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The Role of Stem Cells in Ovarian Cancer: A Review

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Abstract: Ovarian cancer causes the most deaths among gynecological cancers. One of the major reasons for its lethality despite improved surgical techniques and improved chemotherapeutic agents in recent years is the recurrence due to chemoresistance. There have been several proposed mechanisms for chemoresistance of which the existence of cancer stem cells has recently been brought to attention. Cancer stem cells represent a small proportion of cells within a tumor with the ability to proliferate in order to increase the number of cancer stem cells, as well as the ability to differentiate into heterogeneous-nontumorigenic cancer cell types. Also in ovarian cancer as in other types of cancer, different populations of cancer stem cells were isolated from tumors. In literature the cancer stem cells are represented by different cell populations expressing different phenotypes and characteristics. The most commonly used markers for their isolation are CD44, CD133, CD24, CD117 and ALDH, which are often used in combinations. *In vitro* and *in vivo* studies confirmed that these isolated cells behave as cancer stem cells and use different mechanisms to minimize the effect of chemotherapeutic agents. Also proteins, which are generally known as pluripotent stem cell markers of normal stem cells, such as NANOG, OCT-4A, SOX-2 and c-MYC, have an important role in ovarian cancer development and chemoresistance. Additionally, it was demonstrated that the expression of the mentioned markers in ovarian tumors can be used as a prognostic tool, where usually the over-expression of a specific marker correlates with a poor prognosis. However, the conclusions of the prognostic value of cancer stem cell markers are still under debate. The reason for vague conclusions may lay in the fact that stem cells expressing the same markers can be also found in "healthy" ovaries, which could represent a bias in cancer studies. Even more, since stem cells located in "healthy" ovaries, including small stem cells resembling very small embryonic-like stem cells, represent the most of the cells that divide and proliferate, they seem to be the best candidate to undergo malignant transformation and could serve as tumor initiating cells. Therefore, the knowledge on stem cells, expressing the pluripotency-related markers in "healthy" ovaries is a prerequisite to better understand the ovarian cancer.

Keywords: Ovary, stem cells, cancer stem cells, chemoresistance.

INTRODUCTION

It is estimated that 239,000 women of all ages were diagnosed with ovarian cancer all over the world in 2012, which represents approximately 3.6% of all diagnosed cancers (except non-melanoma skin cancer) in women. This put ovarian cancer in 7th place of incidence among all cancers [1]. It was also estimated that 152,000 women died in 2012 due to ovarian cancer, and this represents approximately 4.3% of deaths caused by all cancers (except non-melanoma skin cancer) in women [1]. Ovarian cancer is the most lethal gynecological cancer, because it is often diagnosed at advanced stages when the treatment is not effective anymore. The diagnosis is delayed due to the diversity of symptoms [2], although there are symptoms such as unusual bloating, fullness and pressure, abdominal or back pain, and fatigue, which could indicate there is a possibility for the development of ovarian cancer [3]. But even if diagnosis is not delayed, the treatment is complicated. Usually it is successful in the short term, but in the long term it is often unsuccessful due to recurrence. The main problem for recurrence of ovarian cancer probably lies in cancer stem cells, which are resistant to chemotherapy [4].

TYPES OF OVARIAN CANCER

Ovarian cancer is a very complex disease. It can originate from different sources, and therefore the ovarian tumors are diverse and can be distinguished in several ways. They can be divided based on the site of their occurrence/location and are usually represented as epithelial ovarian tumors, germ cell tumors, and sex-cord stromal tumors. Most of these tumors are represented by epithelial ovarian tumors, which are also the most lethal [5]. They can be divided into five main types: high-grade serous (70%), endometrioid (10%), clear cell (10%), mucinous (3%), and

