Стволовые свойства опухолевых клеток асцитической жидкости у больных раком яичника: ключ к управлению распространением процесса

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РЕЗЮМЕ

Рак яичника принято рассматривать как наиболее злокачественную и агрессивную опухоль женской репродуктивной системы, что во многом связано с ранним развитием злокачественного асцита и перитонеального канцероматоза. Опухолевые клетки, представляющие первичный очаг, а также содержащиеся в составе асцитической жидкости, крайне гетерогенны с морфологической, иммуногистохимической и молекулярно-генетической позиций. Значимую роль в процессах самообновления опухоли, ее дифференцировки, метастазирования и развития химиорезистенстности играют опухолевые стволовые клетки.

Настоящий обзор направлен на обобщение имеющихся данных о стволовых опухолевых клетках рака яичников и их роли в опухолевой прогрессии. При написании обзора проведен биоинформационный поиск в универсальных базах данных PubMed, NCBI, Google Scholar и eLibrary с применением следующих ключевых слов для поиска: cancer stem cells, ovarian cancer, malignant ascites, hemoresistance и т.п.

Представленные данные позволяют всесторонне охарактеризовать роль стволовых свойств опухолевых клеток рака яичника. Изложена актуальная информация о молекулярно-биологических параметрах стволовых опухолевых клеток рака яичника, представляющих клеточный компонент злокачественного асцита, с приведением данных собственных исследований. Отражены современные представления о механизмах формирования клеточных сфероидов и их вкладе в прогрессирование опухолевого процесса.

Опухолевые стволовые клетки являются крайне перспективной мишенью в создании будущих терапевтических стратегий, основанных на изучении сигнальных путей в стволовых клетках рака яичников, механизмах образования сфероидов, а также вкладе иммунных клеток в приобретение стволовых свойств опухоли.

Ключевые слова: рак яичника, опухолевые стволовые клетки, злокачественный асцит, опухолевые сфероиды, химиорезистентность

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INTRODUCTION

Ovarian cancer (OC) is an extremely malignant and the most aggressive tumor among all neoplasms of the female reproductive system. Tumor progression is accompanied by early development of malignant ascites with metastatic spread of the tumor to the abdominal organs [1]. The modern stem cell theory of cancer postulates that cancer stem cells (CSCs) are responsible for self-renewal, differentiation, metastasis, and development of chemotherapy resistance. By their nature, CSCs are capable of symmetric and asymmetric division with subsequent differentiation of tumor subclone(s), which contributes to phenotypic and functional heterogeneity in the hierarchical organization of tumors [2].

CANCER STEM CELL MARKERS IN OVARIAN CANCER

It should be noted that identification of CSC properties in cancer patients is rather challenging due to the lack of a universal marker or panel of markers. Currently, there is a wide variety of proteins whose expression is considered as a sign of stem cell-like properties in these cells (Table 1).

Table 1

Cancer stem cell markers in ovarian cancer

Marker	Description	Reference
CD133 (prominin-1)	Glycosylated transmembrane protein	[3]
CD44	Hyaluronic acid receptor	[4, 5]
CD24	P-selectin ligand	[6]
CD177	Type III tyrosine kinase receptor	[7]
MyD88	TIR domain-containing cytosolic adaptor protein	[8–10]
EpCAM	Calcium-independent homotypic epithelial cell adhesion molecule	[11]
ALDH1	Enzyme catalyzing oxidation of aldehydes to carboxylic acids	[12, 13]
CXCR4	CXCL12 chemokine receptor	[14–16]
Nanog	Transcription factor	[17–19]
SOX2	Transcription factor	[20, 21]
OCT4	Transcription factor	[22]

A number of diagnostic markers proved useful for isolating ovarian CSC subpopulations, including CD133+ [23, 24] CD133+ ALDH+ [25], CD44+ CD117+ [7], EpCAM [26].

EpCAM, a calcium-independent homotypic epithelial cell adhesion molecule, is a type I transmembrane glycoprotein expressed by subpopulations of normal epithelial cells and numerous stem cells, including ovarian CSCs [11, 27]. It was shown *in vivo* that EpCAM-positive tumor cells isolated from the remaining ovarian carcinoma cell population have greater tumorigenic potential in comparison with EpCAM-negative tumor cells [28].

CD133 is a glycosylated transmembrane protein which is encoded by the *PROM1* gene. The physiological function of this protein is not fully understood to date, but it was shown that this receptor is actively involved in modulating tumor spread and developing drug resistance of the tumor. CD133 is one of the most studied CSC markers of ovarian, colon, prostate, and lung cancer [3]. Y.J. Lee et al. (2016) demonstrated a correlation of CD133 expression with tumor differentiation. The CD133 expression score in grade III tumors (high-grade tumors) was significantly higher than in grade I tumors (low-grade tumors) [5].

ALDH1 is a member of the family of enzymes that catalyze oxidation of aldehydes to carboxylic acids. Metabolic activity of this enzyme was detected by the ALDEFLUOR assay in identifying CSCs in a number of solid tumors. High ALDH1 expression is significantly associated with poor clinical outcomes in serous ovarian cancer. Currently, ALDH is used as a CSC marker in ovarian cancer [12, 13].

CD44 is a receptor for hyaluronic acid and many other components of the extracellular matrix. CD44 is responsible for cell – cell interactions, adhesion, and cell migration. The accumulated data indicate that CD44, especially a CD44v isoform, is a CSC marker in various tumors including ovarian carcinomas. CD44 is also involved in the regulation of stem cell-like properties including self-renewal, tumor initiation, metastasis, and chemotherapy and radiotherapy resistance. In addition, there is ample evidence that CD44 expression, especially of the CD44v isoform, correlates with poor patient survival. This is significantly an unfavorable prognostic marker. In turn, the CD44v isoform can be a promising target for targeted therapy [4, 5].

CD24 is a ligand of P-selectin, an adhesion receptor on activated endothelial cells. It is often co-expressed in CD44 and CD133-positive tumor cells in ovarian carcinomas. CD24-positive tumor cells have a higher metastatic potential compared to CD24-negative cell populations. It is important that CD24 induces EMT that leads to the formation of a highly proliferative mesenchymal CSC phenotype as well as the development of drug resistance of the tumor through the activation of the PI3K / Akt, NF-κB, and ERK signaling cascades [29].

Recent studies of ascitic fluid in ovarian cancer patients by multicolor flow cytometry showed that the cellular composition of ascitic fluid is heterogeneous. A big proportion of ascites tumor cells is represented by atypical / hybrid cell forms with stemness traits, as well as Epcam+CD45-CD44+CD24+CD133+/- CSCs both with and without EMT [30]. In addition, we found that the number of ascites tumor cells with Epcam+CD45-CD44-CD24+CD133-Ncadherin+ and Epcam+CD45-CD44-CD24+CD133+Ncadherin+

phenotypes as well as the number of atypical / hybrid Epcam+CD45+CD44+CD24+/-CD133+/-Ncadherin+/- cells have a positive correlation with the carcinomatosis index [31]. It should be noted that these cells are CD24-positive.

