Cancer stem cells markers in ovarian cancer: Clinical and therapeutic significance (Review)

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Abstract. Ovarian cancer is a gynecological neoplasm that can be found in women, which, due to diagnostic difficulties, is often detected at advanced stages when treatment becomes a significant problem. Moreover, in a number of cases there is a cancer recurrence and resistance to standard chemotherapy treatment. It has been suggested that cancer stem cells (CSCs) that were not eradicated during therapy may be responsible for this. For this reason, effective therapeutic methods eliminating CSCs are being studied, such as therapy targeting CSCs markers. In addition, numerous studies have also drawn attention to the usefulness of CSCs markers in predicting disease progression and assessing patient's prognosis as well as their importance in the development of treatment resistance. The present review presented research on selected CSCs markers, which may be of significant prognostic and therapeutic importance in ovarian cancer.

Contents

1. Introduction

Ovarian cancer (OC) is one of the 10 most common types of cancer in women in the world. In 2020, ovarian cancer was ranked eighth in terms of incidence and mortality with over 313 000 new cases and over 207 000 deaths (1).

Among ovarian cancers (carcinomas), there are cancers originating from epithelial cells (the most common), germ cells and stromal cells (2). According to the World Health Organization (WHO) classification of female genital tumours from 2020, at least five main types of ovarian carcinomas are identified based on histopathology, immunoprofile and molecular analysis. Among them, high-grade serous carcinoma (HGSC) is the most common ovarian cancer accounting for about 70% of all ovarian carcinomas, the second most common histotype is endometroid carcinoma (EC, 10%), clear cell carcinoma (CCC, 6-10%), low-grade serous carcinoma (LGSC, 5%) and mucinous carcinoma (MC, 3-4%) (3).

An important diagnostic and therapeutic problem is a diagnosis of ovarian cancer patients at advanced stages with metastatic sites within the peritoneal cavity, retroperitoneum and even in distant organs (4). In such cases patients have a much lower chance of recovery and the five-year survival rate is less than 30% (5,6). Early diagnosis of ovarian cancer is difficult due to the lack of appropriate markers and definitive screening tools as well as non-specific symptoms accompanying this cancer. These include: bloating and abdominal pain, early satiety or fullness, changes in bowel habits or frequent urination. Women with those symptoms may seek medical help too late and even be treated without identifying the specific causes of their symptoms (7).

Treatment depends on the diagnosed stage of ovarian cancer (8). The standard therapy involves surgical treatment, which is maximal cytoreductive debulking, and the platinum-based chemotherapy (8,9). Unfortunately, tumours relapse in over 70% of cases, despite an initially good response to treatment by the majority of patients (4). In recent years, Poly(ADP-ribose) polymerase inhibitors (PARPi) have been approved for the treatment of ovarian cancer as drugs that maintain therapy following the completion of first-line platinum-based chemotherapy (10,11).

It is believed that cancer stem cells (CSCs) that have not been eliminated during treatment and are responsible for the development of resistance to chemotherapy and are able to replenish their population, may contribute to recurrence of the cancer, which might be even more aggressive (4).

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Hence, effective methods of cancer treatment based on the elimination of CSCs are being sought. High hopes are raised by CSCs-targeting therapies, which in combination with traditional methods of treatment may give a better therapeutic effect (12).

2. Cancer stem cells (CSCs)

Models of tumorigenesis. The two main models try to explain the origin, progression and heterogeneity of tumours: the stochastic model (or clonal evolution model) and the hierarchical (or CSC) model (13-15). According to the stochastic model, each tumour cell is biologically homogeneous and has the same developmental potential as well as the ability to promote tumour progression (16,17). This model assumes that the acquisition of oncogenic mutations in normal differentiated somatic cells results in hyperplasia and contributes to clonal expansion (14,17). The accumulation of genetic and epigenetic alterations in cells may increase tumour aggressiveness, invasiveness and treatment resistance which in turn leads to tumour progression and increases tumour heterogeneity (13).

The hierarchical model states that only a distinct population of cancer cells features tumorigenic potential-these cells are referred to as cancer stem cells (CSCs). According to this model, tumour initiation starts when a normal stem cell escapes regulation and becomes cancer stem cell-the first abnormal cell assumed to be the cell-of-origin (14,17). Moreover, this model says that there is a differentiation hierarchy of cells in tumour that includes CSCs responsible for maintaining the whole populations of cells in tumour (13,15,16,18).

However, there is an alternative model of cellular plasticity that combines these two models by assuming that cancer cells can interconvert between stem cell and differentiated states (13). Cell dedifferentiation capacity may be inherited (hierarchical model) or acquired through mutations (stochastic model) (14). According to the plasticity model, differentiated tumour cells can reacquire stem cell characteristics by intrinsic processes of these cells and/or stimuli within the tumour microenvironment (13).

