Proliferative activity of Ehrlich carcinoma cells after use of nanocomplexes

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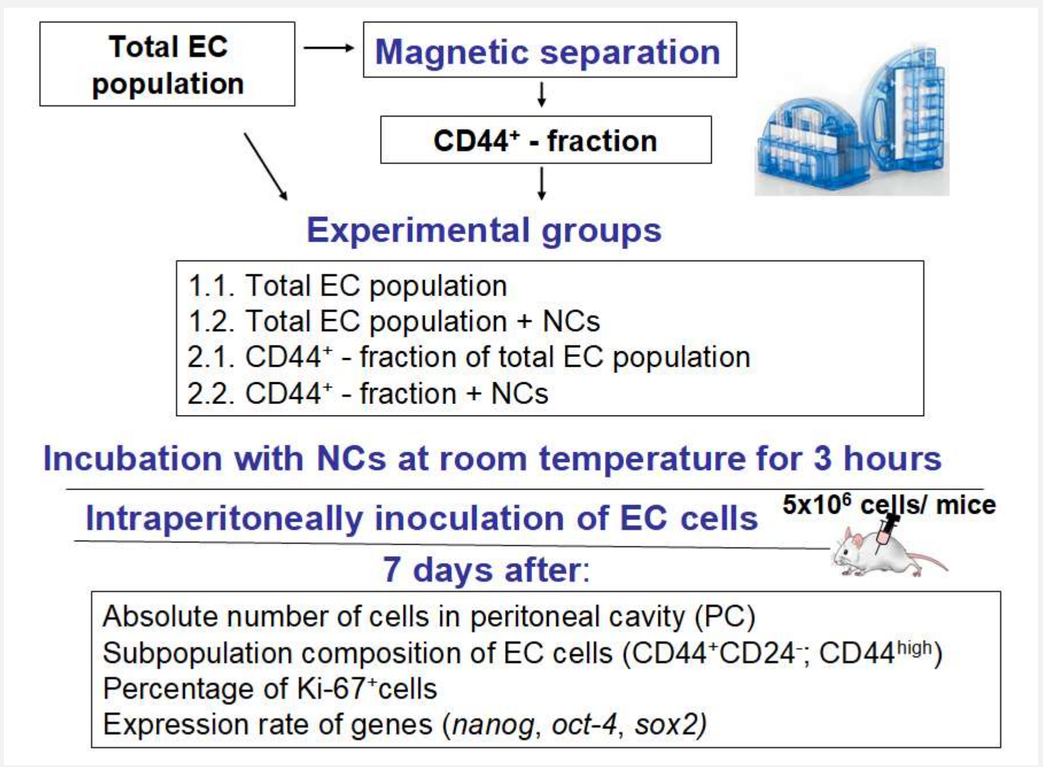
The development of criteria for evaluating the effectiveness of antitumor therapy is a key task of oncology. One of them may be determining the proliferative status of tumor cells using the marker Ki-67, a protein present in actively dividing cells. In addition, the completeness of implementation by tumor cells of their tumorigenic potential depends on the expression rate of the CD44 marker. Cells with the $CD44^+CD24^{-/\,low}$ phenotype are cancer stem cells (CSCs) with unlimited ability to proliferate and self-maintenance. This function of CSCs is realized by the interaction of CD44 with its ligand, hyaluronic acid, which promotes epithelial-mesenchymal transition (EMT). EMT is believed to be controlled by the activity of a number of stemness genes, in particular *nanog*, *oct4*, *sox2*.

The search for compounds capable of targeting the cells with the highest oncogenic potential is underway. In this regard the use of orthovanadates of rare earth metals in nanoform is promising.

A convenient model to investigate an antitumor activity of the novel therapeutic agents is Ehrlich carcinoma (EC), a heterogeneous pool of which contains highly proliferating $CD44^+$ cells and less potent precursors.

This research was aimed to compare the ability of nanocomplexes (NCs) containing spherical nanoparticles $GdYVO_4:Eu^{3+}$ and cholesterol to change the proliferative status and stemness activity of tumor cell genes formed by cells of the total EC population and the most highly proliferating $CD44^+$- fraction.