MyD88 is a TIR domain-containing cytosolic adaptor protein involved in signal transduction from Toll-like receptors. Activation of the TLR4 / MyD88 / NF-κB signaling pathway enhances the aggressive tumor phenotype and worsens the clinical outcome in patients with ovarian cancer. Expression of this protein is often detected in CSCs [8–10].

CD177 is a type III tyrosine kinase receptor, which activates phosphorylation by initiating transcriptional processes in various cell types. It is involved in the regulation of cell apoptosis, differentiation, proliferation, chemotaxis, and adhesion. The receptor is often expressed in hematopoietic stem cells (HSCs), myeloid progenitor cells, pro-B cells, progenitor cells, as well as in CSCs [7].

CXCR4 is a chemokine receptor. The receptor is involved in cell chemotaxis in response to CXCL12 chemokine binding and is used as one of the markers of ovarian CSCs. It is assumed that CXCR4 is associated with the induction of ovarian cancer metastasis, as well as poor overall survival of patients [14–16].

NANOG as a transcription factor is one of the most important markers used to identify CSCs. It is reported that NANOG mRNA was detected in pluripotent mouse and human stem cells, but not in differentiated cells (Chambers et al., 2003). Nanog expression is known to be statistically higher in CSCs compared to tumor cells without stemness traits. NANOG is responsible for morphofunctional plasticity and self-renewal of embryonic stem cells through interaction with other transcription factors, such as SOX-2 and Oct-4. These genes attach to Octamer / SOX elements in the NANOG promoter, which leads to activation of NANOG transcription (Rodda et al., 2005). NANOG was found to maintain CSC traits through activation of various signaling pathways, including TGIF-β, Wnt / β-catenin, JAK / STAT, Notch, and Hedgehog (Alemohammad et al., 2020). NANOG overexpression was found in tumors from embryonic cells, which correlates with cell proliferation, tumor recurrence, clonal tumor evolution, oncogenicity, invasiveness, and resistance to treatment, such as chemotherapy and radiotherapy [17–19].

SOX2 is a member of the SOXB1 transcription factor family, and its three main domains are the N-terminal domain, the high mobility group (HMG) domain, and the transactivation domain. SOX2 is an important marker of CSCs. It was observed that SOX2 is overexpressed in spheroids as well as in subsequent generations of cancer cell spheroids. SOX2 expression is closely associated with chemoresistance and a poor prognosis in patients with ovarian cancer [20, 21].

Oct4 transcription factor is expressed in embryonic stem cells as well as in ovarian CSCs [22].

The analysis of the key stemness-related genes in CSC subpopulations found new promising markers closely related to the development of ovarian carcinoma including LCP2, FCGR3A, COL1A1, COL1A2, MT-CYB, CCT5, and PAPPA [32].

REGULATION OF STEM CELL-LIKE PROPERTIES OF OVARIAN CANCER CELLS

To date, many mechanisms of regulation of ovarian stem cell-like properties have been described. S. Bai et al. (2021) showed that epidermal growth factor-like protein 6 (EGFL6) acts as a stem cell regulatory factor, promotes asymmetric division of ALDH-positive ovarian CSCs, and thereby increases tumor cell proliferation *in vitro* and tumor growth *in vivo* [33]. EIF5A2 factor positively regulates ovarian cancer cell stemness through the E2F1 / KLF4 pathway [34]. The L1CAM / FGFR1 / SRC / STAT3 signaling pathway is considered as a new driver of stemness in ovarian cancer. L1CAM was shown to potentiate several stemness-related properties in ovarian cancer cells, including spheroid formation and tumor initiation *in vivo* [35]. FOXK2-driven activation of IRE1α leads to unconventional splicing of XBP1 and activation of SOX2, OCT4, NANOG, and ALDH1A1 stemness pathways [36].

Interestingly, the CSC phenotype is regulated in particular by the tumor microenvironment. It has been shown that activation of NF-κB signaling leads to increased activity of the Wnt signaling pathway, which leads to dedifferentiation of tumor cells from non-stem cells into CSCs [37]. Heterospheroids including polarized CD206+ M2 macrophages showed increased aldehyde dehydrogenase (ALDH) activity, which suggests an interaction between CSCs and macrophages promoting tumor cell activation and self-renewal of CSCs [38].

Cells of the tumor microenvironment secrete factors that contribute to the acquisition of stemness traits in tumor cells, such as KIT ligand and R-spondin as ligands for CD117 [39] and LGR5 [40], respectively.

INDIVIDUAL CELLS WITH STEM CELL-LIKE PROPERTIES IN ASCITIC FLUID IN OVARIAN CANCER

A study by S.O. Genning et al. (2021), which examined stem cell populations in ascites, showed that 95.5% of CSCs had a CD44+ / CD133- phenotype and 4.5% had a CD44- / CD133+ phenotype. The population of CD44+ / CD133+ cells was minor (0.2%) [41]. In other studies, ascites cells with a high level of CD44 and CD133 expression, which were obtained from ovarian cancer patients, had a great potential for self-renewal and long-term proliferation [42–45].

The co-expression of CD133 and CD44, as well as the expression of each marker individually was the highest in tumor cells that were present in ascitic fluid of primary human ovarian cancer. In addition, the expression of CD97, CD104, CD107a, CD121a, and CD307c was significantly higher in CD133+CD44+ tumor cells of malignant ascites than in primary tumor cells or metastatic ovarian tumors [5]. In studies by M. Jäger et al. (2012) double staining of ascites cells in samples obtained by cytospin revealed the presence of CD133+ / EpCAM+ cells in 100% of the studied patients [46].

Stemness-related markers can be expressed not only by classical tumor cells, but also hybrid cells found in ascitic fluid. Their presence is characteristic of ovarian carcinoma. M.Z. Akhter et al. (2018) state that the entire EpCAM+CD45+ population is highly invasive and consists of ovarian CSCs (CD133+ and CD117+CD44+) [47]. Another striking finding of this study is that CSC phenotypes are primarily restricted to the EpCAM+CD45+ compartment. This does not allow to deny the existing hypothesis that CSCs arise due to dysregulation of tissue-specific stem cells [48].

Similar results were obtained in the studies by E.V. Kaigorodova et al. (2020) that showed high heterogeneity of EpCAM+ cells in ascitic fluid of ovarian cancer patients. A high concentration of these cells was represented by atypical and hybrid forms of EpCAM+CD45+ cells with stemness traits [30, 49]. In addition, another study demonstrated a positive correlation between the number of EpCAM+CD45+ cells with stemness traits in ascitic fluid and the carcinomatosis prevalence index in ovarian cancer patients [31]. It was also revealed that the number of atypical / hybrid EpCAM+CD45+ cells in ascitic fluid in patients with borderline ovarian tumors was significantly lower than in patients with serous ovarian carcinomas ($p = 0.02$) [50]. A review by E.V. Kaigorodova et al. (2022) suggests theories of hybrid tumor cell formation, their varieties and characteristics and shows the role of cancer-associated macrophage-like cells (CAMLs) and circulating hybrid cells (CHCs) as tumor biomarkers [51].