The origin of cancer stem cells. Cancer stem cells (CSCs) represent a small subpopulation of cells in tumour mass (19). The origin of these cells has still not been clearly elucidated. One of the hypotheses suggests that CSCs originate from normal adult stem cells that have acquired epigenetic and genetic changes (20). On the other hand, CSCs may derive from mature differentiated cells through various mechanisms, including genomic instability, horizontal gene transfer and microenvironmental changes (21). A differentiated cancer cells may de-differentiate into CSCs in response to different factors, such as stress and hypoxia, wounding or ionizing radiation (22). Various studies suggest that the epithelial-mesenchymal transition (EMT) is involved in dedifferentiation and cells that have undergone this process exhibit a more CSC-like phenotype allowing them to self-renew and differentiate into all cell types in the tumour (23). It is believed that CSCs can also arise as a result of cell fusion and metabolic reprogramming of non-CSCs into CSCs during the cancer development (21,22).

Characteristics of cancer stem cells. The assumption that CSCs originate from normal stem cells (NSCs) that have accumulated transforming mutations may be supported by the fact that these cells share many features (13). Both CSCs and NSCc have the capacity for self-renewal through mitotic divisions. Symmetric divisions give rise to two sister stem cells, while asymmetric divisions give rise to one daughter stem cell and one differentiated cell (24). The ability of CSCs to divide asymmetrically enables these cells to both self-renew their population and initiate a neoplastic process (25). However, NSCs are able to control and regulate self-renewal, while CSCs have lost this capacity (26). Moreover, CSCs and NSCs are regulated by similar signalling pathways such as Wnt, Hedgehog or Notch (26). Most of these pathways are essential for stemness properties of NSCs, such as the ability to self-renew, differentiate, proliferate and develop various organs during embryogenesis. However, genetic mutations and epigenetic changes may cause dysregulation of these pathways in the CSCs, leading to uncontrolled self-renewal and impaired differentiation of these cells (27).

Both CSCs and NSCs have the ability to differentiate into multiple progenitor cell types. However, CSCs can replicate and differentiate in an uncontrolled manner into populations of molecularly and phenotypically altered progenitor cells that may have limitless proliferative and survival potential with more plasticity than progeny of NSCs (13). Additionally, CSCs and NSCs possess high telomerase activity that prolongs their life span, express similar surface receptors and can stimulate angiogenesis (28).

In addition to impaired self-renewal and differentiation abilities, CSCs also have other characteristics that distinguish them from NSCs, such as the ability to form tissues and organs. NSCs develop through organogenesis to form internal organs, while CSCs have tumorigenic properties and form tumour tissues. Moreover, NSCs have normal karyotyping, whereas CSCs have abnormal karyotyping with genetic alterations (28). Characteristics of CSCs are summarised in Fig. 1.

The importance of tumour microenvironment for CSCs. Stem cells division and differentiation take place in a specialised microenvironment (the niche) that regulates self-renew of these cells through cell-cell communication or secretion of paracrine factors (28,29). CSCs are population of cancer cells within the cancer microenvironment that consists of various cells, including cancer-associated fibroblasts (CAFs), mesenchymal stem cells (MSCs), endothelial cells (ECs) and immune cells (the macrophages, T-cells and natural killer (NK) cells, factors secreted by these cells, such as cytokines and growth factors as well as the extracellular matrix (ECM) (28,29). ECM is a noncellular component of tumour microenvironment composed of glycosaminoglycans, collagens, metalloproteases, hyaluronic acid, polysaccharides, glycoproteins, proteoglycans and other proteins (30).