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low-grade serous carcinomas (<5%) [6], while by the WHO guidelines these tumors are divided into eight histological types (serous, endometrioid, mucinous, clear cell, transitional cell, squamous cell, mixed epithelial, and undifferentiated) [7]. It was also proposed to divide epithelial ovarian tumors into type I and type II tumors regarding their way of formation [8]. Shih and Kurman [8] explain that type I tumors are low-grade neoplasms, such as low-grade serous carcinoma, mucinous carcinomas, endometrioid carcinomas, malignant Brenner tumors, and clear cell carcinomas; and type II tumors are high grade neoplasms, such as high-grade serous carcinoma, malignant mixed mesodermal tumors (carcinosarcoma), and undifferentiated carcinoma. Despite that better survival for type I tumors was observed [9, 10], some data indicates that type I tumors are more chemoresistant [11]. This could be explained with the quiescence of initiating tumor cells or with genotypic differences (mutations of certain genes) between type I and type II tumors. Despierre et al. [12] showed that type I tumors are often subjected to somatic sequence mutations, since 49% of them were *KRAS* or *PIK3CA* mutated. It is worth to mention that the frequency of *KRAS* or *PIK3CA* somatic sequence mutations is distinct also between subtypes of type I tumors. This study also showed these mutations are rare for type II tumors (less than 3% for *KRAS* or *PIK3CA*) [12]. On the other hand it was shown that type II tumors are chromosomally unstable, and *TP53* mutated in more than 95% of cases [13], but type I tumors are chromosomally stable and rarely *TP53* mutated [14]. The mutations of these genes are not specific for ovarian germ cell tumors and sex-cord stromal tumors, since they were reported just in some individual cases [15–18]. Ovarian germ cell tumors develop more often in girls and young women. They represent approx. 25% of all ovarian tumors but only a few percent of all ovarian malignancies [19]. Histologically they can be divided in dysgerminomas, yolk sac tumors, embryonal carcinomas, polyembryomas, teratomas, choriocharcinomas, and mixed forms [19]. These tumors can be distinguished morphologically by experienced histopathologists, although distinguishing between subtypes may represent a hurdle. This can be resolved with the analysis of serum tumor markers such as β-hCG, α-fetoprotein, lactic dehydrogenase, and CA-125. Despite the fact that these tumor markers are not specific, they can offer some additional prognostic information, since some combinations of these elevated markers may indicate a malignant ovarian germ cell tumor or can help to monitor the success of treatment or possible recurrence [20]. Same as in ovarian germ cell tumors, the sex-cord stromal tumors are rarely malignant [21]. They represent approximately 7% of all ovarian tumors and are usually nonaggressive [21], although they can cause endocrinologic instabilities. The hormonal balance is altered, because the tumors may consist of cells, which are able to produce hormones. The origin and location of these cells (theca, granulosa, Leydig, and Sertoli cells) is also the basis for the WHO classification of sex-cord stromal tumors in relation to pure stromal tumors, pure sex cord tumors, and mixed sex cord-stromal tumors [22].

OVARIAN CANCER TREATMENT

There are different approaches to treating ovarian cancer. The selection of treatment mainly depends on the type and stage of the diagnosed ovarian cancer. When a presumably nonaggressive type is diagnosed, such as borderline or early-stage ovarian cancer, surgical removal of the affected tissue is recommended and can be efficient [23]. When a more developed cancer or a more aggressive type is suspected, a combination of surgery and chemotherapy is preferred. Most often this is the choice, especially in epithelial ovarian cancers. The standard chemotherapy (which usually follows surgery) is based on platinum- and taxane - agents, which can be used in different combinations and are also typically used for chemotherapy in other cancers [24]. Widely used platinum agents are carboplatin and cisplatin. Cisplatin acts cytotoxically to cancer cells by its interaction with their DNA to form DNA adducts (intrastrand crosslink adducts), and this activates several signal transduction pathways, including p53 (activation of p53 normally leads to apoptosis) [25]. Unfortunately, cisplatin is not ideal therapy, since it is neurotoxic, nephrotoxic, and ototoxic. This could lead to complications in treatment [26]. Based on data, also carboplatin treatment is not without side effects (higher hematologic toxicity), although it is still less toxic in other aspects than cisplatin treatment, and it provides a better quality of life [27, 28]. Structurally, it is a derivative of cisplatin and also has a similar mechanism of action [29]. It was shown that chemotherapy with platinum-based agents is more effective when in combination with taxane agents [30]. Taxanes are diterpenes, and two widely used taxane agents are paclitaxel and its analogue, docetaxel. Their mechanism of action is based on their ability to bind to microtubulin subunits, which changes the balance in the microtubule assembly and blocks mitosis, consequently causing apoptosis [31]. Despite the similarities in structure and mechanism of action between paclitaxel and docetaxel, there are differences in their molecular pharmacology, pharmacokinetics, and pharmacodynamic profiles [32]. For instance, docetaxel has a wider cell cycle activity, a more direct antitumoral effect, and a longer retention time in the tumor [32]. All these drugs are effective, but it can also happen that chemotherapy fails and cancer recurs. There are different mechanisms for chemotherapy resistance, but they can be organized into four groups: pre-target resistance, on-target resistance, post-target resistance, and off-target resistance [33]. For cisplatin they were thoroughly reviewed by Galluzi et al. [33] and explained as mechanisms that influence the processes before cisplatin binds to DNA (pre-target resistance); that