The most comprehensive and complex study of ascites cells in patients with ovarian cancer was performed by B. Izar et al. (2020) [52]. The authors conducted RNA sequencing (scRNA-seq) of individual cells (approximately 11,000 cells) from 22 ascites samples obtained from 11 ovarian cancer patients, while they comprehensively characterized the ascites ecosystem in high-grade serous ovarian cancer (HGSOC). Eighteen different cell clusters were annotated, encompassing epithelial cells (5 clusters labeled by EPCAM, cytokeratins, kallikreins), macrophages (4 clusters labeled by CD14, AIF1, CSF1R, CD163) cancer-associated fibroblasts (CAFs) (4 clusters labeled by PDPN, DCN, and THY1), dendritic cells (2 clusters labeled by CD1C, CD1E, CCR7, CD83), B cells (CD19, CD79A/B), T cells (CD2, CD3D/E/G), and red blood cells (GATA1, hemoglobin). Signaling pathways that differed in malignant cells of each patient were identified among the five tumor clusters. One cluster of cells contained distinct markers of stem (ALDH1A3 and CD133 / PROM1) and mesenchymal (FN1, ACTA2, and MYL9) cells, as well as AXL and its only known ligand GAS6, which is associated with drug therapy resistance [53].

COMPLEXES OF CELLS WITH STEM CELL-LIKE PROPERTIES IN ASCITIC FLUID IN OVARIAN CANCER

There is limited information about the study of stem cell-like properties of individual cells. At the same time, complexes of cells found in ascites, which also have stemness traits, are widely described. Indeed, it is common to distinguish single tumor cells, cell aggregates, and cell spheroids among the cellular component of malignant ascites in ovarian cancer [54].

Moreover, formation of essential spheroids is considered as a stemness trait. The process of spheroid formation can be easily observed when cells are cultured *in vitro*. In patients, however, it is not always possible to establish whether they were formed from a single detached cell due to its proliferation, following aggregation of individual cells or a release of cell complexes from the primary tumor.

S.A. Bapat et al. (2005) isolated two tumorigenic

clones (A2 and A4-T) of CD44+ ovarian stem-like cancer cells that were capable of forming spheroids in ascitic fluid. When these cell lines were further cultured, NESTIN and NANOG were overexpressed in A2 and A4-T monolayers, while a decrease in the level of spheroid expression from these cell lines was noted. The described phenomenon gave the authors a reason to believe that the formation of spheroids represents an event of differentiation. In addition, the spheroids showed expression of markers that may indicate differentiation into the ovarian surface epithelium (cytokeratin 18 and vimentin), granulosa (cytokeratin 18 and E-cadherin), or germ cells (alkaline phosphatase, etc.). The differentiation into germ cells was aberrant [55].

In contrast to the results obtained during the study of CD44+ / CD24- breast cancer stem cells, the data obtained by H. Jiang et al. (2012) show that in ascites transformation of CSCs occurs with formation of a tumor subclone, referred to as side population (SP) cells. This cell population is more differentiated and does not correspond to primary cancer cells (non-SP cells), which is probably due to epithelial – mesenchymal transition (EMT) [27]. In addition, the authors showed that SP ovarian cancer cells showed a lower invasive potential. In contrast, non-SP ovarian cancer cells presumably have greater migration and invasive properties. The authors of the study made a reasonable assumption that SP cells can be responsible for the interaction between the tumor and tumor microenvironment, which is also determined by their lateral / edge localization in the tumor focus [28].

A number of questions remain, including whether the metastatic potential of single cells and spheroids is the same.

MECHANISMS OF SPHEROID FORMATION IN ASCITIC FLUID IN OVARIAN CANCER

One hypothesis suggests that multicellular spheroids arise from single cells that aggregate in the abdominal cavity [56]. It can be assumed that not all cells have the ability to aggregate and, perhaps, aggregation occurs only among cells with certain properties. For example, CD44 expression through homotypic interactions is known to mediate tumor cell aggregation and polyclonal metastasis in patient-derived xenograft models of breast cancer [57]. The intercellular adhesion molecule E-cadherin appears to play a crucial role in spheroid formation. Indeed, higher expression of E-cadherin was generally associated with denser and more compact spheroids [58]. MUC16 and integrin β1were also shown to be involved in spheroid formation [59]. The role of endogenous fibronectin (FN1) in the process of metastasis was previously demonstrated in experimental models of ovarian cancer. Using *in vitro* model, H.A. Kenny et al. [60] and M.P. Iwanicki et al. [61] showed that FN1, which is either secreted by mesothelial cells or ovarian cancer cells, is necessary for tumor spheroids formed by ovarian cancer cells to survive in the absence of fixation and in an unsuitable metabolic environment.

An alternative mechanism of spheroid formation discussed by some authors is that cells separate from the primary tumor in whole groups (layers of cells), subsequently forming spheroids [62–64]. The authors report that spheroids predominantly form as a result of multicellular detachment from the primary tumor and are responsible for the development of peritoneal carcinomatosis. In addition, it was shown that detached spheroids after implantation and proliferation of tumor cells form morphological structures corresponding or similar to the patterns of the primary tumor, while possessing immunophenotypic heterogeneity.

The proportion of tumor cells in spheroids is poorly understood. Indeed, ascites spheroids in ovarian cancer are usually described as heterogeneous cell complexes consisting of a small number of tumor cells and various types of non-tumor cells [65–68]. In addition, the proportion of cancer cells in the entire ascites varies in patients and is reported to range from 1% [69] to approximately 8% [52] of the total cellular component of malignant ascites.

The role of fibroblasts and macrophages in the formation of tumor spheroids is described in sufficient detail in the review by M. Rakina et al., which also discusses the specific functions of fibroblasts, macrophages, and T cells in tumor proliferation and implantation in the peritoneum [70].

In Fig. 1, we presented our own scheme of the way tumor spheroids, which are a part of malignant ascites in ovarian cancer, are formed. On the basis of the literature data and our own studies, we can assume two main mechanisms of tumor spheroid formation. The first mechanism is due to the proliferation of single CSCs followed by the formation of a spherical structure. The second mechanism involves the detachment of a cell layer containing stem cells from the primary tumor (Fig. 1). Subsequently, tumor fibroblasts and M2 macrophages are attached to the spheroid, and adhesion of non-stem cells to the formed structure occurs, followed by the dissemination through the abdominal cavity.