Experiment Design



Results

After 7 days of *in vivo* culturing, the tumorigenic potential of $CD44^+$-fractions was 15 times higher than that of the total EC population, evidenced by the absolute number of cells in PC. Pre-treatment of cells of the total population of EC with NCs led to inhibition by 65% of tumor growth intensity, while after similar treatment of $CD44^+$- fraction the corresponding value decreased by 90% (Table).

The most important role in implementing tumorigenesis process is the CD44 marker expression rate. It was demonstrated that the number of $CD44^+CD24^-$ and $CD44^{high}$ cells in ascites formed by the $CD44^+$-fraction was significantly higher than in total EC population.

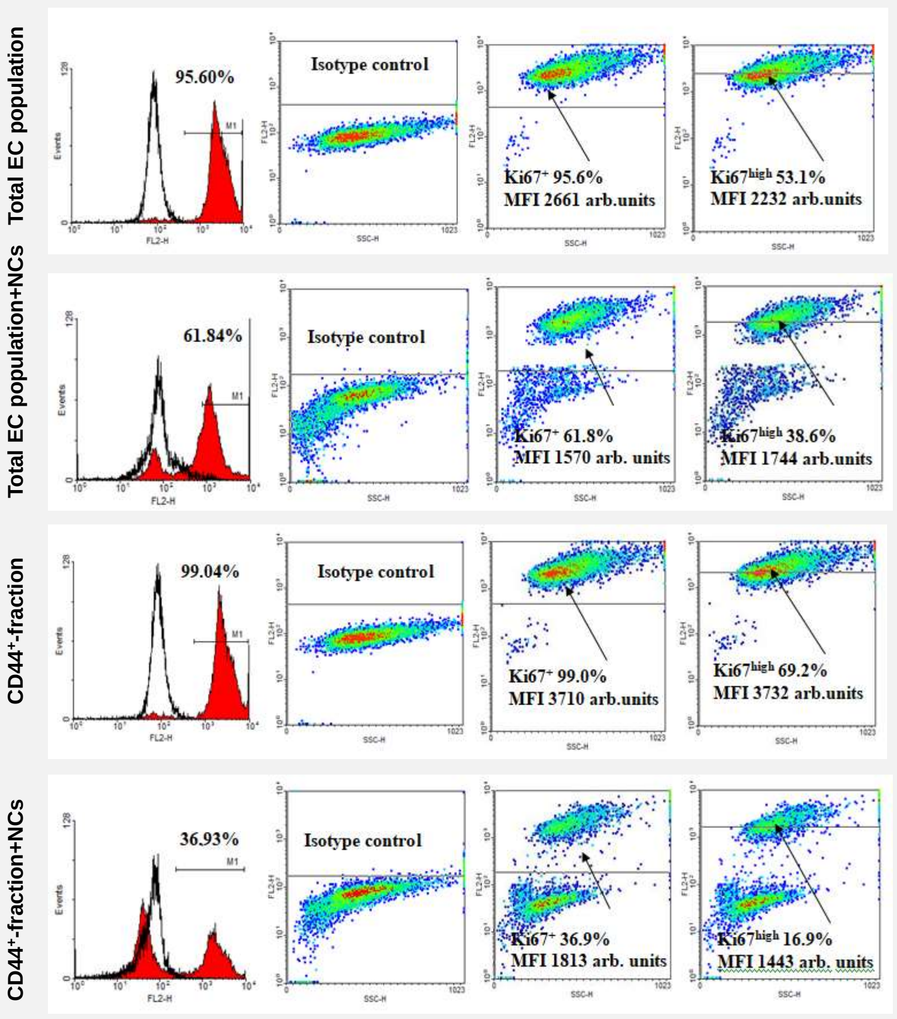
Indices of tumor growth intensity and subpopulation composition of EC cells after incubation with NCs *in vitro* and further *in vivo* culturing

Group	Total EC population		CD44+- fraction	
	EC	EC +NCs	CD44+- fraction	CD44+- fraction +NCs
Absolute number of cells in the PC, x10^7	52.3±7.6	18.3±1.2	826.5±6.5	91.4±4.9*
Tumor growth intensity, %	100	34.9±4.7*	100	11.1±0.8*
Rate of tumor growth inhibition, %	-	65.0±4.5	-	88.9±5.8
Percentage of $CD44^+CD24^-$cells, %	3.8±0.5	0.6±0.1*	5.6±0.7	0.5±0.04*
Percentage of $CD44^{high}$ cells, %	0.4±0.1	0.02±0.1*	1.2±0.1	0.05±0.1*
Percentage of Ki-67^+ cells, %	95.6±7.4	61.8±5.7*	99.0±8.5	36.9±3.7*
Percentage of Ki-67^{high} cells, %	53.1±4.8	38.6±4.6*	69.2±7.4	16.9±3.5*

