^6 cells/mouse in volume of 0.3 ml and cultured for 7 days in the peritoneal cavity (PC). To day 7 of *in vivo* culturing there was determined an absolute number of cells in PC taking into account the volume of ascitic fluid and the concentration of cells per ml. EC development was considered as sufficient if in the PC of mice an absolute number of cells was at least 3.5×10^8 [21].

Nanoparticles of orthovanadates ($ReVO_4$: Eu^{3+} (Re = Y, Gd, La)) of various geometric shapes were synthesized as developed by Klochkova [5]. The solid phase of aqueous colloidal solution were the NPs of: spherical shape $GdYVO_4$: Eu^{3+} with 2–3 nm average diameter, spindle-like $GdVO_4$: Eu^{3+} with an average size of 5×40 nm and rod-like $LaVO_4$: Eu^{3+} — 12×250 nm shapes.

Pre-treatment of EC cells with nanoparticles. Pre-washed with saline "stabilized" EC cells were incubated *in vitro* with an aqueous colloidal solution of NPs of spherical, spindle and rod-like shapes in 9:1 ratio so that the final concentration of NPs in the samples was 0.875 and 4.38 g/l. Incubation time (3 hr) provided their maximum accumulation in cells [3]. Choice of NPs concentration was determined by the fact that incubation of cells with orthovanadate molecules in concentration below 0.8 g/l is inadequate to detect luminescence in the cells, and the one higher than 4.4 g/l leads to a significant reduction in the number of viable non-malignized cells [22].

In the research there were following variants of EC cells' treatment: 1 — 1×10^7 EC cells in 0.9 ml of 5% glucose + 0.1 ml spherical NPs diluted in 5% glucose solution (0.875 g/l); 2 — 1×10^7 EC cells in 0.9 ml solution of 5% glucose + 0.1 ml spherical NPs diluted in 5% glucose solution (4.38 g/l); 3 — 1×10^7 EC cells in 0.9 ml of 5% glucose + 0.1 ml spindle-like NPs diluted in 5% glucose solution (0.875 g/l); 4 — 1×10^7 EC cells in 0.9 ml of 5% glucose + 0.1 ml spindle-like NPs diluted in 5% glucose solution (4.38 g/l); 5 — 1×10^7 EC cells in 0.9 ml of 5% glucose + 0.1 ml rod-like NPs diluted in 5% glucose solution (0.875 g/l); 6 — 1×10^7 EC cells in 0.9 ml of 5% glucose + 0.1 ml rod-like NPs diluted in 5% glucose solution (4.38 g/l); the control were EC cells, incubated in 5% glucose solution with no treatment of NPs.

After pre-treatment the EC cells were washed thrice by adding the normal saline (1:1) and subsequent centrifugation of excessive NPs. Then the EC cells were intraperitoneally injected with 3×10^6/mouse with physiological solution so that the volume of injected suspension did not exceed 0.3 ml and following the cells were cultured for 7 days in PC.

Phenotypic characteristics of the EC cells of all the studied groups were assessed to day 7 of development with a flow cytometer FACS Calibur (Becton Dickinson, USA) using monoclonal antibodies (BD Biosciences, USA) to the CD44 (FITC) (№ 553133, clone IM7) and CD117 (FITC) № 553352, clone 2B8) and CD24 (PE) (№ 553 262, clone M1 / 69). As the control there were used the samples with adding non-immune FITC and PE labelled monoclonal antibodies of the same isotype (BD Biosciences), (№ 553 988, A95-1 and clone number 553 989, clone A95-1), as the antibodies to the tested marker. Using CD44 (FITC) and CD24 (PE) monoclonal antibodies there was performed an immune phenotypic double staining. The cells with an average fluorescence value of CD44-marker more than 10^3 (on a logarithmic scale) were referred to $CD44^{hi}$-subpopulation. The results were accounted and analyzed with the software WinMDi 2.9 (Joseph Trotter, La Jolla, USA).

In each of the studied groups the following parameters were determined:

$CD44^{hi}$/ $CD117^+$ index as a ratio of relative content of $CD44^{hi}$-cells to the one of $CD117^+$-cells;

inhibition rate (I_r) of EC growth, which is calculated by the formula:

$$I_r = [(V(c) - V(e)) / V(c)] \times 100\%,$$

where V (c) — absolute number of EC cells in PC of the control group, V (e) — absolute

number of EC cells in PC of experimental group;

survival of animals was estimated to day 20 after intraperitoneal injection of non-treated and treated EC cells with all the types and concentrations of NPs.

