Phenotypic plasticity of ovarian carcinoma cells lead to higher resistance to treatment when cultivated in collagen-based 3D model

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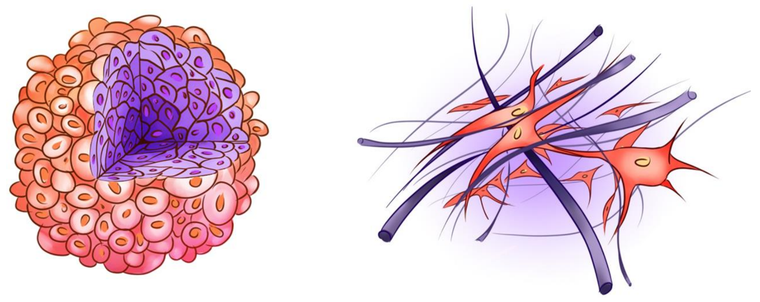
Introduction

Epithelial-mesenchymal transition (EMT) is a process in which epithelial cells transform into mesenchymal cells by change in the expression and metabolism profile and acquire the ability to invade. The cultivation conditions of cancer cells can provoke EMT, which in turn can affect their resistance to therapy. Therefore, when researching and developing anticancer drugs, it is important to take into account that the chosen research model can affect further results related to the resistance of tumor cells to antitumor agents.

The aim of this work was to compare the expression profile of proteins representing the main types of intercellular contacts in human ovarian adenocarcinoma cells in a monolayer, tumor spheroids and collagen hydrogel.

Materials and Methods

Two cell lines of HER2-overexpressing human ovarian adenocarcinoma, SKOV-3 and SKOV-3.ip, were cultured in monolayer and in two types of 3D in vitro models, namely tumor spheroids and a collagen hydrogel-based model (collagen hydrogel).



Characterization of 3D In Vitro Tumor Models

Spheroids were produced using 96-well ultra-low-attachment round-bottom plates. The dynamics of spheroid growth was assessed by disaggregating individual spheroids using trypsin solution and staining dead cells with trypan solution on different days of growth. *Collagen hydrogels* were produced in individual wells of 12-well tissue culture plates. The cells were enclosed in nutrient-rich collagen hydrogels by mixing the cell suspension with the ingredients of the gel. The dynamics of cell growth was assessed by enzymatic destruction of the gel and further staining the suspension with trypan blue solution and counting living and dead cells using a hemocytometer.

Flow Cytometry Analysis of Proteins of Cell–Cell Contacts

To study the level of expression of proteins of interest, the obtained three-dimensional models were also subjected to enzymatic degradation. After that, the cells were stained with antibodies specific to E-cadherin (adhesive contact protein), occludin and ZO-1 (tight junction proteins), desmoglein-2 (desmosome protein) and connexin-43 (gap junction protein) or to the tumor marker receptor HER2 and analyzed by flow cytometry. Also, the expression of these proteins was investigated in monolayer cell cultures.

Cytotoxicity Assay of Doxorubicin and DARPin-LoPE

We analyzed the responsiveness of ovarian adenocarcinoma cells to doxorubicin (DOX) and anticancer targeted toxin DARPin-LoPE. Doxorubicin is a widely used anticancer drug of the anthracyclin family which intercalates into DNA and blocks replication and transcription processes, which leads to cell death. DARPin-LoPE is a novel targeted toxin. Targeting properties of DARPin-LoPE are realized via HER2-specific DARPin and toxicity is realized by low-immunogenic Pseudomonas exotoxin fragment via protein synthesis arrest (Sokolova et al. Int. J. Mol. Sci. 20(10), 2019).

