Obstetrics and Gynecology

Chapter 1

The Role of Cancer Stem Cells (CSCs) in Gynecological Malignancies

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1. Introduction

Cancer in women is a global health problem. The identification of molecular mechanisms related to cancer development will enable the introduction of new effective therapies and oncological gynaecology.

Cancer stem cells (CSCs) are one of the reasons behind the failure of oncological treatment. Most likely, the source of CSCs are somatic stem cells that have been transformed under the influence of various factors: genetic and epigenetic. CSCs are a small, specific subpopulation of cells which account for about 2% of the tumour mass but are resistant to treatment by standard methods: chemotherapy and radiation. These cells play a key role in tumour progression, metastasis and cancer recurrence. The relationship between CSCs and the initiation of some types of malignant tumours has also been described [1,2,3,4]. CSCs are located in the vascular niche near endothelial cells which provides contact with the microenvironment, facilitates CSC survival and supports their properties [5, 6, 7].

CSCs were first described in 1994 in acute myeloid leukaemia [8]. In 2005, Bapat et al., [9] isolated CSCs in ovarian cancer.

CSCs possess unique characteristics [10-14]:

- the ability to self-renew
- can remain inactive as “dormant cells” – mostly in the G0 / G1 phase
- the ability to repair DNA
- promote angiogenesis
- display overexpression of ABC (ATP-binding cassette family) gene coding for transport proteins (e.g. P-gp-glycoprotein P, BCRP-breast cancer resistance family, MRP1- multidrug resistance protein -1),
- are able to use various signalling pathways, including canonical Wnt, Notch, Hedgehog-Shn (sonic hedgehog homolog) and PI3K / AKT / mTOR related to survival, self-renewal, mobility, adhesion, inhibition of apoptosis, proliferation control and resistance to cytostatics.
- Cancer stem cells can be identified and isolated through specific biomarkers for different types of cancer, for primary and relapsed cancers.
- CSCs carry specific molecular portraits which can assist CSC isolation and targeted therapy [5,15,16]. The most frequently detected markers in breast, ovarian, endometrial and cervical cancer include [13,17,18,19,20,21,22]:
- CD44 +, C117 (c-kit). Also known as ovarian cancer-initiating cells, their expression is related to the poor clinical course of many gynaecological cancers.
- CD133 + (promin), ALDH1 (aldehyde dehydrogenase 1). Their expression is associated with resistance to platinum derivatives and taxanes. They are an independent predicting factor for poor prognosis.
- Oct-4 (POUSF 1), Nestin, BMi1 are transcription factors associated with self-renewal of CSCs, resistance to cytostatics and they also correlate with poor prognosis in many cancers.
- According to Massard et al. [23], to avoid failure of oncological treatment resulting from the activity of CSCs, they should be differentiated into mature forms or eradicated.

Research into CSCs is becoming an increasingly strategic factor. The research focuses on signalling pathways such as Wnt, Notch or Shn that determine the survival of CSCs, on ABC transporters, and specific properties of CSCs. The induction of differentiation should lead to the loss of CSC self-renewal abilities (e.g. the described role of retinoids) and the elimination of CSCs and loss of CSC surface markers may be achieved through inhibitors of the signalling pathways used [3,7,20,24].

Molecular and observational studies have established that salinomycin, metformin, as well as some microRNAs are active in therapeutic strategies targeting stem cells.

Salinomycin is an ionophore antibiotic (cation carrier) isolated in 1974 from the bacterial strain *Streptomyces albus*. Salinomycin disrupts the Na + / K + ion gradient in biological membranes, which leads to cell apoptosis.

In 2009, Gupta et al. [25] tested as many as 16,000 chemical compounds against breast cancer CSCs and detected a strong (100 x greater) activity of salinomycin compared to taxol. Many studies have been published on the antitumor activity of salinomycin, including the mechanism of its action in various types of cancer *in vitro* and *in vivo* [26].

Salinomycin has been found to induce apoptosis in CSCs via the Wnt pathway, it is resistant to ABC transporters and, due to its molecular size (75 kDa), is not pumped out of the cell. Salinomycin inhibits the activity of MDR1, influences the differentiation of CSCs by destroying their properties, and is also active in the destruction of CSCs resistant to cytostatics (e.g. taxol and cisplatin) [27, 28, 29, 30].