For statistical analysis there was used the method of descriptive statistics to study the statistical parameters of the distribution of attributes (arithmetic mean, arithmetic error of the mean, standard deviation, median, confidence interval) and statistical analysis to test the hypothesis. The normality of the distribution of quantitative traits was checked using a common criterion of examining the symmetry and kurtosis of zero. In a normal distribution a variable significance of differences between the groups was estimated using Student t-test, and in the absence of the normal distribution of variables there was used the U-Mann-Whitney test. Differences were considered as statistically significant at $P < 0.05$.

Results and Discussion

In therapies of clinical oncology practice both quantitative characteristic and differentiation rate of tumor cells should be considered. Ehrlich ascites carcinoma is a re-transplantable line of undifferentiated cells of the mice's breast cancer and it is also an appropriate model for experimental studies to evaluate the antitumor activity of various therapeutic agents [19]. During BC development as a classic manifestation there have been already characterized the most undifferentiated cells possessing all the features of CSCs. They have a $CD44^{hi}CD24^{-/low}$ phenotype and represent less differentiated cells, the number of which in the basal breast cancer is less than 10% [23]. In orthotopic implantation of 5×10^5 RAS-transformed cells $CD44^{hi}$- and $CD44^{lo}$-cells to the NOD/SCID mice it has been shown that a low tumorigenicity was inherent to $CD44^{lo}$-subpopulation (tumor was formed in 30% of cases), herewith the tumors were morphologically differentiated while $CD44^{hi}$-cells were capable of forming tumors in 100% of cases [24]. A subpopulation of $CD44^+CD24^-$-, as well as of $CD44^{hi}$-cells, is able of tumor generating in a recipient's body. Introducing of mice with just 200 cells of $CD44^+CD24^{-/lo}$ ESA^+ phenotype led to the formation of solid tumors in 100% of cases within 5 months after injection [9]. The cells with $CD44^+CD24^+$ phenotype are more advanced in differentiation as compared with $CD44^+CD24^-$-subpopulation of breast cancer cells and are able of forming new tumors only in 30% of cases when injected in a dose of 1×10^5 cells/mouse [25]. Despite an adequate study of the historical experience of the cultivation of EC cells *in vivo* the question about the subpopulation composition of the tumor is still disputable.

Considering the EC as an experimental model of breast cancer, Goltsev et al. [26] confirmed that isolated by immune-magnetic sorting fraction of $CD44^+$-cells has a much higher potential for tumorigenicity compared with $CD44^-$ subpopulation. The authors showed that in the culture of EC induced with $CD44^+$-fraction, there was observed a preferential formation of the cells with $CD44^+$ and $CD44^{hi}CD24^-$ phenotype.

This research results suggest the presence in a heterogeneous population of EC of the cells with the signs of CSCs of various differentiation levels and of those which can be attributed to the accessory-regulatory elements of microenvironment. Concentrations of the cells with these characteristics in a common pool of EC (control) are presented in the Table.

It is known that $CD44^-$ — molecule is one of the most characteristic markers of CSCs responsible for their self-renewal and proliferation [23, 27]. No less important function of tumor cells, i.e. ability to metastasize is determined by the presence on their surface of glycoprotein CD24. The rise in the synthesis rate of CD24 is accompanied by an increase in the "aggressiveness" of the tumor due to enhancing the "cell-to-cell" and "cell-to-extracellular matrix" cooperation. It has been found that the most undifferentiated CSCs of breast cancer do not express CD24-receptor [12]. Cancer stem cells with $CD44^{hi}CD24^{-/lo}$ phenotype are capable of unlimited self-renewal, representing the highest level in the hierarchy of differentiation [9]. However, with the development of tumor in its certain sites a hypoxia develops that leads to an increased synthesis of hypoxia-inducible factor (HIF), serving as a signal for producing CD24. Increasing of the amount of CD 24 — receptors on the more differentiated tumor cells contributes to their dissemination in places with more favorable conditions for the development of new tumor sites [28]. Thus the subpopulation of $CD44^-CD24^+$ is more differentiated and has the minimal tumorigenic activity [10].

Quite interesting is the established in our research fact of the presence in EC of the $Sca\text{-}1^+$ — cells. Sca-1 is a classic marker of

hematopoietic stem cells in bone marrow of mice [29], also expressed by the populations and other progenitor cells, including breast cancer progenitors [30]. The Sca-1^+-cells of BALB-neuT mice BC are shown to have an enhanced ability to form spheres *in vitro* and tumor-generating activity when re-inoculating *in vivo* [30]. Li et al. [31] showed that in the breast tissue of healthy C57BL/6 mice the number of Sca-1^+-cells did not exceed 20%, while in transgenic mice with the development of BC due to the *wnt* oncogene activation their number was 3 times higher.

