# Nanocomplexes based on rare-earth metal orthovanadates are able to change activity of self-maintenance genes in Ehrlich carcinoma cells

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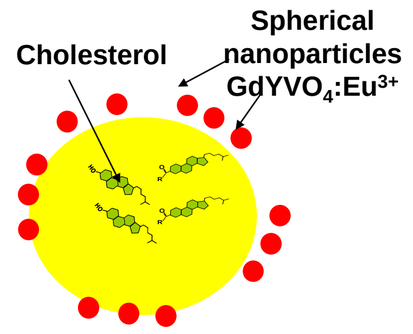
The research of the team of scientists of the Department of Cryopathophysiology and Immunology of the Institute for Problems of Cryobiology and Cryomedicine together with the Institute for Scintillation Materials of the NAS of Ukraine is focused on finding the ways to treat oncopathology using different types of nanomaterials. The search for methods to selectively recognize and inactivate the cancer stem cells (CSCs) with the $CD44^+$ phenotype having tumorigenic activity is an urgent task. An important aspect of the manifestation of the functional potential of CSCs is the expression of pluripotency genes, which can change both in the process of differentiation of these cells and under the influence of various factors. It was shown that hybrid nanocomplexes (NCs) are able to inhibit the functional activity of Ehrlich carcinoma cells (EC) [Goltsev A.N., 2017]. The implementation mechanism of this effect may be related to the modulation of the expression of self-maintaining genes in tumor cells, which was the aim of study.

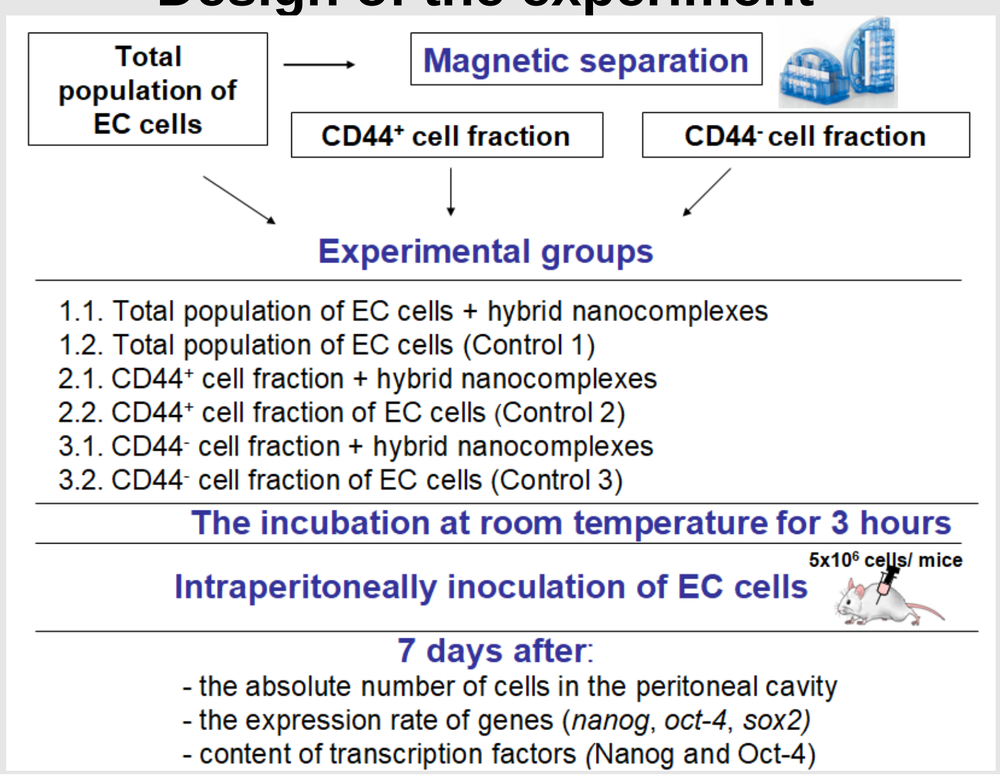


**Schematic representation of NC**




The hybrid nanocomplex is the product of aqueous dispersion of cholesterol (0,55 g/l) and NPs (1.3 g/l) of rare earth orthovanadates of $GdYVO_4:Eu^{3+}$ composition.