Metformin - a biguanide derivative, is used in type 2 diabetes. It has been shown that in diabetic patients, this drug reduces morbidity and mortality from various neoplasms by more than 30% compared to patients with diabetes treated with other drugs [31]. Metformin exhibits pleiotropic activity: it inhibits mTOR signalling, has anti-angiogenic activity, reduces cyclin D1 activity, stops the cell cycle in the GO / G1, G2 / M and S phase, lowers the expression of anti-apoptotic Bcl-2 and increases pro-apoptotic Bax. In addition, it lowers the expression of markers typical for ovarian CSCs - such as CD44 +, Nanog and ALDH +. A growing body of evidence suggests that it sensitizes cancer cells to chemical treatments and other DNA damaging drugs by selectively targeting their CSCs [32,33,34].

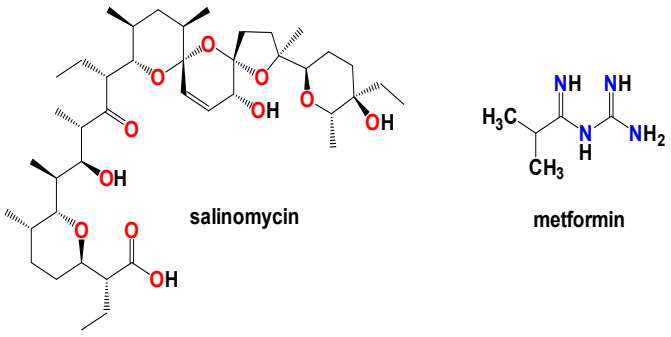

Figure 1: Structure of salinomycin and metformin.

MicroRNAs – miRNAs are short, non-coding RNAs of an average length of 22 nucleotides, key post-transcriptional regulators of about 60% of genes. Research has established that in the context of cancer, miRNAs can act as oncogenes and as suppressors. The miR-34 and miR-200 families have been reported to be suppressors of cancer development, especially miR-34a.

Loss of miRNA expression influences cancer progression through invasion, metastasis and drug resistance as well as anti-apoptotic activity. These processes related to the initiation and development of cancer are caused by the regulation of the properties of CSCs - including drug resistance and self-renewal [3,34,35,36].

2. Breast Cancer

According to the world registry Globocan, breast cancer is the most common malignant neoplasm in women [37]. The high rates of aggressiveness, drug resistance, and recurrence of this cancer are attributed to the presence of CSCs. Due to the heterogeneity of breast cancer, the applied chemo- and targeted therapies are continually being developed, with particular focus on the properties of CSCs [4,38].

Studies on established MCF-7 cell lines and highly aggressive triple-negative cancer lines (MDA-MB-231) showed a promising effect of salinomycin derivatives. Salinomycin esters induced cell apoptosis of both types of cancer to a greater extent than salinomycin alone. Moreover, these compounds showed lower toxicity in relation to normal breast cells [39].

Gao et al. [40] also used a new drug delivery system on breast cancer cell lines (MCF-7 and MCF-7-MS) - nanoparticles containing salinomycin and docetaxel (DTX). According to the authors, the assessment of the toxicity of nanoparticles was more potent than any drug *in vitro*. Similarly, other studies used keratin nanoparticles loaded with salinomycin combined with photodynamic therapy (chlorine e6). CSCs have been eradicated, mainly by limiting their self-renewal capacity [41].

Shen et al. [42] also applied a nanotherapy strategy to eliminate CSCs. The nanoparticles contained the chemotherapeutic agent camptothecin and all-trans retinoic acid (ALTRA), which induces CSC differentiation. Studies in a mouse model showed the effectiveness of this drug combination: tumour growth inhibition, relapse and metastasis prevention.

Therapies targeting breast cancer CSCs are developing. Salinomycin use was pioneered by Naujokat, who applied salinomycin for the first time in a 40-year-old woman with disseminated breast cancer with good results [43].