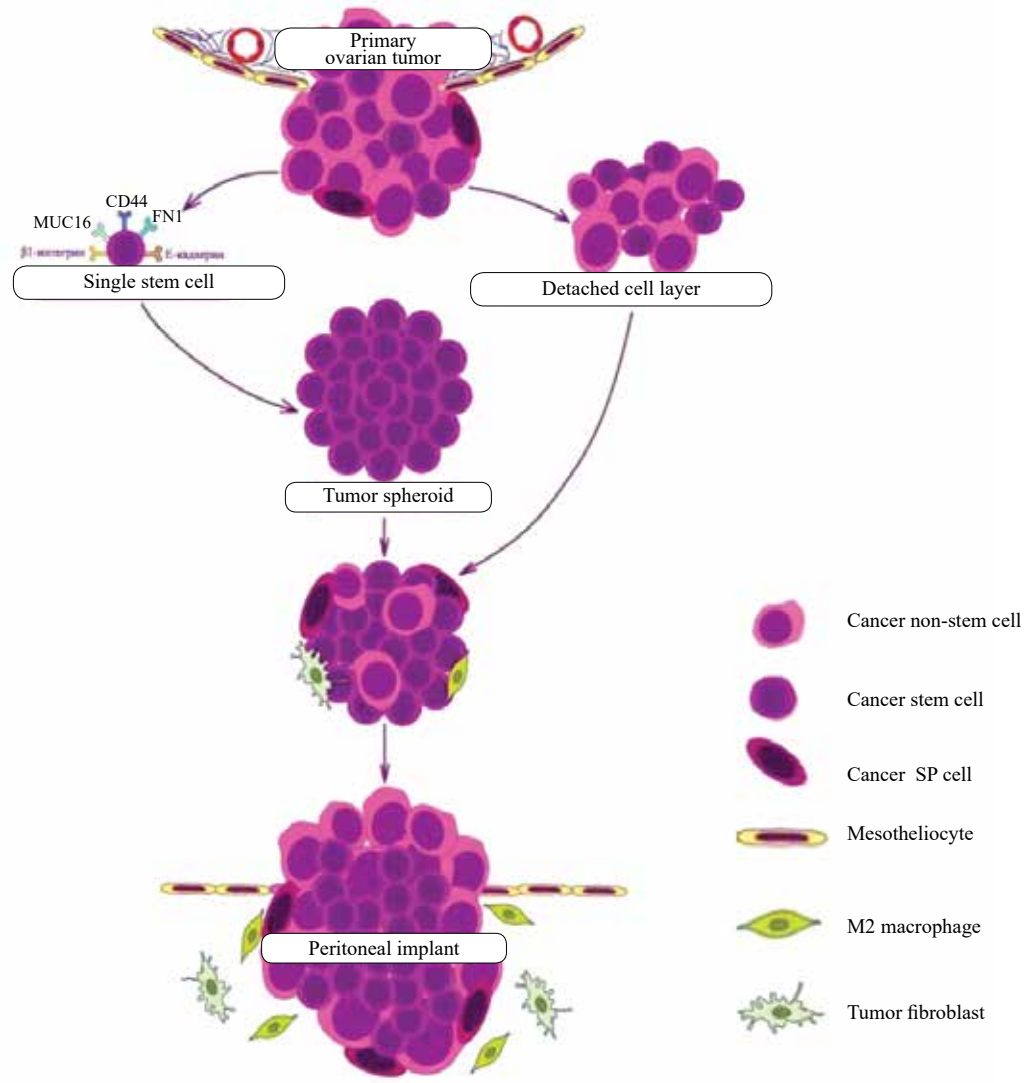

Fig. 1. A contemporary view on the formation of tumor spheroids as a part of malignant ascites in ovarian cancer

CHEMORESISTANCE OF CSCS AND STRATEGIES TO OVERCOME IT IN OVARIAN CANCER

The property of excreting cytotoxic substances from their cytoplasm is characteristic of CSCs, and on the basis of this property it is possible to identify them. This property was used in the isolation of CSCs by fluorescence-assisted cell sorting (FACS). In *in vitro* and *in vivo* experimental models, cells were incubated with a dye and separated into different fractions using FACS based on their ability to retain the dye. It was shown that cells that excreted most of the dye had more pronounced stemness traits compared to the rest of the cells. This method was first used to isolate tumor-initiating cells in acute myeloid leukemia [71]. Resistance to cisplatin, topotecan, and docetaxel was described in tumor cells forming spheroids [72].

Stimulation of CSC differentiation can be a rather promising approach to ovarian cancer therapy. Chemotherapy regimens targeting CSCs may be ineffective since the possibility of proliferation and dedifferentiation of daughter CSCs can replace the population of eradicated therapy-sensitive CSCs. Currently, there are developed approaches to differentiation therapy, for example, using all-trans-retinoic acid in the treatment of acute promyelocytic leukemia [73]. Similar strategies using bone morphogenetic proteins proved effective in experimental therapy of gliomas, which led to a decrease in the number of CSCs [74].

Some studies discussed that fasudil treatment for lateral osteosarcoma in murine models induced de-differentiation of some CSCs caused by the implantation of a cell-line with an inhibited *c-Myc* gene (osteosarcoma-mimicking cells). These cells were capable of trilinear differentiation (into osteocytes, chondrocytes, and adipocytes). Some of the resistant tumor cells with stem cell-like properties were transformed into adipocytes under the effect of chemotherapy, thereby contributing to tumor pathomorphosis [75]. In the literature, there are no data on differentiating agents for ovarian CSCs, but it was shown that mullerian inhibitory substance (MIS) or its mimetic SP600125 specifically inhibit CD44+CD24+Epcam+ CSCs in ovarian cancer cell lines derived from ascites cells [27].

The point of impact for systemic therapy for ovarian cancer, which is aimed at suppressing stem cell-like properties can be various signaling pathways regulating stemness. For example, one of the mechanisms for the development of chemoresistance of spheroids derived from the culture of tumor cells in malignant ascites was demonstrated *in vitro*. It consists in the transition of these cellular structures into the quiescent stage (G0 phase) by reducing the synthesis of B-protein kinases due to inhibition of *AKT* (alpha serine / threonine-protein kinase) gene, which led to increased expression of p130/ RBL2 and p27Kip1 and a decreased SKP2 level. Subsequently, it was shown that after spheroid adhesion on the surface optimal for implantation, activation of the AKT signaling pathway occurs, thereby triggering tumor cell invasion and proliferation [76]. Two AKT inhibitors, capivasertib and ipatasertib, are currently undergoing phase III clinical trials for cancer treatment [77].

A strategy based on destroying or preventing spheroid formation also seems quite promising. Inhibition of another known Hedgehog signaling pathway by cyclopamine resulted in the induction of a 10-fold decrease in spheroid formation in ovarian cancer cell lines [78]. Nectin-4 peptide 10 (N4-P10) is known to lead to rapid disruption of spheroid formation in ovarian cancer [78]. The study by S. Rafehi et al. showed that spheroid formation from cells isolated from ascites of ovarian cancer patients was impaired by SB-431542, which made the cells susceptible to carboplatin-induced cell death [79].

Interaction of tumor and non-tumor cells within spheroids could be another possible application point for therapy. Paracrine activation of Wnt during the interaction of CSCs and M2 macrophages represents a positive feedback loop, which probably contributes to the formation of a more aggressive tumor cell phenotype [38], which makes the Wnt pathway a potential target for CSC suppression. In addition, studies showed that catumaxomab eliminates CD133+ / EpCAM+ CSCs by activating T cells in ascites in advanced ovarian cancer [80].

In Figure 2, we presented a scheme describing several strategies to overcome CSC chemoresistance in ovarian cancer.