Recently the attention of researchers has been attracted to the cells making-up the tumor microenvironment, providing the functioning of CSCs due to secretion of cytokines, chemokines and growth factors [16, 32]. One of the candidates for the role of the tumor microenvironment are $CD117^+$-cells. In ovarian cancer there was noted an increase in the expression rate of CD117/CD73-marker the very in accessory fibroblast stromal cells, which is a bad prognostic sign [33]. Depending on concentration the $CD117^+$-cells are able of blocking or activating the targeted genes in different cells of a heterogeneous pool of CSCs [32]. The established by us fact of $CD117^+$-subpopulation presence in a heterogeneous pool of EC (Table) suggests that they belong to the tumor accessory-regulatory elements with the corresponding function as for CSCs.

Application of cytostatics and/or radiation therapy when treating the cancer pathology leads to a redistribution of tumor subpopulation composition by including the cascades of activation /inhibition of proliferative activity of stem cells, initiating/inactivating the tumor process. Presented in the table data indicate that pretreatment of EC cells with NPs and subsequent their inoculation into the peritoneal cavity of animal led to the marked changes in the formation of subpopulations of progenitor cells of various levels of differentiation. Thus, after using the spherical NPs in both concentrations (variants 1 and 2) in the *in vivo* generated pool of EC cells the content of all the subpopulations decreased which were CD44 positive. In a greater extent there was reduced the content of $CD44^+CD24^-$-cells. Pretreatment of the cells with spherical NPs at a concentration of 0.875 g/l has almost no effect on the formation of the most "mature" $CD44^-CD24^+$-population, and a rise in the concentration of NPs up to 4.38 g/l resulted in its decreased content (Table).

Pretreatment of EC cells with the NPs of spindle-like shape at lower concentration (variant 3) resulted in the maximal increase in the content of $CD44^{hi}$-cells (3.5-fold)

Phenotypical markers and index of $CD44^{hi}/CD117^+$ cells ratio of Ehrlich carcinoma prior to and after pre-treatment of nanoparticles based on orthovanadates

Index	Control	Pre-treatment variants					
		1	2	3	4	5	6
		Spherical NPs (0,875 g/l)	Spherical NPs (4,38 g/l)	Spindle-like NPs (0,875 g/l)	Spindle-like NPs (4,38 g/l)	Rod-like NPs (0,875 g/l)	Rod-like NPs (4,38 g/l)
$CD44^{hi}$,%	0.14± 0.009	0.05± 0.004*	0.09± 0.006*#	0.47± 0.03*	0.06± 0.004*#	0.07± 0.005*	0.04± 0.006*#
$CD44^+CD24^-$,%	3.38± 0.21	0.75± 0.05*	0.69± 0.05*	1.45± 0.10*	0.52± 0.04*#	0.50± 0.04*	0.26± 0.02*#
$CD44^+CD24^+$,%	2.17± 0.02	0.62± 0.04*	0.87± 0.06*#	1.19± 0.08*	0.33± 0.02*#	0.49± 0.03*	0.28± 0.02*#
$CD44^-CD24^+$,%	5.33± 0.42	4.97± 0.31	2.85± 0.20*#	1.45± 0.10*	0.52± 0.04*#	2.40± 0.20*	3.61± 0.32*#
Sca-1^+,%	90.32± 5.33	87.96± 6.21	91.30± 6.41	77.80± 3.41*	92.61± 6.50#	90.90± 6.42	83.12± 5.83#
$CD117^+$,%	7.81± 0.52	1.02± 0.07*	0.36± 0.04*#	0.38± 0.03*	1.07± 0.07*#	1.68± 0.15*	0.18± 0.02*#
$CD44^{hi}/CD117^+$,arb. units	0.02± 0.001	0.05± 0.002*	0.25± 0.03*#	1.24± 0.07*	0.06± 0.005*#	0.04± 0.002*	0.20± 0.02*#

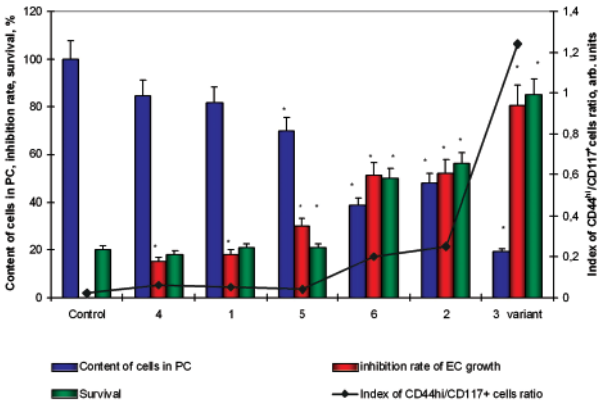
Notes: differences are statistically significant if compared to the control (*); between different concentrations of one type of nanoparticles (#), ($P < 0.05$).