Recently, the *in vivo* eradication effect of recombinant adenovirus (Ad-apoptin-hTERTp-E1a, Ad-VT) on BCSCs (breast CSCs) was described. Researchers believe that these results form the theoretical basis for future treatment of breast cancer [44].

3. Ovarian Cancer

Ovarian cancer is the leading cause of mortality from gynaecological cancers. About 70% of cases are diagnosed in the advanced clinical stage, which requires chemotherapy.

Despite an initially good response to treatment, disease relapses mean that the 5-year survival in the advanced stage is only 25% [20, 37].

Salinomycin has been shown (*in vitro* and *in vivo*) to eradicate the CSCs of cisplatin-resistant ovarian cancer. The mechanism of this activity was based on cell apoptosis caused by an increase in the expression of the death receptor DR5 and caspase 8 - the death executive enzyme [45]. The same research group also found a different way to eliminate cisplatin-resistant ovarian CSCs. In *in vitro* studies and an animal model, salinomycin inhibited the nuclear transcription factor NF-kB leading to apoptosis of CSCs [29].

Zhang et al. [46] used salinomycin at various concentrations and intervals (24-72 hours) on six different ovarian cancer cell lines, including those resistant to cisplatin and xenograft (*in vivo*). They showed that salinomycin significantly inhibited the growth of human cisplatin ovarian cancer cell lines as well as tumour growth in the xenograft by inducing apoptosis which was strongly associated with MAPKp38 activation.

Salinomycin has also been found to eradicate ovarian cancer cells through apoptosis. Apoptosis occurs through the activation of caspase 3 (which can also be cascade-activated via the aforementioned caspase 8) and an increase in the expression of the pro-apoptotic Bax gene as well as a decrease in the expression of the anti-apoptotic Bcl-2. During the 24-hour incubation, salinomycin killed 40% of cancer cells, but did not affect normal cells [47].

Lee et al. [28] investigated the effect of salinomycin on ovarian cancer stem cells isolated from ascites in women with advanced ovarian cancer. The surface markers of these CSCs were labelled CD44 + and CD17 + and treated with salinomycin or salinomycin and paclitaxel. The ability to form a spheroid forming assay (which is an essential feature for the survival of CSCs) was observed for 14 days. Adding paclitaxel alone or salinomycin alone was less effective in reducing CSC viability, inhibiting CSC growth and the formation of spheroids than when salinomycin was combined with paclitaxel. The authors concluded that salinomycin in combination with paclitaxel has the potential to treat ovarian cancer effectively.

In other studies, it has been shown that metformin inhibits ovarian cancer stem cells (*in vitro* and *in vivo*) and reduces the expression of markers typical to ovarian CSCs: CD44 +, Nanog and ALDH + [48,49]. This was confirmed by observational studies that showed a significant increase in progression free survival (PFS) and overall survival (OS), as well as a reduction in the risk of ovarian cancer recurrence in women with type 2 diabetes treated with metformin compared to women treated with other antidiabetics [50,51].

In 2020, the results of a phase II clinical trial with metformin as a drug targeting ovarian CSCs were announced. Patients with stage II-IV ovarian cancer were treated with neoadjuvant metformin, surgery and then adjuvant chemotherapy with metformin, or neoadjuvant

metformin and chemotherapy, while in the following stages, they were treated with surgery and complementary chemotherapy with metformin. Determination of CSCs with markers CD133 + and ALDH + and observation of PFS and OS indicated that metformin is an active drug against ovarian CSCs and causes increased sensitivity to cisplatin [52].

Targeting the Notch3 pathway, bound to the transmembrane protein, is a novel and novel method of eradication of ovarian CSCs [53]. Gamma-secretase inhibitors (GSI) against Notch receptors have been used in preclinical and clinical studies. It has been suggested that Notch3 is associated with carboplatin resistance and may be both a biomarker and a target in future therapies [23].

4. Endometrial Cancer EC

Endometrial cancer is one of the most common malignant neoplasms of the female genital organs. CSCs have been shown to play a significant role in the progression, metastasis, therapeutic resistance, and recurrence of this cancer [54].