Results

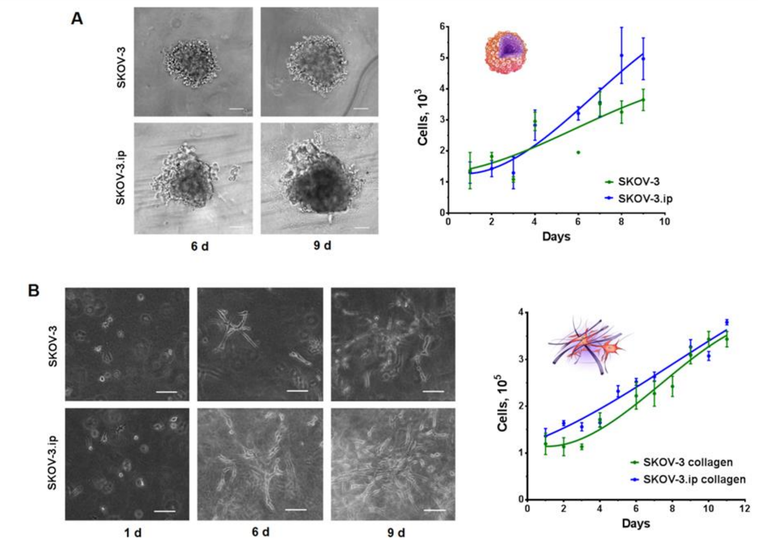


Figure 1. Growth of SKOV-3 and SKOV-3.ip cells in 3D in vitro models.