directly influence DNA–cisplatin adducts (on-target resistance); that influence signal transduction pathways, which lead to apoptosis (post-target resistance); and that influence the processes that are not in relation to cisplatin treatment (off-target resistance). On the contrary, some mechanisms of resistance in ovarian cancer in taxane treatments were proposed but are less defined. It was shown for paclitaxel resistance, that its cellular accumulation could be decreased with P-glycoprotein export [34], or the mechanism of resistance could be a consequence of microtubule changes (due to mutations, post-translational modifications or changes in the level of regulatory proteins) [35]. Also some biomarkers to predict taxane resistance were determined. It was shown that overexpression of class III β-tubulin, Sox2, and nuclear survivin [36], as well as the lower expression levels of the mitochondrial mimitin and 14-3-3 ζ/δ proteins, were predictive of taxane resistance [37]. Besides these mechanisms there are numerous factors, for example DNA methylation, histone modification, and microRNAs, which are associated with chemotherapy resistance [38], however recently more and more evidence shows that the main reason for aggressiveness, resistance, and recurrence of ovarian cancer are cancer stem cells (CSCs) [39, 40].

CANCER STEM CELLS

General overview

In recent years there has been a tremendous improvement in cancer studies, and especially the role of cancer stem cells has been more and more emphasized. A lot of effort is being invested to determine the cells, or population of cells, that initiate the development of tumors/cancer and that are responsible also for the self-renewal of tumors (cancer recurrence). These cells can be named cancer stem cells, cancer initiating stem cells, tumor initiation stem cells, or therapy resistant cells [41, 42]. Clarke et al. [41] defined them as a small subset of cancer cells, which constitute a reservoir of self-sustaining cells with the exclusive ability to self-renew and maintain the tumor. They also explain these cancer stem cells are able both – to proliferate to increase the cancer stem cell pool and to differentiate into the heterogeneous nontumorigenic cancer cell types, which represent the majority of cells in the tumor. It is not clear if CSCs develop from normal tissue stem cells, which have undergone malignant transformation, or from somatic differentiated cells, which "dedifferentiate" and undergo malignant transformation [43]. The data show that cancers of distinct subtypes within an organ may derive from cells of different origin [44]. Although the normal stem cells and progenitors represent most of the cells that divide and proliferate in tissues and organs, they are theoretically the most suitable source of transformation [45]. While the data on the existence and the properties of cancer stem cells grow, a new model of tumor development was introduced, so called hierarchical model [43]. It explained that tumors could develop just from CSCs, and CSCs only could form metastasis. On the contrary, the stohastic (clonal) model of tumor development suggests that all cells can form tumors and metastasis [46]. In this model malignant cells are a homogenous population at the beginning. Later, due to the influence of endogenous and exogenous factors, they develop into a heterogeneous population (for example, due to mutations, changes in gene expression, cytokines, interactions between cells, influence of niche) [46]. In contrast, the hierarchical model suggests that malignancy is organized similarly as in normal tissues, where normal stem cells are capable of self-renewal to form two daughter cells [45]. One daughter cell is identical to a mother cell, and the other is a so-called progenitor cell, which is more limited in her developmental potential. Despite the fact these models differ, some data indicates the heterogeneity of cells in a tumor is the result of cancer development by both models at the same time [47–49]. It is possible to determine the proportion of CSCs within tumors, but reported numbers are quite different between studies. This is probably due to comparing the tumors derived from different sources/tissues and using different assays. For example, in human melanoma the estimations for proportion of CSCs ranges from 1 per thousand [50] to 41% [51] and in mouse leukemia around 10% [52]. A common problem in determining CSCs is also confusion in the selection of a proper marker of CSCs (as discussed in the next chapter in detail for ovarian CSCs). While in leukemia the selection of surface antigens of hematopoietic stem cells, CD34 and CD38, is a reliable decision [53, 54], these markers are more diverse and less reliable in solid tumors. For instance, the surface antigen CD133 was used as a marker of CSCs derived from different tissues, such as from a brain tumor [55, 56], from ovaries [57, 58] and from colon cancer [59, 60], even though Shmelkov et al. [61] showed that also normal colonic epithelium cells express CD133. Additionally, it was identified that CD133+ tumor cells probably gave rise to a more aggressive CD133(−)subset, which were (same as CD133+ cells) capable of tumor initiation in NOD/SCID mice. Similarly, contradictory results were described also in melanoma studies for using CD271 as a CSCs marker [51, 62].