The components of tumour microenvironment provide ideal conditions for maintaining the properties of CSCs, such as self-renewal, proliferation and differentiation as well as generation of heterogeneous cancer population (30). Moreover, the tumour niche supports initiation, growth, invasion and metastasis of tumour cells and also plays an important role in therapy resistance, mostly by supporting stem-related signaling pathway maintenance in CSCs (29). The CSCs are surrounded
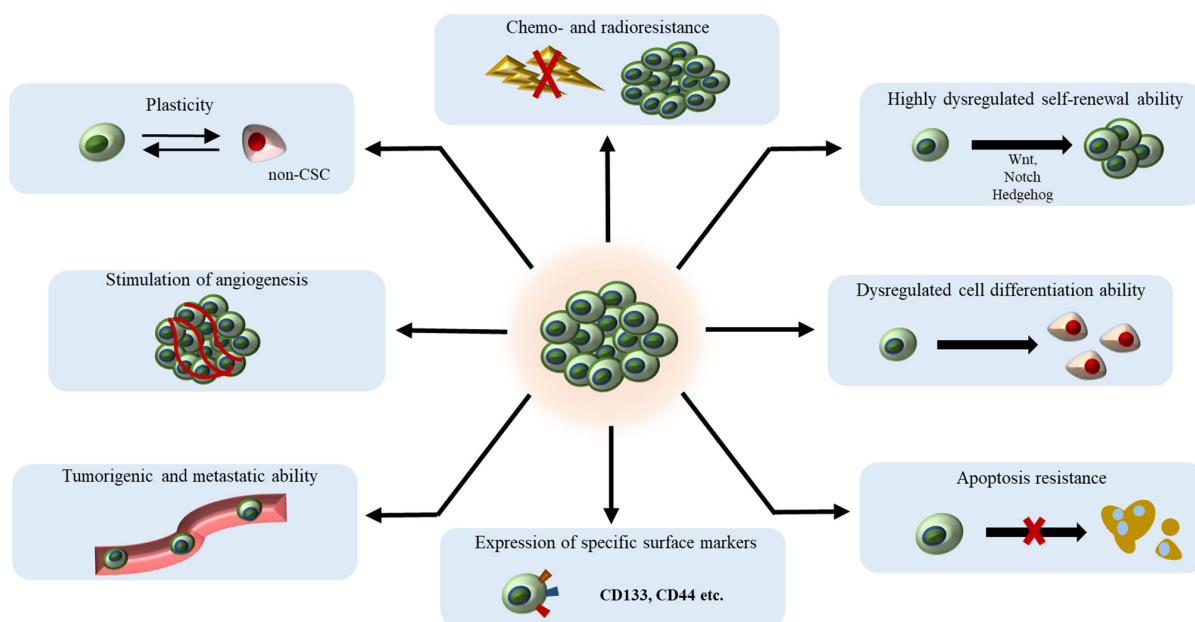
Figure 1. Characteristics of CSCs including dysregulated self-renewal and differentiation abilities, apoptosis resistance, expression of specific surface markers, tumorigenic and metastatic ability, stimulation of angiogenesis, plasticity, chemo- and radio-resistance (21,26,28). CSCs, cancer stem cells.

by cancer niche cells, which secrete factors promoting survival and plasticity of CSCs, as well as increasing drug resistance. Additionally, ECM is a physical barrier that protects CSCs from chemotherapeutic agents (29).

However, the relation between CSCs and their niche can be bidirectional. It is suggested that CSCs may promote the recruitment and activation of niche components by producing factors such as proinflammatory cytokines and chemokines. Furthermore, it has been shown that CSCs may differentiate into functional ECs, which in turn may transdifferentiate into MSCs (29).

Within tumours CSCs are usually located near hypoxic regions. Hypoxia plays a role in the maintenance of CSCs characteristics. It is also involved in chemo- and radioresistance (29). In hypoxic conditions CSCs produce vascular endothelial growth factor (VEGF), which further induces angiogenesis (14).

Methods of CSCs identification and isolation. The specific properties of CSCs are used in methods of isolating these cells from a tumour mass or cell culture (31). There are several *in vitro* assays to identify CSCs, such as the detection of surface markers, assessment of the activity of aldehyde dehydrogenase (ALDH)-Aldefluor assay, sphere-forming assay or Hoechst dye exclusion assay (32). Methods that use the expression of surface markers of CSCs to isolate these cells include fluorescence-activated cell sorting (FACS), based on flow cytometry and using fluorescently labeled antibodies, and magnetic activated cell sorting (MACS) using antibodies coupled with superparamagnetic nanoparticles (31). Moreover, polymerase chain reaction analysis is also used to isolate CSCs by identifying the markers expressed on the cell surface (21,32).

On the other hand, CSCs may also be identified by the flow cytometry-based ALDEFLUOR assay which measure the activity of intracellular marker-ALDH, which is increased in these cells (31-33).

The ability of CSCs to form spheres is also used to identify and isolate these cells. Culturing cells harvested from tumour specimens in a serum-free medium supplemented with basic fibroblast growth factor (bFGF) and epithelial growth factor (EGF) results in the formation of non-adherent spheres by immature cells (21,31,32). Another method of isolating CSCs is based on the ability of cells, termed as the side population (SP), to export a fluorescent dye, such as Hoechst 33342 or Rhodamine 123, by ABC transporters (21,31,32). However, some ABC transporters expressed on CSCs, such as ABCB1 or ABCG2, are also expressed on non-CSCs (21). Moreover, this method is limited by the toxicity of the dyes (21,32).