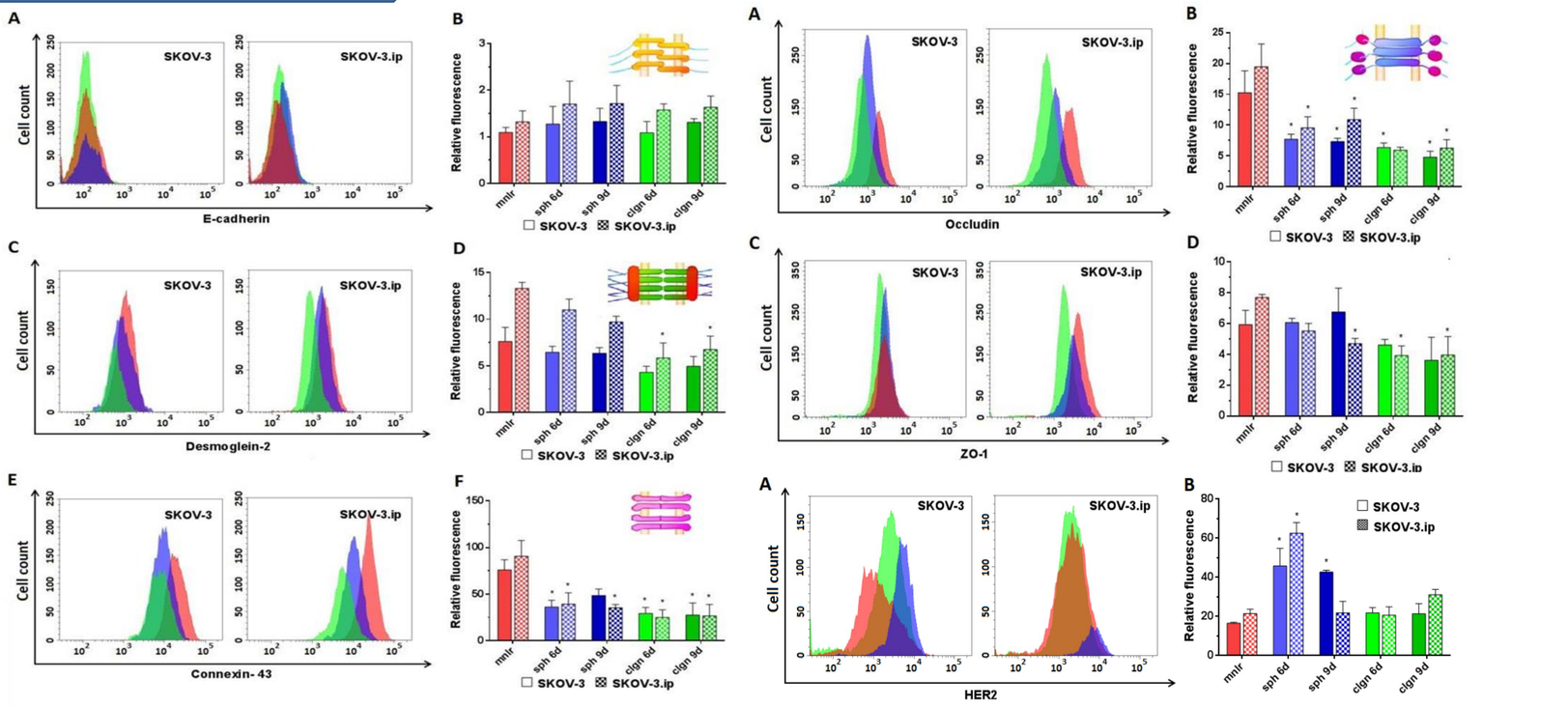


Figure 2. Expression level of analyzed proteins of adherent junctions (E-cadherin), desmosomes (desmoglein-2), gap junctions (connexin-43), tight junctions (occluding, ZO-1) and Human Epidermal growth factor Receptor 2 (HER2) in SKOV-3 and SKOV-3.ip cells cultured in monolayer and 3D in vitro models

* Relative fluorescence value (RF) calculated as a ratio of mean fluorescence intensity of cells stained with specific antibodies to mean fluorescence intensity of cells stained with antibodies of isotypic control. RF value equal to 1 indicates that no analyzed protein is present in the cells.

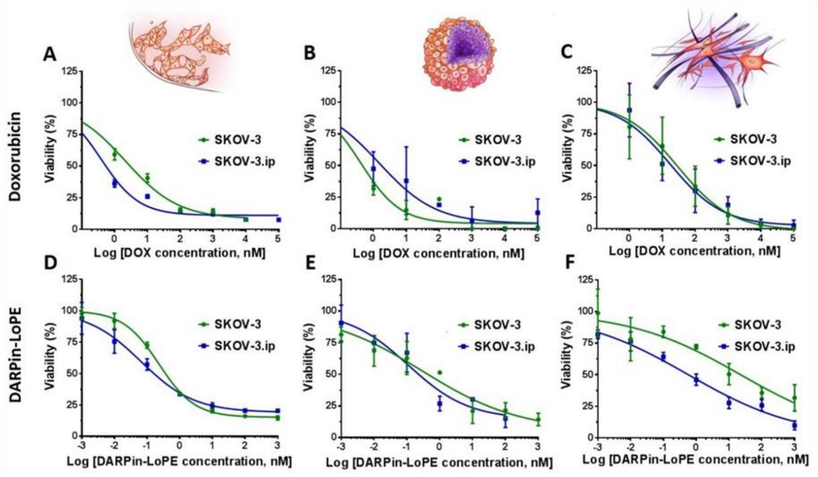


Figure 3. Cytotoxicity of doxorubicin and targeted toxin DARPin-LoPE against SKOV-3 and SKOV-3.ip cells cultured in monolayer and 3D in vitro models

I. **Three-dimensional models were characterized by extremely low expression of basic molecules of adherens junctions E-cadherin and demonstrated a simultaneous decrease in desmosomal protein desmoglein-2, gap junction protein connexin-43 and tight junction proteins occludin and ZO-1. The reduction in the level of contact proteins was most pronounced in collagen hydrogel.**
II. **Culturing cells in a hydrogel also resulted in higher resistance to treatment with doxorubicin or a protein toxin specific for the HER2 tumor marker.**

Conclusions

A decrease in the level of contact proteins during cell cultivation in a 3D model can be a sign of the acquisition of a more mesenchymal phenotype by tumor cells. We believe that the epithelial-mesenchymal transition may be induced by three-dimensional cultivation and altered features of the microenvironment, which, in turn, can change the responsivity to treatment. Therefore, the use of three-dimensional models is promising for predicting the response of tumor cells to the action of various anticancer compounds.

The results of this work are published (Kutova et al. Biology. 9(12), 2020).

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