One of the CSCs special features is dormancy. It is a state where CSCs are in a lengthy hibernation without growth, proliferation, or formation of tumors for years or even decades [63, 64]. The exact mechanisms for dormancy are not clear, but it seems it is a result of a balanced combination of extracellular and stromal microenvironment, autophagy, and epigenetics [64]. Due to this feature, these cells are more resistant to anti-proliferative chemotherapy, and additionally metastasis-initiated tumors can grow long after radical surgery or chemotherapy. Some of the other mechanisms that enable the resistance of CSCs are high expression of anti-apoptotic proteins, resistance

to DNA damage, and usage of ABC efflux pumps [65]. Based on this knowledge, it would be ideal to develop a treatment, which would act directly on CSCs and neutralize them. One strategy is to induce CSCs to exit the dormant state and then to treat them with chemotherapy. This approach was used by Saito et al. [66]. They used NOD/SCID/IL2rgamma(null) mouse model of human acute myeloid leukemia (AML) and showed AML stem cells were dormant (cell cycle was arrested). To "wake up" these dormant cells they used granulocyte colony-stimulating factor followed by cell cycle-dependent chemotherapy, which resulted in a higher rate of apoptosis. After such treatment more secondary recipients survived after transplantation of leukemia cells compared to chemotherapy alone. A second strategy to neutralize CSCs would be to target their self-renewal mechanisms. Whereas these mechanisms are similar to CSCs and normal stem cells, this treatment needs to be carefully targeted and is not widely used for now [67]. Another strategy would be to regulate the functioning of ABC efflux pumps. This could be done in various approaches, such as with chemotherapy through competitive and allosteric modulators; with chemotherapy mediated by nanoparticle targeting; with targeting transcriptional regulation of ABC transporters; with microRNA therapeutics; with targeting signaling pathways involved in the regulation of ABC transporters; and with combinational targeting with CSCs targeting agents and transporter modulating drugs or dual targeting with a single agent [68].

OVARIAN CANCER STEM CELLS

More and more data supports the theory that ovarian cancer is a (cancer) stem cells related disease. This theory is based primarily on the fact that, despite ovarian tumor response to chemotherapy and its reduction, tumors most often grow back. Like other cancers, for ovarian cancer there are still no generally accepted populations of stem cells referred to ovarian cancer, although there are some important markers, which are presumed to be expressed in ovarian cancer.

Pluripotency-related markers

Various stem cell markers, otherwise typical for normal stem cells, were identified to be expressed in ovarian cancer and in OCSCs (ovarian cancer stem cells). Special interest is lately dedicated to the expression of pluripotent stem cell (PSC) markers, such as NANOG, SOX-2, OCT-4A, c-MYC, which expression is increasingly used as diagnostic and prognostic factor. Most of these markers have been confirmed in the ovarian tissue using immunohistochemistry or molecular genetic analyses. More and more data indicates that especially NANOG has a key role in the development of cancers in various tissues/organs. The expression of NANOG, which is also one of the key transcription factors for maintaining self-renewal of pluripotent stem cells, was strongly correlated with the clinical stage and grade of ovarian serous cystadenocarcinoma, namely the proportion of NANOG positive samples increased with raising the grade and the clinical stage of this carcinoma (100% of the samples were positive for stage IV, and 96.67% for grade III) [69]. Additionally, the expression of NANOG in ovarian serous carcinoma was correlated with shorter overall survival of patients [70]. The mechanisms for such significant impact on the clinical outcome seem to be multilayered. Using ovarian cancer cell lines, it was shown that the knockdown of NANOG increases the expression of E-cadherin, caveolin-1, FOXO1, FOXO3a, FOXJ1 and FOXB1, resulting in reduced proliferation, migration, and invasion of ovarian cancer cells [71]. Similarly, Liu et al. [72] showed that low expression of NANOG accompanied with increased expression of E-cadherin and decreased expression of vimentin, β-catenin, and Snail, hinders cell migration and invasion capacity of ovarian cancer cells. Additionally, besides the expression of β-catenin and Snail, which are involved in epithelial-mesenchymal transition (EMT), they showed that EMT and drug resistance in epithelial ovarian cancer can be regulated when Stat3 pathway is activated with NANOG. The expression of NANOG coincides also with the expression of other PSC markers. Virant-Klun et al. [73] demonstrated NANOG, SOX-2, and SSEA-4 positive small cells are located among epithelial cells in the ovarian surface epithelium and as a single cell or groups of cells/clusters in ovarian sections of borderline ovarian cancer and serous ovarian carcinoma patients. He et al. [74] showed spheres derived from primary high-grade serous ovarian carcinoma overexpressed OCT-4, NANOG and SOX-2. If only 10,000 of these cells were injected into NOD/SCID mice, new tumors would form. Additionally, these sphere-forming cells exhibit high chemoresistance to cisplatin and paclitaxel. Di et al. [75] detected a small proportion of ovarian tumor cells and ascites cells that expressed, not only *NANOG* and *OCT4A*, but also *c-MYC*. It was interesting that confocal laser scanning microscopy revealed that the c-MYC, which is normally localized in the nucleus, was found also in cytoplasm. A similar phenomenon was already observed in NANOG, which was found to be localized in cytoplasm of tumor cells from various tissues/organs – for instance, in lung cancer cells [76], in nasopharyngeal carcinoma cells [77], in colorectal tumor cells [78], and in mesenchymal stem cells located in cervical cancer stroma [79].