When identifying and isolating CSCs, it should be kept in mind, that CSCs share many features with NSCs and are similar e.g., in the expression of specific surface markers or the utilization of common signaling pathways. In contrast, when CSCs are transplanted into animals they can form a tumour, while NSCs do not have this ability (32). In the method of isolating CSCs *in vivo*, that is serial transplantation assay in animal model, tumour cells are transplanted into immunocompromised mouse. Such *in vivo* assays are regarded as the gold standard in the identification of CSCs (32).

3. CSCs in ovarian cancer

Cancer stem cells (CSCs) are a small subpopulation of cancer cells within ovarian tumour tissue (24). Bapat *et al* (34) were the first to confirm the existence of cells with the characteristics of NSCs in ovarian cancer, which are capable of driving tumorigenesis. Ovarian CSCs are thought to be responsible for tumour growth, metastasis and recurrence, as well as resistance to standard treatments such as chemotherapy (35). It is believed that the involvement of CSCs in metastasis in ovarian cancer is related to their ability to resist *anoikis*, which allows them to survive in non-adherent conditions and then to adhere in other than primary locations and

create secondary tumours there (36). Moreover, the metastasis formation may be influenced by the ability of CSCs to undergo the process of EMT which is an example of the plasticity of these cells (36).

An important therapeutic problem in patients with ovarian cancer is the frequent recurrence of the disease, even if an initial response to the treatment is promising. Moreover, patients may become resistant to chemotherapy, which results in treatment failure or even death (37). The mechanisms underlying the development of chemoresistance are not entirely clear, but it is suggested that CSCs may play a role in cancer recurrence following chemotherapy. There are several mechanisms implicated in chemoresistance of ovarian CSCs, including increased drug effects, CSCs quiescence (essential for self-renewal function), enhanced DNA repair, autophagy, etc. (37).

Various markers are used to identify CSCs in ovarian cancer. However, due to a tumour heterogeneity it is difficult to describe ovarian CSCs phenotype. Among the characteristic markers of CSCs in ovarian cancer, there are: CD133, CD44, CD24, CD117, or ALDH1 (4,38). Recent findings indicate that some markers may be of diagnostic and prognostic importance in ovarian cancer (38). In addition, scientists' attention is drawn to the use of CSCs markers in targeted personalised therapies (39).

4. Clinical significance of CSCs markers in ovarian cancer

This review presents selected CSCs markers used in ovarian cancer research with particular emphasis on their prognostic value and association with chemoresistance in this cancer.

CD133. CD133 is one of the most well-known markers of CSCs, used to isolate and study these cells in different types of cancer, including ovarian cancer (40). Zhou *et al* (41) performed meta-analysis of eight studies including a total of 1051 women with ovarian cancer to investigate the association between the expression of CD133 and clinicopathological outcomes as well as to determine the prognostic value of CD133 in ovarian cancer. Their analysis showed that the presence of CD133 expression was highly correlated with poor two-year overall survival (OS), which may indicate the prognostic importance of this marker related to the worse prognosis in patients with ovarian cancer. Moreover, they showed that the expression of CD133 correlated with tumour stage, but was not associated with other clinical parameters, such as patients' age, tumour grade, histological type and response to treatment (41). Another meta-analysis performed by Tao *et al* (42) indicated that the expression of CD133 correlated with FIGO stage and was statistically associated with tumour differentiation grade, which may suggest the involvement of CD133 in the malignant progression of ovarian cancer.

Different results were obtained in the study of Onisim *et al* (43), who did not observe an association between the expression of CD133 and progression free survival (PFS) or OS in patients with serous ovarian carcinoma. They also found that the expression of CD133 in tumour cells was not significantly associated with clinicopathological parameters, such as age, serum CA125, peritoneal carcinomatosis, malignant ascites or tumour grade (43).

In the study by Ruscito *et al* (44) it was shown that there was a significant shift from higher frequency of CD133+ cells in patients with primary high-grade serous ovarian cancer (HGSOC) to lower levels in the paired recurrent samples. Moreover, all primary ovarian cancer CD133+ patients were diagnosed at FIGO III/IV stage and had significantly worse progression-free survival (PFS) as well as OS (44). In turn, in the study by Steg *et al* (45), who examined matched primary and recurrent tumour pairs from patients with high grade ovarian adenocarcinomas, it was shown that the average number of CD133-positive cells was significantly higher in the samples of recurrent tumours than in primary tumours. Moreover, the expression of CD133 was significantly increased in tumours collected from recurrent platinum-resistant patients (45). Liu *et al* (46) showed that the absence of CD133 expression in patients with primary epithelial ovarian cancer was significantly associated with high platinum sensitivity in patients with and without central nervous system (CNS) metastases. Their results also indicated a positive association between the expression of CD133 in primary tumours and increased risk of CNS metastases (46). The association between the expression of CD133 and chemoresistance was also shown in another study (47).