compared to the control (Table). Herewith there was found a decrease of all other EC subpopulations, including the most "conservative" Sca-1^+-population, the content of which was not significantly changed in any of the groups. It likely appears that in concentration of 0.875 g/l the spindle-like NPs namely via the inhibiting the function of Sca-1^+-cells were capable of blocking the differentiation potential of CSCs ($CD44^{hi}$), resulting in rising the concentrations on the background of lowering the content of more differentiated cells. Increase in the concentration of spindle-like NPs up to 4.38 g/l (variant 4) *vice versa* contributed to a two-fold decrease in the content of $CD44^{hi}$-cells if compared with the control, and almost to an eight-fold one if compared with the variant 3. It is important that in a concentration of 4.38 g/l the NPs are equally (in 6.5 times if compared to the control) caused a reduction of $CD44^+CD24^-$ and $CD44^+CD24^+$-cells of EC and in 10 times for the most differentiated subpopulation ($CD44^-CD24^+$).

Previously with the methods of fluorescence microscopy and spectroscopy, we have shown that the rod-like NPs in contrast to spherical ones were unable to visualize the EC cells [5]. However, the Table shows, that pretreatment of EC cells with these NPs (variants 5 and 6) causes a significant reduction of all the assessed cell subpopulations, except Sca-1^+. Furthermore, the pretreatment of the EC cells with rod-like NPs in concentration of 4.38 g/l causes the formation of tumors with a minimum content of both the most potent CSCs ($CD44^{hi}$) and microenvironmental cells ($CD117^+$) in comparison with the same indices for other variants of pretreatment.

This can be explained by the existence of several ways via which the NPs can indirectly disorder the cell membrane integrity. These mechanisms include the destabilization of membrane, the damage because of generation of reactive oxygen species etc. [34]. Herewith the "damaging" activity of NPs is noted to be determined by their charge. It has been demonstrated that macromolecules with a symmetric tree-like structure having a charge, can electrostatically interact with biological membranes, forming therein the openings of 15–40 nm diameter [35]. We can assume that the used in this research rod-like NPs can thereby interact with the membranes of tumor cells.

Effect of antitumor therapy is often associated with elimination rate of CSCs in a heterogeneous pool of tumor cells. It has previously been shown that preventive administration of native and cryopreserved fetal liver cells to C3H mice with predetermined development of breast cancer actually resulted in the reduced content of CSCs with $CD44^{hi}$- and $CD44^+CD24^-$ phenotype in mammary glands. This prevented the formation of palpable breast tumors at 16-month-old animals [14]. However, under the impact of nanomaterials on the tumor the oncology activation/inhibition may be determined by functional state of not only CSCs, but also by accessory-regulatory cells of the microenvironment, such as $CD117^+$. This was clearly demonstrated in the variant, where in spite of significant (about 3.5-fold) increase in the concentration of $CD44^{hi}$ -cells, there was a sharp (higher than 20-fold compared to the control) reduction of the concentration of $CD117^+$-cells. It is important that such a redistribution of the EC subpopulation composition was expressed in maximum increase of the index of the $CD44^{hi}/CD117^+$ ratio, resulting in maximum inhibition of tumor growth by 80.34% (Figer). The extent of EC growth inhibition in variants 2 and 6 was approximately equal (51.0% and 52.0%) and significantly lower than in group 3. However, tumor growth inhibition in variants 2 and 6 was accompanied by a decrease in the content of both $CD44^{hi}$- and $CD117^+$-cell subpopulations of EC cells (Table). It is of interest, that



Content of cells in peritoneal cavity, inhibition rate of EC growth, survival of animals and index of $CD44^{hi}/CD117^+$ cells ratio after pre-treatment with nanoparticles of various shapes and concentrations

The number of cells in PC in the control is assumed as 100%; * — differences are statistically significant if compared to the control ($P < 0.05$); axis X shows the variants of treatment of the cells with NPs in an ascending order of their anti-tumor activity.

in variant 6 there was observed the most pronounced reduction of $CD117^+$-cells using all the types and concentrations of NPs. The index of the $CD44^{hi}/CD117^+$ ratio in variants 2 and 6 was roughly the same (0.25 ± 0.03 and 0.20 ± 0.02, respectively). Tumor growth inhibition after treatment with NPs seems to be caused rather by a change in the concentration of relationship of different subpopulations of progenitors, directly or indirectly affecting the functional status of each other (see Table) than elimination of certain subpopulations of EC cells. The validity of this supposition is confirmed by the fact that the pretreatment variants 1, 4, 5, although caused a statistically significant reduction in the number $CD44^{hi}$-cells (CSCs) and their accessory-regulatory microenvironment ($CD117^+$), but failed to provide an inhibition of tumor growth and did not result in an improved survival of animals. The index of the $CD44^{hi}/CD117^+$ ratio in these three cases did not have statistically significant differences.