While described PSC markers are a useful tool for the diagnosis of ovarian cancer and as a prognostic tool, they are a less useful tool for the isolation of OCSCs from ovarian tumors. Therefore, the expression of these markers in ovarian cancer has not been related to any special population of stem cells in cancerous ovaries until now. As discussed already, there is confusion in the field of cancer stem research as to how to select the most appropriate marker for isolation from tumors. Also in this aspect, OCSCs are no exception.

Established markers of ovarian cancer stem cells

Researchers use a variety of markers (often in combinations), which are widely recognized as general surface stem cell markers, to isolate OCSCs from ovarian tumors, but there is still no solid conclusion as to which marker is most appropriate. For this reason we will hereinafter describe and discuss the most frequently used markers, and we will try to elucidate which marker, or combination of markers, are most promising for the isolation of OCSCs from ovarian tumors.

CD44

Probably the marker used most often in OCSCs research is CD44 (also known as HCAM). It is a surface adhesion molecule, and it is expressed in most human tissues where it connects cells with other cells or with extracellular matrix [80]. Its main ligand is hyaluronic acid, but it can bind also osteopontin, serglycin, collagens, fibronectin, and laminin [80]. It had been correlated to ovarian cancer already before the theory of cancer stem cells emerged. In 1993 the researchers showed that when CD44 expression is down-regulated, the epithelial ovarian cancer cells can be released from tumors into peritoneal space in ascites. When they bind to mesothelium, the level of CD44 expression is normal again [81, 82]. Further studies confirmed that the expression of CD44 is important for binding ovarian cancer cells to mesothelium [83], although it is not clear as to which splice variant is responsible for this action [84]. It was shown that the expression of CD44s, CD44-v4, -v5, -v6, -v9, and -v10 splice variants could be considered as predictors for survival in epithelial ovarian carcinoma [85]. For instance, the expression of CD44-v6 is up-regulated in recurrent ovarian serous cancer and in metastasis sites [86]. Additionally, expression of CD44-v10 in the primary tumor was correlated with improved survival, but on the other hand, if this splice variant was expressed in metastases, it predicted decreased survival. Also, other studies indicated that CD44 was up-regulated during the development of ovarian tumors, but when the tumors progressed it was down-regulated and indicated a poor prognosis [87, 88]. While these initial studies were focused more on exploring if there is any importance of CD44 expression in ovarian tumors, further studies focused more on isolating CD44(+) cells from ovarian tumors and exploring their properties. Zhang et al. [89] used FACS to isolate CD44(+)CD117(+) cells from a primary human ovarian tumor. They estimated that only 0.2% of ovarian tumor cells are CD44(+)CD117(+) and demonstrated that only 100 of these cells are enough to propagate the original tumor. The analysis also showed that 100 000 of CD44(−) CD117(−) cells were not able to propagate tumors. If ovarian tumors were propagated as spheroids, after 2 months between 79% and 82% of spheroid cells were CD44(+)CD117(+). It seems these cells have a high tumorigenic potential, but according to Cheng et al. [90] it is possible to suppress tumorigenicity of CD44 (+) /CD117(+) stem cells isolated from a human primary ovarian tumor using microRNA-199a (miR-199a). This microRNA causes down-regulation of CD44 expression and inhibits the expansion of OCSCs by affecting their cell cycle regulation. Even more, miR-199a down-regulates the expression of the *ABCG2* gene, which is known to be responsible for chemo-resistance and increases the sensitivity of OCSCs to cisplatin, paclitaxel, and adriamycin. Similarly as Zhang et al. [89], Alvero et al. [91] observed over 90% of CD44(+) cells in spheroids derived from ascites of epithelial ovarian cancer patients. They also estimated that a proportion of CD44(+) cells is 6% in the primary ovarian tumor, 19% in metastatic tumor, and 18% in ascites, respectively. Detailed gene transcription analysis showed that in CD44(+) cells, Toll Like Receptor 4 (*TLR4*) and Myeloid Differentiation Factor 88 (*MyD88*) were up-regulated (compared to CD44(−) cells). When the CD44(+) and CD44(−) cells were exposed to TRL4 ligand paclitaxel, apoptosis was triggered in CD44(−) cells. Alternatively, CD44(+) cells responded with improved NF-κB activity leading to a higher cytokine production and consequently to chemo-resistance. It was also shown by others using *in vitro* model that paclitaxel even helps to enrich CD44(+)MyD88(+) OCSCs population, and that surviving CD44(+)MyD88(+) OCSCs obtain more aggressive phenotype with improved chemo-resistance and migratory potential [92]. Since there are many shortcomings in monitoring the influence of chemotherapeutics on OCSCs *in vitro*, Chen et al. [93] used a special 3D culture system to mimic *in vivo* conditions in the best possible manner and demonstrated that CD44(+) CD117(+) cells are more chemo-resistant in a 3D culture system as in a 2D culture system. In their experiment a human epithelial ovarian cancer SKOV-3 cell line was used, and chemotherapeutics, such as ocetaxel, cisplatin, and carboplatin, were tested.