The presented results may indicate a relationship between the expression of CD133 and chemoresistance in women with ovarian cancer and the potential use of this marker in personalized targeted therapy.

CD44. The prognostic value and clinical significance of CSCs surface marker CD44 in patients with ovarian cancer is controversial. Different authors in their reviews point out that there are some conflicted data on CD44 expression and its correlation with prognosis in ovarian cancer (48,49).

The meta-analysis performed by Lin and Ding (50) included 18 studies conducted in total on over 2,000 patients with ovarian cancer. Their study revealed that the expression of CD44 in ovarian cancers was significantly associated with a high TMN stage and with a poor five-year OS, while was not significantly correlated with disease-free survival (DFS). They also showed that there was no significant correlation between the expression of CD44 and tumour grade, lymphatic metastasis, patients' age, residual tumour size, ascites volume as well as response to chemotherapy (50). Another meta-analysis conducted by Tao *et al* (42) showed that overexpression of isoform CD44s was associated with poor OS and worse DFS as well as with chemotherapy resistance in ovarian cancer patients. However, there was no association between overexpression of isoform CD44v6 and poor OS (42).

In the studies of Zhou *et al* (51) it was found that in patients with ovarian cancer the high expression of CD44 was associated with higher histological grade and more advanced FIGO stage. Moreover, they showed that high expression of CD44 was significantly associated with worse OS and DFS suggesting that CD44 may be a potential prognostic marker (51). High expression of CD44 has also been demonstrated in the samples of chemotherapy resistant epithelial ovarian cancer tissue, which may indicate the usefulness of this marker in targeted therapy (52).

Zhu *et al* (53) showed that CD44/myeloid differentiation factor 88 (MyD88) co-expression in patients with epithelial ovarian carcinoma (EOC) was associated with tumour

progression, metastasis and recurrence. Moreover, the authors' findings suggest that CD44/MyD88 co-expression is an independent prognostic factor related to poor DFS and OS (53).

The researchers' attention is also focused on the clinical significance of CD44 variant 6 (CD44v6). It was found that CD44v6 is highly expressed in ovarian cancer patients, suggesting that CD44v6 may promote incidence and progression of this cancer (54). In addition, the study by Tjhay et al (55) showed that an increased number of CD44v6-positive cancer cells in primary tumours was associated with a shortened OS in patients with advanced epithelial ovarian cancer (stage III-IV). The authors also found that CD44v6-positive cancer cells show metastatic potential and they are associated with tumour chemoresistance (55). Motohara et al (56) found that the expression of CD44v6 was an independent risk factor for distant metastatic recurrence in patients with ovarian cancer. Moreover, increased expression of this marker in primary ovarian tumours was associated with shorter OS (56).

ALDH1. Different studies results indicate a relationship between high expression of ALDH1 and poor prognosis and clinical outcome in patients with ovarian cancer (57-59). However, there is also a study in which the expression of ALDH1 was associated with favourable prognosis in ovarian cancer (60). The long-term follow-up retrospective study by Huang et al (61) showed that high expression of ALDH1 in ovarian cancer cells was associated with histological subtypes, early FIGO stage, well differentiation grade and better survival. However, in multivariate analysis, the expression of ALDH1 in tumour cells was not an independent risk factor for OS. Their study revealed that high expression of ALDH1 in ovarian cancer cells may portends favourable prognosis (61).

The clinicopathological characteristics and prognostic significance of ALDH1 in ovarian cancer were evaluated by Zhao et al (62) in a meta-analysis of 18 studies including over 2 500 patients. Their results indicated that elevated expression of ALDH1 was significantly associated with poor OS but not with DFS. They also found that ALDH1 was most frequently elevated in patients with poor clinicopathological characteristics and was associated with FIGO stage, lymph node metastasis and distant metastasis (62). Another meta-analysis, published in the same year, showed that overexpression of ALDH1 was correlated with poor OS as well as with worse DFS (42).

Ayub et al (63) demonstrated that in patients with advanced epithelial ovarian cancer the enrichment of ALDH1 expression after treatment was associated with poor response to chemotherapy. Another study showed that the expression of isoform ALDH1A1 was associated with poor response to platinum-based therapy in patients with high-grade ovarian serous carcinoma (64).