Thus, the obtained results demonstrate the ability of the synthesized NPs of rare-earth orthovanadates elements of spherical, spindle- and rod-like shapes to inhibit tumor in the EC experimental model. Tumor growth inhibition rate depends on the shape and concentration of NPs. Inhibition of the tumor growth was accompanied by an altered ratio of tumor cells of various levels of differentiation with different phenotypic markers in the total pool of EC. The greatest inhibitory effect possessed by spindle-like NPs in concentration of 0.875 g/l, which was followed by increasing the maximum correlation index $CD44^{hi}/CD117^+$.The highest degree of increase in the index of $CD44^{hi}/CD117^+$ ratio coincides with the maximum survival of tumor-bearing mice. This parameter can be used in determining the prognosis of cancer pathology and the effectiveness of its treatment.

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ЗАСТОСУВАННЯ НАНОЧАСТИНОК НА ОСНОВІ ОРТОВАНАДАТІВ РІДКІСНОЗЕМЕЛЬНИХ ЕЛЕМЕНТІВ ДЛЯ ІНАКТИВАЦІЇ РОСТУ АДЕНОКАРЦИНОМИ ЕРЛІХА

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Метою роботи було дослідження особливостей росту аденокарциноми Ерліха *in vivo* після інкубації з наночастинками на основі ортованадатів рідкісноземельних металів сферичної, веретеноподібної і стрижнеподібної форми за різних концентрацій. Методом імунофлуоресценції здійснено кількісне оцінювання пухлинних прекурсорів різного ступеня диференціювання за наявністю фенотипових маркерів CD44, CD24, CD117 і Sca-1. Показано гальмування пухлинного процесу після передобробки клітин аденокарциноми Ерліха наночастинками усіх форм та вивчених концентрацій. Найбільшою мірою до гальмування росту пухлини були здатні веретеноподібні наночастинки за концентрації 0,875 г/л, що зумовлювало максимальну виживаність мишей-пухлиноносіїв. Показано істотний перерозподіл вмісту в зростаючій пухлині прекурсорів з вищезазначеними фенотиповими маркерами після передобробки перевивних клітин аденокарциноми Ерліха вивченими наночастинками. Встановлено прогностичну значущість коефіцієнта співвідношення $CD44^{hi}$ до $CD117^{+}$-клітин в оцінці ефективності протипухлинної терапії.

Ключові слова: наночастинки, стовбурові ракові клітини, аденокарцинома Ерліха.

ПРИМЕНЕНИЕ НАНОЧАСТИЦ НА ОСНОВЕ ОРТОВАНАДАТОВ РЕДКОЗЕМЕЛЬНЫХ ЭЛЕМЕНТОВ ДЛЯ ИНАКТИВАЦИИ РОСТА АДЕНОКАРЦИНОМЫ ЭРЛИХА

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Целью работы было исследование особенностей роста аденокарциномы Эрлиха *in vivo* после инкубации с наночастицами на основе ортованадатов редкоземельных металлов сферической, веретеноподобной и стержнеподобной форм в разных концентрациях. Методом иммунофлуоресценции осуществлена количественная оценка опухолевых прекурсоров разной степени дифференцировки по наличию фенотипических маркеров CD44, CD24, CD117 и Sca-1. Показано торможение опухолевого процесса после предобработки клеток аденокарциномы Эрлиха наночастицами всех форм и изученных концентраций. В наибольшей степени торможению роста опухоли способствовали веретеноподобные наночастицы в концентрации 0,875 г/л, что обусловливало максимальную выживаемость мышей-опухоленосителей. Показано существенное перераспределение содержания в растущей опухоли прекурсоров с вышеуказанными фенотипическими маркерами после предобработки перевиваемых клеток аденокациномы Эрлиха изученными наночастицами. Установлена прогностическая значимость коэффициента отношения $CD44^{hi}$ к $CD117^{+}$-клеткам в оценке эффективности противоопухолевой терапии.

Ключевые слова: наночастицы, стволовые раковые клетки, аденокарцинома Эрлиха.