CD133

Another established marker of OCSCs is CD133. It is also known as AC133 and Prominin-1. It was first described as a surface antigen specific for human hematopoietic stem cells [94, 95]. Today it is used as a specific marker to demonstrate the presence of normal stem cells [96–101] and cancer stem cells [102–106] in various tissues. The expression of CD133 was observed also in normal, small, putative ovarian stem cells, which share some similarities with very small embryonic-like stem cells [107]. It was first described as OCSCs marker by Ferrandina et al. [57]. They used FACS to isolate CD133(+) cells from ovarian carcinoma samples, which showed these cells had increased proliferative potential and formed larger colonies than CD133(−) cells. Additionally, the percentages of CD133(+) cells were lower in normal ovaries, benign ovarian tumors, and omental metastases when compared to ovarian carcinoma. Further studies showed that CD133

expression is epigenetically regulated by histone modifications and promoter methylation [58]. Promoter methylation increased in CD133(−) daughter cells that were derived from CD133(+) cells, while in CD133(+) daughter cells the promoter was mostly unmethylated. This study also showed CD133(+) cells were more resistant to cisplatin chemotherapy and formed more aggressive tumors with a lower number of cells when injected subcutaneously in BALB/cAnNCr-nu/nu female mice. An increased tumorigenic potential of CD133(+) cells derived from primary ovarian tumors [108], platinum resistance, and an increased risk of metastases [109] was demonstrated also in other studies, although it was found that both the CD133(+) and CD133(−) cells derived from human serous ovarian cancer can form tumors in NOD/SCID mice [110]. In some cases tumors were formed exclusively from CD133(−) cells [110]. As a result of this heterogeneity, it is unclear if the presence of CD133(+) cells in ovarian tumors could offer any prognostic information. Although different staining patterns were observed within this group, Ferrandina et al. [111] observed CD133(+) cells only in 31% of ovarian tumors. In 60% of CD133(+) tumors, a diffuse cytoplasmic pattern was observed, and in the remaining 40% of cases, apical cytoplasmic pattern was observed. Despite the trend towards a worse prognosis for cases with diffuse cytoplasmic pattern, the difference was not statistically significant. Similarly, Ricci et al. [112] found no correlation with response to therapy, progression free survival, and overall survival for CD133. The latest meta-analysis of CD133 expression in ovarian cancer offered some inconclusive results [113]. It was demonstrated that overexpression of CD133 correlates with the tumor stage and with a reduced 2-year survival. On the contrary, there was no correlation with patients' age, tumor grade, histological type, or response to treatment. The prognostic value of CD133 expression in ovarian cancer was evaluated also in combination with ALDH (aldehyde dehydrogenase) enzymatic activity, but no correlation was found [112].