CD133/ALDH1. The study conducted by Ricci et al (65) found that neither CD133 expression nor ALDH enzymatic activity were correlated with response to therapy, PFS and OS in ovarian cancer. The authors suggest that those markers do not provide additional predictive/prognostic information in ovarian cancer patients (65). On the other hand, Silva et al (66) showed that the presence of ALDH+CD133+ cells in debulked primary tumour specimens

correlated with reduced disease-free survival and OS in ovarian cancer patients. Similarly, in the aforementioned study by Ruscito et al (44) it was found that the co-expression of CD133/ALDH1 in patients with primary HGSOC, rather than the expression of a single marker, was an independent prognostic factor associated with poor PFS and OS.

CD24. CD24 is a sialoglycoprotein that has been identified as an independent prognostic marker of survival in patients with ovarian cancer (67). CD24 is localised in lipid rafts through its glycosylphosphatidylinositol anchor, but also its diffuse cytoplasmic accumulation is observed in cancer cells (67). Kristiansen et al (68) found that cytoplasmic expression of CD24 was a prognostic factor for poor survival in ovarian cancer, while membranous expression had no influence on patients survival. In the study by Nakamura et al (69) it was shown that the expression of CD24 was significantly associated with progression-free survival and overall survival in patients with ovarian cancer. Moreover, the authors found that the expression of CD24 was correlated with the FIGO stage and the presence of peritoneal and lymph node metastasis. Additionally, CD24 induced the EMT phenomenon in ovarian cancer, which was involved in resistance to chemotherapy (69). Also, according to Soltész et al (70) high expression of CD24 in serous ovarian cancer patients' tissue samples was associated with advanced FIGO stages.

CD117. Meta-analysis conducted by Yang et al (71) included seven studies enrolling over 1200 patients with epithelial ovarian cancer. They showed that the expression of CD117 was significantly correlated with FIGO stage, histological type, tumour differentiation grade and age. Moreover, high expression of CD117 was significantly correlated with poor OS, but there was no statistically significant association between this marker expression and DFS (71). The study by Luo et al (72) showed that the expression of CD117 is also statistically correlated with response to chemotherapy and CD117+ patients were less sensitive to chemotherapy than CD117- patients.

CD105 (endoglin). It has been shown that the expression of CD105 was associated with poor survival in patients with ovarian cancer (73). Furthermore, it is suggested that CD105 plays a role in ovarian cancer metastasis (74). Zhang et al (52) found that moderately and highly differentiated ovarian cancer tissue samples exhibited decreased expression of CD105 compared with poorly differentiated samples. Moreover, early-stage (I and II) ovarian cancer tissue samples exhibited decreased expression of CD105 compared with advanced stage (III) samples. Additionally, there were increased protein expression of CD105 in drug-resistant epithelial ovarian cancer tissue samples compared with drug-sensitive samples (52). Ziebarth et al (75) found that inhibition of CD105 increased cisplatin sensitivity in epithelial ovarian cancer.

CD106 (VCAM-1). The study conducted by Huang et al (76) showed that overexpression of VCAM-1 in high grade serous ovarian cancer cells was associated with poor prognosis. Moreover, the authors found that high expression of VCAM-1

Table I. Association of chemoresistance with type of ovarian cancer.

CSCs marker	(Refs.)	Type of ovarian cancer
CD133	Steg *et al* (45)	High grade ovarian adenocarcinomas
	Liu *et al* (46)	Epithelial ovarian cancer (serous, mucinous, endometroid, clear cell, mixed epithelial, undifferentiated) with and without CNS metastases
	Liu *et al* (47)	Ovarian cancer cell lines (CSC-like SKOV3 spheres, CSC-like IGROV1-spheres)
CD44	Tao *et al* (42)	Meta-analysis (patients with different types of ovarian cancer)
	Zhang *et al* (52)	Epithelial ovarian cancer, OVCAR3 cell line, PTX-resistant OC3/TAX300 cells
ALDH1	Ayub *et al* (63)	Epithelial ovarian cancer
	Roy *et al* (64)	High grade serous ovarian cancer
CD24	Nakamura *et al* (69)	Caov-3 (human ovarian mucinous adenocarcinoma cancer cell line)
CD117	Luo *et al* (72)	Ovarian serous adenocarcinoma
CD105	Zhang *et al* (52)	Epithelial ovarian cancer, OVCAR3 cell line, PTX-resistant OC3/TAX300 cells
	Ziebarth *et al* (75)	Epithelial ovarian cancer (cell lines)
CD106	Zhang *et al* (52)	Epithelial ovarian cancer, OVCAR3 cell line, PTX-resistant OC3/TAX300 cells
	Huang *et al* (76)	High grade serous ovarian cancer
EpCAM	Tayama *et al* (78)	Epithelial ovarian cancer-tissue samples (serous, clear cell, endometroid, mucinous, other). Human ovarian cancer cell lines. Animal study
SOX2	Li *et al* (83)	Tissue specimens (patients diagnosed with ovarian cancer). SKOV3 and SKOV3/TAX cells (paclitaxel-resistant human ovarian adenocarcinoma cell line)
Nestin	Qin *et al* (85)	Serous ovarian cancer