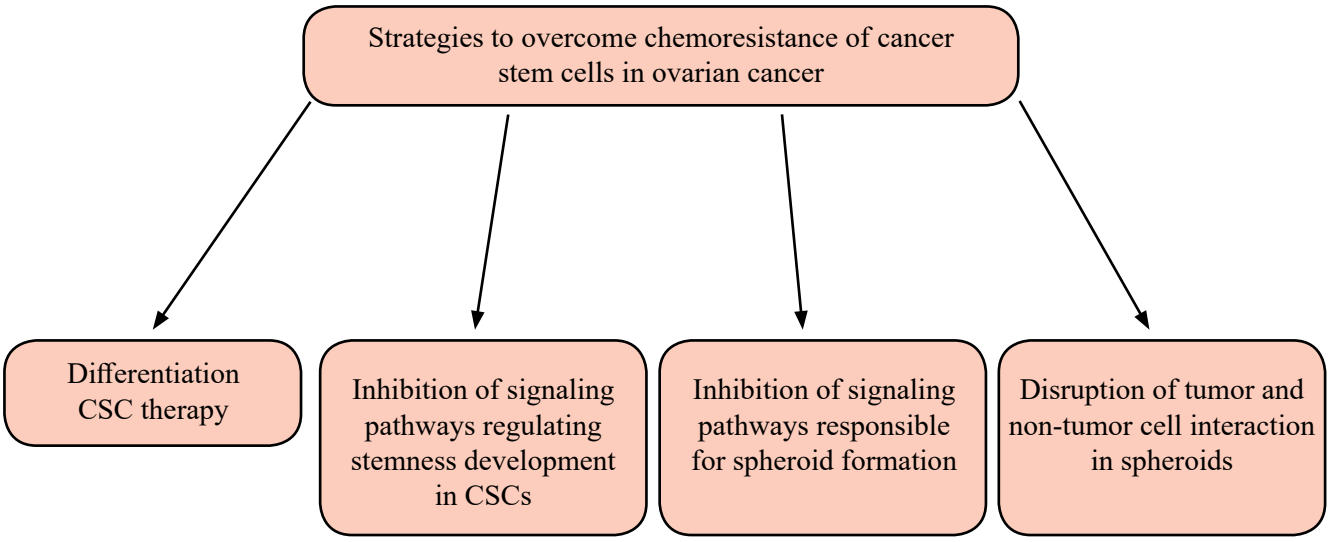

Fig. 2. Strategies to overcome chemoresistance of cancer stem cells in ovarian cancer

CONCLUSION

Future ovarian cancer treatment strategies will be based on the study of signaling pathways in ovarian CSCs, mechanisms of spheroid formation, as well as the contribution of immune cells to the acquisition of stem cell-like properties by tumor cells. It is also important to highlight the possibility of using prognostic biomarkers based on the determination of CSCs. This can be extremely promising in modifying approaches to predict ovarian cancer outcomes and individualize chemotherapy with current treatment regimens.

Considering that CSCs can mediate chemoresistance in ovarian cancer, evaluation of the stem cell-like properties of tumor cells in ascites will allow to quickly predict the efficacy of ongoing therapy in patients. However, identification of CSCs remains the main challenge. Numerous studies show that subpopulations of ovarian cancer cells were found to express stemness markers at very different levels in various combinations, with none of these markers being obligatory. These data confirm the phenomenon of tumor plasticity, which researchers have begun to be study recently, and which requires further research in clinical practice.

REFERENCES

1. Motohara T., Masuda K., Morotti M., Zheng Y., El-Sahhar S., Chong K.Y. et al. An evolving story of the metastatic voyage of ovarian cancer cells: Cellular and molecular orchestration of the adipose-rich metastatic microenvironment. *Oncogene*. 2019;38(16):2885–2898. DOI: 10.1038/s41388-018-0637-x.
2. Ahmed N., Abubaker K., Findlay J.K. Ovarian cancer stem cells: Molecular concepts and relevance as therapeutic targets. *Mol. Asp. Med.* 2014;39:110–125. DOI: 10.1016/j.mam.2013.06.002.
3. Horst D., Kriegl L., Engel J., Kirchner T., Jung A. Prognostic significance of the cancer stem cell markers CD133, CD44, and CD166 in colorectal cancer. *Cancer Investigation.* 2009;27(8):844–850. DOI: 10.1080/07357900902744502.
4. Yan Y., Zuo X., Wei D. Concise Review: Emerging Role of CD44 in Cancer Stem Cells: A Promising Biomarker and Therapeutic Target. *Stem Cells Translational Medicine.* 2015;4(9):1033–1043. DOI: 10.5966/sctm.2015-0048.
5. Lee Y.J., Wu C.C., Li J.W., Ou C.C., Hsu S.C., Tseng H.H. et al. A rational approach for cancer stem-like cell isolation and characterization using CD44 and prominin-1(CD133) as selection markers. *Oncotarget.* 2016;7(48):78499–78515. DOI: 10.18632/oncotarget.12100.
6. Gao M.Q., Choi Y.P., Kang S., Youn J.H., Cho N.H. CD24+ cells from hierarchically organized ovarian cancer are enriched in cancer stem cells. *Oncogene.* 2010;29(18):2672–2680. DOI: 10.1038/onc.2010.35.
7. Zhang S., Balch C., Chan M.W., Lai H.C., Matei D., Schilder J.M. et al. Identification and characterization of ovarian cancer-initiating cells from primary human tumors. *Cancer Res.* 2008;68(11):4311–4320. DOI: 10.1158/0008-5472.CAN-08-0364.
8. Li Z., Block M.S., Vierkant R.A., Fogarty Z.C., Winham S.J., Visscher D.W. et al. The inflammatory microenvironment in epithelial ovarian cancer: a role for TLR4 and MyD88 and related proteins. *Tumour Biology: the Journal of the International Society for Oncodevelopmental Biology and Medicine*. 2016;37(10):13279–13286. DOI: 10.1007/s13277-016-5163-2.
9. Burns K., Clatworthy J., Martin L., Martinon F., Plumpton C., Maschera B. et al. Tollip, a new component of the IL-1RI pathway, links IRAK to the IL-1 receptor. *Nature Cell Biology*. 2000;2(6):346–351. DOI: 10.1038/35014038.
10. López J., Valdez-Morales F.J., Benítez-Bribiesca L., Cerbón M., Carrancá A.G. Normal and cancer stem cells of the human female reproductive system. *Reproductive Biology and Endocrinology*. 2013;11:53. DOI: 10.1186/1477-7827-11-53.
11. Spizzo G., Went P., Dirnhofer S., Obrist P., Moch H., Baeuerle P.A. et al. Overexpression of epithelial cell adhesion molecule (Ep-CAM) is an independent prognostic marker for reduced survival of patients with epithelial ovarian cancer. *Gynecologic Oncology*. 2006;103(2):483–488. DOI: 10.1016/j.ygyno.2006.03.035.
12. Chang B., Liu G., Xue F., Rosen D.G., Xiao L., Wang X. et al. ALDH1 expression correlates with favorable prognosis in ovarian cancers. *Modern Pathology: an Official Journal of the United States and Canadian Academy of Pathology.* 2009;22(6):817–823. DOI: 10.1038/modpathol.2009.35.
13. Deng S., Yang X., Lassus H., Liang S., Kaur S., Ye Q. et al. Distinct expression levels and patterns of stem cell marker, aldehyde dehydrogenase isoform 1 (ALDH1), in human epithelial cancers. *PLoS One*. 2010;5(4):e10277. DOI: 10.1371/journal.pone.0010277.
14. Cioffi M., D'Alterio C., Camerlingo R., Tirino V., Consales C., Riccio A. et al. Identification of a distinct population of CD133(+)CXCR4(+) cancer stem cells in ovarian cancer. *Sci. Report*. 2015;5:10357. DOI: 10.1038/srep10357.
15. Kajiyama H., Shibata K., Terauchi M., Ino K., Nawa A., Kikkawa F. Involvement of SDF-1alpha/CXCR4 axis in the enhanced peritoneal metastasis of epithelial ovarian carcinoma. *International Journal of Cancer.* 2008;122(1):91–99. DOI: 10.1002/ijc.23083.
16. Krohn A., Song Y.H., Muehlberg F., Droll L., Beckmann C., Alt E. CXCR4 receptor positive spheroid forming cells are responsible for tumor invasion *in vitro. Cancer Letters.* 2009;280(1):65–71. DOI: 10.1016/j.canlet.2009.02.005.
17. Rodda D.J., Chew J.L., Lim L.H., Loh Y.H., Wang B., Ng H.H. et al. Transcriptional regulation of nanog by OCT4 and SOX2. *J. Biol. Chem.* 2005;280(26):247317. DOI: 10.1074/jbc.M502573200.
18. Alemohammad H., Asadzadeh Z., Motafakker Azad R., Hemmat N., Najafzadeh B., Vasefifar P. et al. Signaling pathways and microRNAs, the orchestrators of NANOG activity during cancer induction. *Life Sci*. 2020;260:118337. DOI: 10.1016/j.lfs.2020.118337.
19. Lin F.K., Chui Y.L. Generation of induced pluripotent stem cells from mouse cancer cells. *Cancer Biother. Radiopharm*. 2012;27(10):694–700. DOI: 10.1089/cbr.2012.1227.