ALDH

Another important marker of OCSCs belongs to the ALDH enzyme family, which consists of 19 enzymes and can be found in all cellular compartments, where catalyze NAD(P)+ dependent oxidation of various aldehydes [114]. While aldehydes are toxic, this mechanism guards cells. Despite that there are 19 isoforms of ALDH and each isoform has its own specifics, the isoform ALDH1A1 is most often correlated with cancer stem cells found in various tissues, likewise with OCSCs [115]. Kryczek et al. [116] detected ALDH expression (besides other OCSCs markers) in the majority of analyzed fresh ovarian cancer specimens. Interestingly, the expression of these markers was lost when primary tumor cells were cultured *in vitro*, even though the expression of ALDH (and CD133) was recovered by the *in vitro* serum-free and sphere cultures, as well as by the *in vivo* passage in the immune-deficient xenografts. Additionally, these spheroids expressed the most important pluripotent stem cell markers (SOX2, OCT3/4 and NANOG) and were able to proliferate long-term *in vitro*. Another study showed that ALDH positive cells isolated using ALDEFLUOR assay showing a high tumor-initiating ability and a high expression of pluripotent stem cell marker SOX-2 [117]. But when SOX-2 was knockdown, these cells were not able to initiate tumors anymore. The role of ALDH in OCSCs is not clear, but it was suggested that OCSCs expressing ALDH1A maintain chemotherapeutic resistance by changing the regulation of the cell cycle checkpoint and the DNA repair network signaling [118]. Furthermore, cells isolated from ovarian clear cell carcinoma and showing high ALDH expression had an upregulated expression of antioxidant enzymes, where the most important was the up-regulation of *Nrf2* (a key transcriptional factor in the antioxidant system) [119]. With this mechanism CSCs are able to lower the level of reactive oxygen species and thus provide the defending system against chemotherapeutics [119]. The tumor initiating potential of ALDH and CD133 positive cells was demonstrated also by Silva et al. [120]. They showed that 1000 ALDH positive and CD133 negative cells isolated from human epithelial ovarian cancer is able to grow tumors when transplanted in an immunodeficient mouse. Moreover, only 11, of both ALDH positive and CD133 positive cells, isolated from the same ovarian tumors and transplanted in an immunodeficient mouse, are able to form tumors. Using Aldefluor assay, Deng et al. [121] were able to show that high ALDH1 expression is significantly associated with poor clinical outcomes in serous ovarian cancer, and ALDH positive tumor cells are resistant to chemotherapy. A poor prognosis in patients with ovarian cancer and a high ALDH expression was confirmed also in meta-analysis, where more than 1000 patients were included [122].

CD24

Described markers of OCSCs (CD44, CD133, ALDH) are most often used in combinations with markers CD24 and CD117, as already presented in the text above. These two markers are usually immunohistochemically studied. They are rarely used alone for isolation of OCSCs from ovarian tumors. CD24 is a glycosylphosphatidylinositol-linked cell surface protein and is usually not expressed in normal ovarian surface epithelium and adenomas, but it is detected in invasive ovarian carcinomas, where two different staining patterns have been observed [123]. Membranous staining was observed in 84% of cases, and cytoplasmic staining in 59% of cases [123]. Additionally, cytoplasmic staining was correlated with higher invasiveness of tumor cells and with shortened patient survival [123–125]. When 5000 CD24(+) cells were isolated from ovarian serous or mucinous adenocarcinoma using FACS and transplanted into nude mice, they formed tumor

xenografts. However, the same number of CD24(−) did not form any tumors [126]. This study also demonstrated that these cells were otherwise in a quiescent state and chemo-resistant. Further, they had an elevated expression of some stemness genes (*nestin, beta-catenin, Bmi-1, Oct4, Oct3/4, Notch1 and Notch4*).

CD117

CD117 (known also as c-kit) is a tyrosine kinase receptor protein, which its expression was evaluated also as a prognostic factor in ovarian cancer patients. It is not expressed in normal ovarian surface epithelium [127], but its expression was observed in early ovarian tumors. Even if the expression decreased in advanced stages of tumors, that was correlated with a shorter survival time [128, 129]. Additionally, the CD117 expression was correlated with the progression of ovarian cancer despite chemotherapy [130, 131].