SSEA1, stage-specific embryonic antigen-1; EpCAM, epithelial cell adhesion molecule; ALDH1, aldehyde dehydrogenase 1; SOX2, sex-determining region Y-box 2.
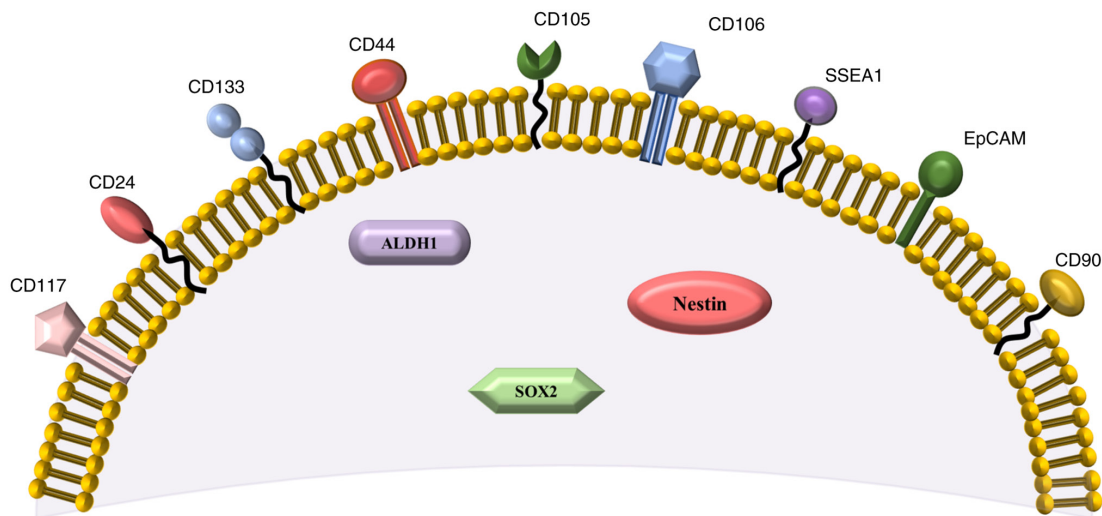

Figure 2. Surface and intracellular markers of ovarian cancer stem cells presented in the present review. The surface markers include: CD133, CD44, CD117, CD24, CD105, CD106, CD90, SSEA1 and EpCAM. The intracellular markers include: ALDH1, SOX2 and Nestin (39,52,84,90). SSEA1, stage-specific embryonic antigen-1; EpCAM, epithelial cell adhesion molecule; ALDH1, aldehyde dehydrogenase 1; SOX2, sex-determining region Y-box 2.

was related to advanced age at diagnosis and poor response to surgery and chemotherapy. Their data suggest that VCAM-1 may be a prognostic factor and novel therapeutic target for ovarian cancer (76). Scalici *et al* (77) found that mesothelium expression of VCAM-1 in patients with epithelial ovarian cancer was associated with shorter PFS and OS. In the study by Zhang *et al* (52) it was shown that high expression of CD106 was associated with drug resistance.

EpCAM. The study by Tayama *et al* (78) showed that an increased expression of EpCAM was associated with poor prognosis in patients with ovarian cancer and correlated with shortened PFS and OS. Moreover, they also found that EpCAM was associated with chemoresistance to platinum-based chemotherapy (78). Spizzo *et al* (79) also showed that overexpression of EpCAM was significantly correlated with decreased OS in patients with epithelial

ovarian cancer. However, different results were obtained by Woopen *et al* (80) who showed that epithelial ovarian cancer patients with overexpression of EpCAM had better prognosis than patients with a weak or no expression of this marker. EpCAM overexpression was associated with a more favourable OS, better PFS and high response to platinum-based chemotherapy (80).

SOX2. The association between the expression of SOX2 and poor prognosis in ovarian cancer was shown by Zhang *et al* (81). They found that the expression of SOX2 was associated with decreased DFS durations, but there was no association between SOX2 expression and OS. Moreover, there was significant association between the expression of SOX2 and high-grade serous carcinoma. Their data showed that there was no significant correlation between the expression of SOX2 and response to chemotherapy (81). Bååth *et al* (82) found that within the group of patients with non-radical debulking surgery, there were shorter OS and PFS for patients with SOX2-positive tumours. Moreover, Li *et al* (83) investigated that the SOX2 was overexpressed in paclitaxel-resistant cells.