20. Kaufhold S., Garbán H., Bonavida B. Yin Yang 1 is associated with cancer stem cell transcription factors (SOX2, OCT4, BMI1) and clinical implication. *J. Exp. Clin. Cancer Res.* 2016;35:84. DOI: 10.1186/s13046-016-0359-2.
21. Stevanovic M., Zuffardi O., Collignon J., Lovell-Badge R., Goodfellow P. The cDNA sequence and chromosomal location of the human SOX2 gene. *Mamm. Genome.* 1994;5(10):640–642. DOI: 10.1007/BF00411460.
22. Peng S., Maihle N.J., Huang Y. Pluripotency factors Lin28 and Oct4 identify a sub-population of stem cell-like cells in ovarian cancer. *Oncogene.* 2010;29(14):2153–2159. DOI: 10.1038/onc.2009.500.
23. Baba T., Convery P.A., Matsumura N., Whitaker R.S., Kondoh E., Perry T. et al. Epigenetic regulation of CD133 and tumorigenicity of CD133+ ovarian cancer cells. *Oncogene.* 2009;28(2):209–218. DOI: 10.1038/onc.2008.374.
24. Curley M.D., Therrien V.A., Cummings C.L., Sergent P.A., Koulouris C.R., Friel A.M. et al. CD133 expression defines a tumor initiating cell population in primary human ovarian cancer. *Stem Cells*. 2009;27(12):2875–2883. DOI: 10.1002/stem.236.
25. Silva I.A., Bai S., McLean K., Yang K., Griffith K., Thomas D. et al. Aldehyde dehydrogenase in combination with CD133 defines angiogenic ovarian cancer stem cells that portend poor patient survival. *Cancer Res*. 2011;71(11):3991–4001. DOI: 10.1158/0008-5472.CAN-10-3175.
26. Wei X., Dombkowski D., Meirelles K., Pieretti-Vanmarcke R., Szotek P.P., Chang H.L. et al. Mullerian inhibiting substance preferentially inhibits stem/progenitors in human ovarian cancer cell lines compared with chemotherapeutics. *Proc. Natl. Acad. Sci. U S A.* 2010;107(44):18874–18879. DOI: 10.1073/pnas.1012667107.
27. Jiang H., Lin X., Liu Y., Gong W., Ma X., Yu Y. et al. Transformation of epithelial ovarian cancer stemlike cells into mesenchymal lineage via EMT results in cellular heterogeneity and supports tumor engraftment. *Mol. Med*. 2012;18(1):1197–1208. DOI: 10.2119/molmed.2012.00075.
28. Motohara T., Masuko S., Ishimoto T., Yae T., Onishi N., Muraguchi T. et al. Transient depletion of p53 followed by transduction of c-Myc and K-Ras converts ovarian stem-like cells into tumor-initiating cells. *Carcinogenesis*. 2011;32(11):1597–1606. DOI: 10.1093/carcin/bgr183.
29. Gao M.Q., Choi Y.P., Kang S., Youn J.H., Cho N.H. CD24+ cells from hierarchically organized ovarian cancer are enriched in cancer stem cells. *Oncogene.* 2010;29(18):2672–2680. DOI: 10.1038/onc.2010.35.
30. Kaigorodova E.V., Fedulova N.V., Ochirov M.O., Dyakov D.A., Molchanov S.V., Chasovskikh N.Yu. Dissimilar tumor cell populations in ascitic fluid of ovarian cancer patients. *Bulletin of Siberian Medicine*. 2020;19(1):50–58 (in Russ.). DOI: 10.20538/1682-0363-2020-1-50-58.
31. Kaigorodova E.V., Ochirov M.O., Molchanov S.V., Rogachev R.R., Dyakov D.D., Chernyshova A.L., et al. Dissimilar populations of EpCam-positive cells in ascitic fluid of ovarian cancer patients: a relationship with the degree of carcinomatosis. *Bulletin of Siberian Medicine.* 2021;20(2):44–53 (in Russ.). DOI: 10.20538/1682-0363-2021-2-44-53.
32. Wang Z., Yang L., Huang Z., Li X., Xiao J., Qu Y. et al. Identification of prognosis biomarkers for high-grade serous ovarian cancer based on stemness. *Front. Genet*. 2022;13:861954. DOI: 10.3389/fgene.2022.861954.
33. Bai S., Ingram P., Chen Y.C., Deng N., Pearson A., Niknafs Y.S. et al. EGFL6 regulates the asymmetric division, maintenance, and metastasis of ALDH+ ovarian cancer cells. *Cancer Res.* 2016;76(21):6396–6409. DOI: 10.1158/0008-5472.CAN-16-0225.
34. Wang K., Wang Y., Wang Y., Liu S., Wang C., Zhang S. et al. EIF5A2 enhances stemness of epithelial ovarian cancer cells via a E2F1/KLF4 axis. *Stem Cell Res. Ther*. 2021;12(1):186. DOI: 10.1186/s13287-021-02256-2.
35. Giordano M., Decio A., Battistini C., Baronio M., Bianchi F., Villa A. et al. L1CAM promotes ovarian cancer stemness and tumor initiation via FGFR1/SRC/STAT3 signaling. *J. Exp. Clin. Cancer Res*. 2021;40(1):319. DOI: 10.1186/s13046-021-02117-z.
36. Zhang Y., Wang Y., Zhao G., Tanner E.J., Adli M., Matei D. FOXK2 promotes ovarian cancer stemness by regulating the unfolded protein response pathway. *J. Clin. Invest*. 2022;132(10):151591. DOI: 10.1172/JCI151591.
37. Schwitalla S., Fingerle A.A., Cammareri P., Nebelsiek T., Göktuna S.I., Ziegler P.K. et al. Intestinal tumorigenesis initiated by dedifferentiation and acquisition of stem-cell-like properties. *Cell.* 2013;152(1-2):25–38. DOI: 10.1016/j.cell.2012.12.012.
38. Raghavan S., Mehta P., Xie Y., Lei Y.L., Mehta G. Ovarian cancer stem cells and macrophages reciprocally interact through the WNT pathway to promote pro-tumoral and malignant phenotypes in 3D engineered microenvironments. *J. Immunother. Cancer*. 2019;7(1):190. DOI: 10.1186/s40425-019-0666-1.
39. Zhang S., Balch C., Chan M.W., Lai H.C., Matei D., Schilder J.M. et al. Identification and characterization of ovarian cancer-initiating cells from primary human tumors. *Cancer Res.* 2008;68(11):4311–4320. DOI: 10.1158/0008-5472.CAN-08-0364.
40. Lau W., Peng W.C., Gros P., Clevers H. The R-spondin/Lgr5/Rnf43 module: regulator of Wnt signal strength. *Genes Dev.* 2014;28(4):305–316. DOI: 10.1101/gad.235473.113.
41. Gening S.O., Abakumova T.V., Antoneeva I.I., Rizvanov A.A., Gening T.P., Gufurbayeva D.U. Stem-like tumor cells and proinflammatory cytokines in the ascitic fluid of patients with ovarian cancer. *Russian Clinical Laboratory Diagnostics*. 2021;66(5):297–303 (in Russ.). DOI: 10.51620/0869-2084-2021-66-5-297-303.
42. Meirelles K., Benedict L.A., Dombkowski D., Pepin D., Preffer F.I., Teixeira J. et al. Human ovarian cancer stem/progenitor cells are stimulated by doxorubicin but inhibited by Mullerian inhibiting substance. *Proceedings of the National Academy of Sciences of the United States of America.* 2012;109(7):2358–2363. DOI: 10.1073/pnas.1120733109.
43. Bourguignon L.Y., Peyrollier K., Xia W., Gilad E. Hyaluronan-CD44 interaction activates stem cell marker Nanog, Stat- 3-mediated MDR1 gene expression, and ankyrin-regulated multidrug efflux in breast and ovarian tumor cells. *The Journal of Biological Chemistry.* 2008;283(25):17635–17651. DOI: 10.1074/jbc.M800109200.