## Design of the experiment



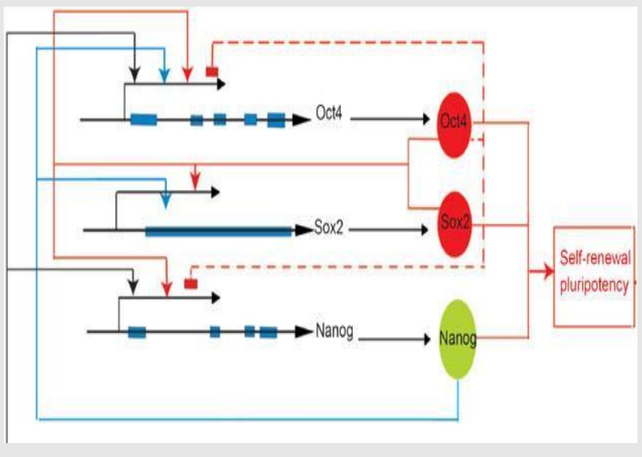

## Results

After 7 days of *in vivo* culturing of the EC cells of all the groups, the inhibitory effect of NCs on tumor growth was noted, and a statistically significant decrease in the intensity of growth rate of the $CD44^+$ fraction was observed.

| Group | Total ECs population | | $CD44^+$ fraction | | $CD44^-$ fraction | |
|---|---|---|---|---|---|---|
| | Absolute number of the cells in PC, $x10^7$ | Intensity growth of the EC, % | Absolute number of the cells in PC, $10^7$ | Intensity growth of the EC, % | Absolute number of the cells in PC, $x10^7$ | Intensity growth of the EC , % |
| Control (ECs) | 55.09±7.56 | 100 | 826.5±6,53* | 100 | 7.82±0,94* | 100 |
| ECs+NC | 18.27±1.23* | 33.16 | 91.40±4.87* | 11.05 | 6.83±2,55 | 87.34 |

Note: * - difference is statistically significant compared with similar indices of EC cells without incubation with NCs (control) ($p < 0.05$).

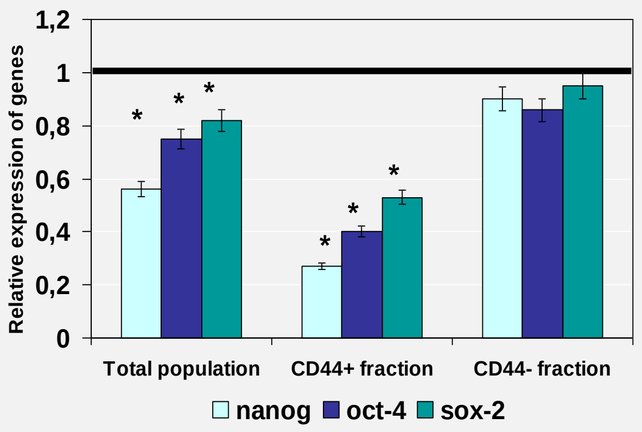
Master regulators such as Nanog, Oct4, Sox2 bind to each other's promoter, and support or limit each other's expression, forming an interconnected autoregulatory network to maintain cancer/embryonic stem cell pluripotency and self-renewal.




Oct4 expression regulates of Nanog expression via binding sites in the promoter of Nanog. Sox2 is involved as cofactor in Oct4 target genes transcription. Oct4 interact with Sox2 to regulate downstream target genes. Binding site exist in Nanog promoter for Oct4-Sox2 complex.



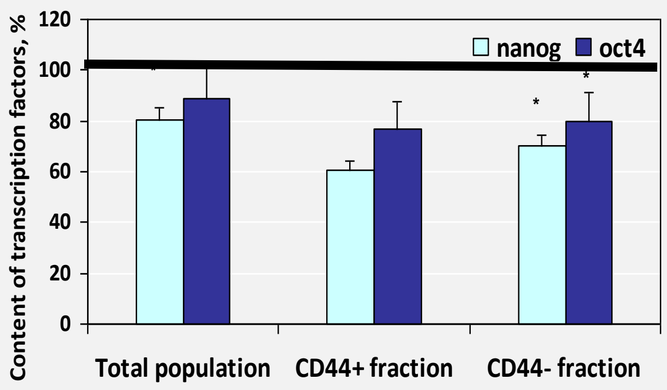
## Relative expression of the genes *nanog, oct-4, sox-2* in EC cells after incubation with NCs *in vitro* and further *in vivo* culturing




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. * - the difference is statistically significant in compared with the control indices; ($p<0.05$)

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.

## Evaluation of Nanog and Oct-4 transcription factors in EC cells after incubation with NCs *in vitro* and further *in vivo* culturing




Notes: The values of expression levels of transcription factors in native EC cells have been taken as "1"; * - the difference is statistically significant in compared with the control indices; ($P < 0.05$)

It has been established that pretreatment of EC cells with NCs caused a decrease in the content of the studied transcription factors in all experimental groups. Based on the minimum level of transcription factors (Nanog and Oct-4) had the highest sensitivity to NCs $CD44^+$ fraction.

## Conclusion

Various extent of changes in expression level of *nanog, oct-4* and *sox-2* genes and content of Nanog and Oct-4 transcription factors after pretreatment of EC cells with NCs was found. Inhibition of pluripotency genes as well as such transcriptional factors as Nanog, oct-4 in $CD44^+$ fraction of tumor cells was maximal and correlated with a decrease in proliferative activity of this fraction cells. Thus, the findings may indicate the implementation of antitumor effect of orthovanadates at the genome level.



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