Nestin. The study by Onisim *et al* (43) showed that the expression of nestin in tumour cells was associated with poorer PFS and OS in patients with ovarian cancer. In another study by Czekierdowski *et al* (84) it was found that in high grade serous ovarian cancer patients with high expression of nestin had worse OS and DFS rates than patients with low expression of nestin. Qin *et al* (85) found that in serous ovarian cancer nestin-positive patients had significantly shorter OS. Moreover, overexpression of nestin was associated with the cisplatin-based chemotherapy resistance (85).

SSEA1. SSEA1 was studied by Davidson *et al* (86) in metastatic high grade serous carcinoma. They found that higher expression of SSEA1 was significantly associated with shorter OS and poorer PFS. Moreover, SSEA1 was significantly overexpressed in post-chemotherapy effusions compared with pre-chemotherapy specimens tapped at diagnosis (86).

Thy-1 (CD90). In the study conducted by Chen *et al* (87) it was found that the expression of CD90 was significantly decreased in ovarian tumour tissues and lower expression of CD90 was correlated with poor survival rate. Moreover, the authors investigated that CD90 decreased the expression of other CSCs markers, such as CD133 and CD24 (87). Different results were obtained by Connor *et al* (88), who found that the expression of Thy-1 (CD90) was associated with poorer clinical outcome in women with ovarian cancer. Their study showed that in high expression of Thy-1 was associated with poorer OS and PFS in women with serous ovarian cancer, while the expression of Thy-1 in endometroid ovarian cancer was associated only with poorer PFS. Moreover, they demonstrated that the expression of Thy-1 is associated with increased proliferative and self-renewal capacity of ovarian cancer cells (88).

All CSCs markers selected for this review are also presented in Fig. 2, according to their surface or intracellular presence. Additionally, association of chemoresistance with type of ovarian cancer is presented in Table I.

5. Therapeutic importance of CSCs markers

Targeting CSCs markers remains a challenge. Most of currently known CSCs surface markers are also expressed on normal stem cells (embryonic and/or adult stem cells) and they are rarely or considerably expressed on various normal tissue cells (89,90). Markers CD133, CD24, CD117, CD90 are expressed on the surface of human embryonic stem cells (hESC) and adult stem cells (89). CD133 is also expressed in epithelial and non-epithelial cells as well as it can be found in many cancers such as breast, lung, ovarian, melanoma, pancreatic, colon, prostate, glioma and hepatocellular cancers (91). EpCAM has been used as an undifferentiated hESC marker and it is also expressed on some normal epithelial cells (89). SSEA-1 is a surface marker for neural stem cells and is related to lung and renal tumours (89). Marker CD44 has been detected in human hematopoietic, mesenchymal and adipose-derived stem cells. Moreover, it is ubiquitously expressed in many normal tissue cells (89). CD106 is expressed by mesenchymal and neural stem cells (52).

Monoclonal antibodies (mAb) that target specific CSCs markers are a promising therapeutic option. Yang *et al* (92) reviewed agents that have been used to target CSCs markers in recent years. For example, anti-CD44mAb (bivatuzumab) was used for the treatment of head and neck squamous cell carcinoma, and EpCAM antibody (adecatumumab) was used in patients with hormone-resistant prostate cancer (92). CSCs markers could also be a target for chimeric antigen receptor (CAR)-T cell therapy (93,94).

6. Conclusion

The role of CSCs in the development and progression of ovarian cancer as well as their association with therapy resistance is still the subject of numerous studies. Unfortunately, due to the heterogeneity and plasticity of these cells, finding a specific phenotype of CSCs that would allow for their better identification remains a challenge. Moreover, identification of such phenotypes could also be helpful in developing new diagnostic and therapeutic strategies in ovarian cancer.

Despite the ambiguous results, the usefulness of CSCs markers in the assessment of prognosis and their relationship with the development of chemoresistance in ovarian cancer patients has been demonstrated. In our review we found that the expression of ovarian CSCs markers CD133, CD44, ALDH1, CD24, CD117, CD105, CD106, SOX2, Nestin and SSEA1 may have a prognostic significance associated with poor prognosis for patients with ovarian cancer. Moreover, the expression of CD133, CD44, ALDH1, CD24, CD117, CD105, CD106, EpCAM, SOX2 and Nestin could be associated with resistance to chemotherapy in ovarian cancer. However, it is advisable to perform further studies that will allow the use of CSCs markers especially in the aspect of tumour recurrence and in the development of personalised targeted therapies.

Availability of data and materials

Not applicable.

Authors' contributions

AMP and PKD conceptualised this review. PKD, DW, MSK and SS searched and selected literature. AMP and PKD prepared and reviewed the original draft. PKD, DW, MSK and SS designed the table and figures. All authors read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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