44. Xia M., Overman M.J., Rashid A., Chatterjee D., Wang H., Katz M.H. et al. Expression and clinical significance of epidermal growth factor receptor and insulin-like growth factor receptor 1 in patients with ampullary adenocarcinoma. *Human Pathology*. 2015;46(9):1315–1322. DOI: 10.1016/j.humpath.2015.05.012.
45. Garson K., Vanderhyden B.C. Epithelial ovarian cancer stem cells: underlying complexity of a simple paradigm. *Reproduction*. 2015;149(2):59–70. DOI: 10.1530/REP-14-0234.
46. Jäger M., Schoberth A., Ruf P., Hess J., Hennig M., Schmalfeldt B. et al. Immunomonitoring results of a phase II/III study of malignant ascites patients treated with the trifunctional antibody catumaxomab (anti-EpCAM x anti-CD3). *Cancer Res*. 2012;72(1):24–32. DOI: 10.1158/0008-5472.CAN-11-2235.
47. Akhter M.Z., Sharawat S.K., Kumar V., Kochat V., Equbal Z., Ramakrishnan M. et al. Aggressive serous epithelial ovarian cancer is potentially propagated by EpCAM+CD45+ phenotype. *Oncogene*. 2018;37(16):2089–2103. DOI: 10.1038/s41388-017-0106-y.
48. Wicha M.S., Liu S., Dontu G. Cancer stem cells: an old idea – a paradigm shift. *Cancer Res.* 2006;66(4):1883–1890. DOI: 10.1158/0008-5472.CAN-05-3153.
49. Kaigorodova E.V., Kovalev O.V., Chernyshova A.L., Vtorushin S.V., Shpileva O.V. Heterogeneity of EpCAM-positive cells in low-grade serous ovarian carcinoma ascitic fluid: a clinical case. *Tumors of Female Reproductive System.* 2021;17(4):90–95 (in Russ.). DOI: 10.17650/1994-4098-2021-17-4-90-95.
50. Kozik A.V., Kaigorodova E.V., Grishchenko M.Yu., Vtorushin S.V., Chernyshova A.L. EPCAM+CD45+ cells in ascitic fluid of patients with ovarian cancer: a relationship with tumor marker levels and tumor grade. *Siberian Journal of Oncology.* 2022;21(5):44–51 (in Russ.). DOI: 10.21294/1814-4861-2022-21-5-44-51.
51. Kaigorodova E.V., Kozik A.V., Zavaruev I.S. Grishchenko M.Y. Hybrid/atypical forms of circulating tumor cells: current state of the art. *Biochemistry Moscow.* 2022;87(4):380–390. DOI: 10.1134/S0006297922040071.
52. Izar B., Tirosh I., Stover E.H., Wakiro I., Cuoco M.S., Alter I. et al. A single-cell landscape of high-grade serous ovarian cancer. *Nature Medicine.* 2020;26(8):1271–1279. DOI: 10.1038/s41591-020-0926-0.
53. Wu X., Liu X., Koul S., Lee C.Y., Zhang Z., Halmos B. AXL kinase as a novel target for cancer therapy. *Oncotarget.* 2014;5(20):9546–9563. DOI: 10.18632/oncotarget.2542.
54. Shield K., Ackland M.L., Ahmed N., Rice G.E. Multicellular spheroids in ovarian cancer metastases: Biology and pathology. *Gynecologic Oncology.* 2009;113(1):143–148. DOI: 10.1016/j.ygyno.2008.11.032.
55. Bapat S.A., Mali A.M., Koppikar C.B., Kurrey N.K. Stem and progenitor-like cells contribute to the aggressive behavior of human epithelial ovarian cancer. *Cancer Res.* 2005;65(8):3025–3029. DOI: 10.1158/0008-5472.CAN-04-3931.
56. Shield K., Ackland M.L., Ahmed N., Rice G.E. Multicellular spheroids in ovarian cancer metastases: biology and pathology. *Gynecol. Oncol.* 2009;113(1):143–148. DOI: 10.1016/j.ygyno.2008.11.032.
57. Liu X., Taftaf R., Kawaguchi M., Chang Y.F., Chen W., Entenberg D. et al. Homophilic CD44 interactions mediate tumor cell aggregation and polyclonal metastasis in patient-derived breast cancer models. *Cancer Discov*. 2019;9(1):96–113. DOI: 10.1158/2159-8290.CD-18-0065.
58. Xu S., Yang Y., Dong L., Qiu W., Yang L., Wang X. et al. Construction and characteristics of an E-cadherin-related three-dimensional suspension growth model of ovarian cancer. *Scientific Reports.* 2014;4:5646. DOI: 10.1038/srep05646.
59. Sodek K.L., Ringuette M.J., Brown T.J. Compact spheroid formation by ovarian cancer cells is associated with contractile behavior and an invasive phenotype. *International Journal of Cancer.* 2009;124(9):2060–2070. DOI: 10.1002/ijc.24188.
60. Kenny H.A., Chiang C.Y., White E.A., Schryver E.M., Habis M., Romero I.L. et al. Mesothelial cells promote early ovarian cancer metastasis through fibronectin secretion. *The Journal of Clinical Investigation.* 2014;124(10):4614–4628. DOI: 10.1172/JCI74778.
61. Iwanicki M.P., Chen H.Y., Iavarone C., Zervantonakis I. K., Muranen T., Novak M. et al. Mutant p53 regulates ovarian cancer transformed phenotypes through autocrine matrix deposition. *JCI Insight.* 2016;1(10):e86829. DOI: 10.1172/jci.insight.86829.
62. Lengyel E. Ovarian cancer development and metastasis. *Am. J. Pathol.* 2010;177(3):1053–1064. DOI: 10.2353/ajpath.2010.100105.
63. Elloul S., Elstrand M.B., Nesland J.M., Tropé C.G., Kvalheim G., Goldberg I. et al. Snail, slug, and smad-interacting protein 1 as novel parameters of disease aggressiveness in metastatic ovarian and breast carcinoma. *Cancer.* 2005;103(8):1631–1643. DOI: 10.1002/cncr.20946.
64. Veatch A.L., Carson L.F., Ramakrishnan S. Differential expression of the cell-cell adhesion molecule E-cadherin in ascites and solid human ovarian tumor cells. *International Journal of Cancer*. 1994;58(3):393–399. DOI: 10.1002/ijc.2910580315.
65. Latifi A., Luwor R.B., Bilandzic M., Nazaretian S., Stenvers K., Pyman J. et al. Isolation and characterization of tumor cells from the ascites of ovarian cancer patients: molecular phenotype of chemoresistant ovarian tumors. *PloS One.* 2012;7(10):e46858. DOI: 10.1371/journal.pone.0046858.
66. Fritz J.L., Collins O., Saxena P., Buensuceso A., Ramos Valdes Y., Francis K. E. et al. A novel role for NUAK1 in promoting ovarian cancer metastasis through regulation of fibronectin production in spheroids. *Cancers.* 2020;12(5):1250. DOI: 10.3390/cancers12051250.
67. Ojasalu K., Brehm C., Hartung K., Nischak M., Finkernagel F., Rexin P. et al. Upregulation of mesothelial genes in ovarian carcinoma cells is associated with an unfavorable clinical outcome and the promotion of cancer cell adhesion. *Molecular Oncology*. 2020;14(9):2142–2162. DOI: 10.1002/1878-0261.12749.
68. Reinartz S., Lieber S., Pesek J., Brandt D.T., Asafova A., Finkernagel F. et al. Cell type-selective pathways and clinical associations of lysophosphatidic acid biosynthesis and signaling in the ovarian cancer microenvironment. *Molecular Oncology*. 2019;13(2):185–201. DOI: 10.1002/1878-0261.12396.
69. Kipps E., Tan D.S., Kaye S.B. Meeting the challenge of ascites in ovarian cancer: new avenues for therapy and research. Nature reviews. *Cancer.* 2013;13(4):273–282. DOI: 10.1038/nrc3432.