The most common signalling pathways activated by endometrial CSCs are Wnt / β catenin, Notch 1, and Hedgehog. North American Network Operations Group Homebox describes encouraging results of selective drugs (the use of chemotherapy with immunotherapy), but little clinical data is available [55].

In vitro and *in vivo* studies on the effects of salinomycin on the cells of the lateral population in endometrial cancer have shown that this drug inhibits Wnt signalling, proliferation, migration and invasion of cells by inducing apoptosis [56].

Studies in the Ishikawa endometrial cancer cell line showed that salinomycin (concentration of 1 μM) caused more than 50% of cell death. This eradication was caused by an increase in the activity of pro-apoptotic genes (BAX, p53 and FASL, and a decrease in the expression of anti-apoptotic genes (Bcl 2, BIRCS) [57].

Another study also on the Ishikawa line showed that cancer cell apoptosis was related to salinomycin levels. Salinomycin was found to affect the expression of mRNA and numerous miRNAs associated with the CSCs of this cancer. Therefore, the action of salinomycin on molecular mechanisms can also be exploited in future target therapies [58].

Other studies confirm the effect of inhibiting the activity of Notch and Wnt / β catenin signalling pathways. In their *in vitro* and *in vivo* studies, Wang et al. [59] reported that miR-34a plays an inhibitory role in endometrial cancer by reducing Notch 1 activation. This effect inhibited proliferation, migration and invasion. In a study by Chen et al. [60], miR-202 inhibited EC migration and invasion by inactivating the Wnt / β catenin pathway.

This mode of activity raises the hypothesis that miR-34a and MIR-202 may be potential targets for prevention and treatment [59,60].

A review by Gadducci et al. [33] describes the beneficial effect of metformin on EC. It restores the sensitivity of cancer cells to both cisplatin and paclitaxel and to progestins. Meirles et al. [61] presented the results of a query from electronic databases on the effect of metformin on EC. Patients with concomitant diabetes treated with metformin had significantly longer OS compared to those treated with other antidiabetic medication or compared to women with EC without diabetes. The above authors' are of the opinion that these results support the importance of future clinical trials concerning the influence of metformin on the treatment of endometrial cancer.

5. Cervical Cancer

Cervical cancer is the most common malignancy among women, especially in countries with an economically lower standard. New therapeutic solutions are required due to the high morbidity and mortality rates. The field of research on CSCs in this neoplasm is developing dynamically. Determination of the expression of CSCs markers in the tissues of patients diagnosed with CIN III and invasive cancer will allow for early recognition of CSC markers and determination of the relationship between their expression and the prognosis.

CD24 + has been reported to be an independent prognostic marker associated with metastasis and shorter survival. OCT4 expression is also an important prognostic factor [62]. Liu and Zheng [63] determined ALDH expression in cervical cancer cells and in an *in vivo* model. They found that ALDH expression is increased in cisplatin-resistant cervical cancer cells. Fan et al. [64] reported that the use of ALTRA (all-trans retinoic acid - vitamin A derivative) in combination with cisplatin, compared to cisplatin alone, significantly inhibited cell proliferation, cell migration and induced apoptosis. The authors concluded that ALTRA significantly enhances the antitumor effect of cisplatin through the eradication of CSCs.

Another described method of CSC elimination in cervical cancer was the use of Pterostilbene - a polyphenol derived from resveratrol. This compound inhibited cancer cells and CSCs with better results than resveratrol [65]. The compound was found to reduce cell survival and metastatic capacity significantly. In addition, Pterostilbene led to a cycle arrest in the S / G2 / M phase, induced cell apoptosis and inhibited the expression of matrix metalloproteinases (MMP 2 and 9).

The activity of pterostilbene was associated with the reduction in the expression of CSCs markers, including CD 133, Oct 4, Nanog and SOX 2, which is a key transcription factor. In the authors' opinion, this plant compound may be a potential antitumor agent acting both on cervical cancer cells and on CSCs.

6. Conclusion

The existence of CSCs is undoubtedly associated with the reduction of the effectiveness of oncological therapies. Developing knowledge about cancer-specific markers and signalling pathways used by CSCs will introduce targeted therapies against CSCs.

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