*- difference is significant in comparison with the similar indices of EC and BM cells without incubation with NPs (Control 1 and Control 2), p <0.05

An evidence that NCs mediates antitumor activity due to changes in functional potential of $CD44^+$ cells is the fact that treatment of cells of total EC population with NCs led to the formation of ascites with a reduced in 18 times number of $CD44^{high}$ cells and a 6-fold decrease of $CD44^+CD24^-$ cells compared with the corresponding group without incubation with NCs. Even more pronounced changes caused by NCs occurred in $CD44^+$-fractions, i.e. the content of the most tumorigenic $CD44^{high}$ cells decreased by 24 times, and the number of $CD44^+CD24^-$ cells did by 11 times.

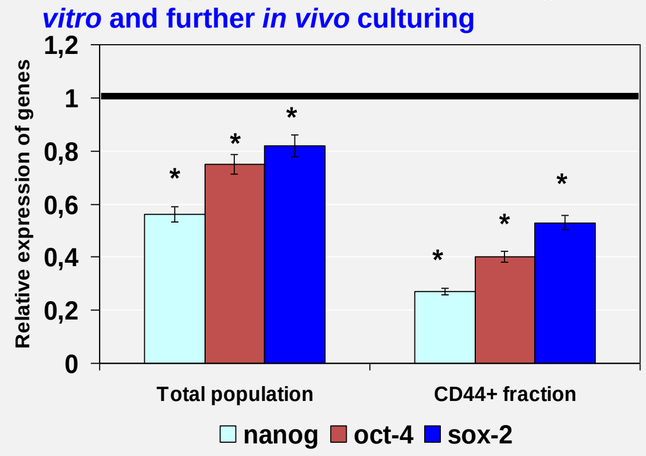
Percentage of Ki-67^+ cells of EC after incubation with NCs *in vitro* and further *in vivo* culturing



Ehrlich ascites carcinoma was found to be an actively proliferating tumor, judging by the number of Ki-67^+ cells, which on day 7 of cultivation of the EC total population was 95%, and $CD44^+$-fractions made 99%. The Ki-67^+ cells formed from the $CD44^+$-fraction had a significantly higher mean fluorescence intensity (MFI) compared to the total population of EC (3710 and 2661 arb. units, respectively).

In the tumor formed from the $CD44^+$-fraction, 69% of cells had a high rate of expression of the marker Ki-67^{high} with MFI 3732 arb.units. A similar rate of cells in total EC population was 53% (MFI 2232 arb.units). This confirms that the $CD44^+$-fraction comprises the cells with higher proliferative activity. Treatment with NCs of $CD44^+$-fraction led to the biggest reduction in the number of $CD44^{high}$ cells and Ki-67^{high} cells in the growing pool of EC, which resulted in almost 90% inhibition of tumor growth.

Relative expression rate of stemness genes of EC cells after incubation with NCs *in vitro* and further *in vivo* culturing



The statistically significant decrease in expression in total population and $CD44^+$ cells of stemness genes (*nanog, oct-4, sox-2*) was observed. Strong decrease in the tumorigenic activity of the $CD44^+$-fraction was noted, which might be associated with maximum inhibition of the key *nanog* gene, determining their self-maintenance.

Notes: The expression of genes in EC cells is represented as relative value where the expression of corresponding genes in EC cells with no treatment by NCs (control) is assumed as 1. The data were normalized according to the expression of the house-keeping gene Rn18s. * - difference is statistically significant in compared with the control indices; ($p<0.05$)

Conclusions

The ability of NCs to the greatest extent to inhibit proliferative activity cells of $CD44^+$ - fraction, judging by the decrease in the content of both $CD44^{high}$ and Ki-67^{high} cells in tumor. One of the ways of implementing the antitumor action of NCs may be inhibition of the activity of stemness genes in tumor cells.

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