70. Rakina M., Kazakova A., Villert A., Kolomiets L., Larionova I. Spheroid formation and peritoneal metastasis in ovarian cancer: the role of stromal and immune components. *International Journal of Molecular Sciences*. 2022;23(11):6215. DOI: 10.3390/ijms23116215.
71. Dick J.E., Bhatia M., Gan O., Kapp U., Wang J.C. Assay of human stem cells by repopulation of NOD/SCID mice. *Stem Cells*. 1997;15Suppl.1:199–203;discussion 204–197. DOI: 10.1002/stem.5530150826.
72. Wang L., Mezencev R., Bowen N.J., Matyunina L.V., McDonald J.F. Isolation and characterization of stem-like cells from a human ovarian cancer cell line. *Molecular and Cellular Biochemistry*. 2012;363(1-2):257–268. DOI: 10.1007/s11010-011-1178-6.
73. De Thé H. Differentiation therapy revisited. *Nat. Rev. Cancer.* 2018;18(2):117–127 DOI: 10.1038/nrc.2017.103.
74. Nayak S., Mahenthiran A., Yang Y., McClendon M., Mania-Farnell B., James C.D. et al. Bone morphogenetic protein 4 targeting glioma stem-like cells for malignant glioma treatment: latest advances and implications for clinical application. *Cancers (Basel).* 2020;12(2):516. DOI: 10.3390/cancers12020516.
75. Arima Y., Nobusue H., Saya H. Targeting of cancer stem cells by differentiation therapy. *Cancer Sci*. 2020;111(8):2689–2695. DOI: 10.1111/cas.14504.
76. Correa R.J., Peart T., Valdes Y.R., DiMattia G.E., Shepherd T.G. Modulation of AKT activity is associated with reversible dormancy in ascites-derived epithelial ovarian cancer spheroids. *Carcinogenesis.* 2012;33(1):49–58. DOI: 10.1093/carcin/bgr241.
77. Hua H., Zhang H., Chen J., Wang J., Liu J., Jiang Y. Targeting Akt in cancer for precision therapy. *Journal of Hematology & Oncology*. 2021;4(1):128. DOI: 10.1186/s13045-021-01137-8.
78. Emami K.H., Nguyen C., Ma H., Kim D.H., Jeong K.W., Eguchi M. et al. A small molecule inhibitor of beta-catenin/CREB-binding protein transcription [corrected]. *Proceedings of the National Academy of Sciences of the United States of America*. 2004;101(34):12682–12687. DOI: 10.1073/pnas.0404875101.
79. Rafehi S., Ramos Valdes Y., Bertrand M., McGee J., Préfontaine M., Sugimoto A. et al. TGFβ signaling regulates epithelial-mesenchymal plasticity in ovarian cancer ascites-derived spheroids. *Endocrine-related cancer.* 2016;23(3):147–159. DOI: 10.1530/ERC-15-0383.
80. Jäger M., Schoberth A., Ruf P., Hess J., Hennig M., Schmalfeldt B. et al. Immunomonitoring results of a phase II/III study of malignant ascites patients treated with the trifunctional antibody catumaxomab (anti-EpCAM x anti-CD3). *Cancer Research*. 2012;72(1):24–32. DOI: 10.1158/0008-5472.CAN-11-2235.

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