STEM CELLS IN HEALTHY OVARIES

In recent years strong experimental evidence emerged showing that normal stem cells can be isolated from healthy mammalian ovaries, however there is a little confusion in the field since several different populations of stem cells were recognized. To avoid confusion and to discuss gathered data in a clear manner, stem cells derived from a different ovarian compartment will be described separately.

Ovarian surface epithelium (OSE)

It is well known that OSE plays a crucial role in the regeneration of ovaries after ovulation, and first studies of OSE were focused mainly to understand this process, though they were not designed to characterize OSE cells from today's perspective of stem cells [132, 133]. Closer to today's understanding of stem cells was a study by Bowen et al. [134], which showed OSE possesses a multipotent character, but it did not show exactly which population of cells plays the main role. On the contrary, studies by a group of Virant-Klun [107, 135–139] were focused directly to find this population of cells. As shown in several consecutive studies, these cells are probably small stem cells (with a diameter from 2 to 4μm), which resemble very small embryonic like-stem cells (VSELs) from bone marrow. These cells were isolated from the OSE of women without naturally present follicles and oocytes (postmenopausal women and women with premature ovarian failure), and they expressed pluripotent stem cell markers such as *SSEA-4, OCT-4, NANOG, SOX-2*, and *c-kit* [107, 135–139]. More importantly, these cells were able to develop into oocyte-like cells *in vitro*, which express some markers characteristic of oocytes and pluripotent stem cells, such as *Oct-4A, Oct-4B, c-kit, VASA, ZP2*, *NANOS, STELLA, CD9, LIN28, KLF4, GDF3*, and *MYC*. Additionally, oocyte-like cells also expressed markers specific for meiosis, such as *SCP1, SCP2, SCP3, BUB1*, and *BUB3* [139]. As shown further these small, VSELs-like stem cells also expressed primordial germ cell markers, such as *PRDM1, PRDM14*, and *STELLA* [107, 138]. These small stem cells resembling VSELs were confirmed to be present also in ovaries of several animal models, such as mice, sheep, rabbits, and pigs [140–142]. Since 70–80% of ovarian tumors originate from OSE, these findings could reveal some reasons for their development and resistance. Based on this data we suggest that described small stem cells derived from OSE can be stimulated to differentiate into oocyte-like cells (into germinal lineage), in one direction or in to tumors, if their endogenous control system gets disrupted (Figure 1). Yet mechanisms triggering such behavior are still not completely clear. One explanation for their tumorigenic behavior can be found in the disruption of the epigenetic control. This mechanism was described in detail in VSELs derived from bone marrow [143].

Small stem cells from adult ovaries resemble VSELs from adult bone marrow discovered by the research group of Ratajczak [144, 145]. In detail, VSELs were first isolated from bone marrow [144], but they were later confirmed and isolated also from other adult tissues [145–147]. By definition they are only 2–4 μ in size. Rarely (approximately 0.02%) and due to their properties, it is challenging to isolate them [144]. They are pluripotent, and one of their special features is quiescence, which seems to be epigenetically regulated [143]. Like for small stem cells derived from human adult OSE [107, 138], it was shown also for VSELs derived from murine bone marrow to express several epiblast/primordial germ cell (PGC) markers [148]. For this reason it was proposed that VSELs could originate from epiblast/migrating PGC-like cells and possess an epigenetic signature of imprinted genes that keep these cells quiescent in adult tissues, as well as prevent them from teratoma formation. A high similarity of small stem cells from adult human ovaries and VSELs from bone marrow and their relation to primordial germ cells indicates the persistence of small stem cells in adult human tissues from the embryonic period of life. As mentioned above concerning epigenetic regulation of VSELs, Shin et al. [143] demonstrated this in VSELs' upregulation of *H19* and $p57^{KIP2}$ and repression of *Igf2* and *Rasgrf1* (as a consequence of hypomethylation/erasure of imprints in paternally methylated and hypermethylation of imprints in maternally methylated regions). Even more, when they co-culture VSELs with myoblastic C2C12 cells, VSELs exit quiescent state and proliferate, forming spheres with the capacity to differentiate into all three germ layers. This shows that if the microenvironment functions properly, VSELs serve as a pool of cells intended for regeneration of tissues. But what would happen if the described epigenetic control of quiescence in VSELs would malfunction, and the cells would proliferate without control? Since small cells were isolated from the OSE, similar to VSELs, the question arises as to if these cells could lead to the development of epithelial ovarian cancer,

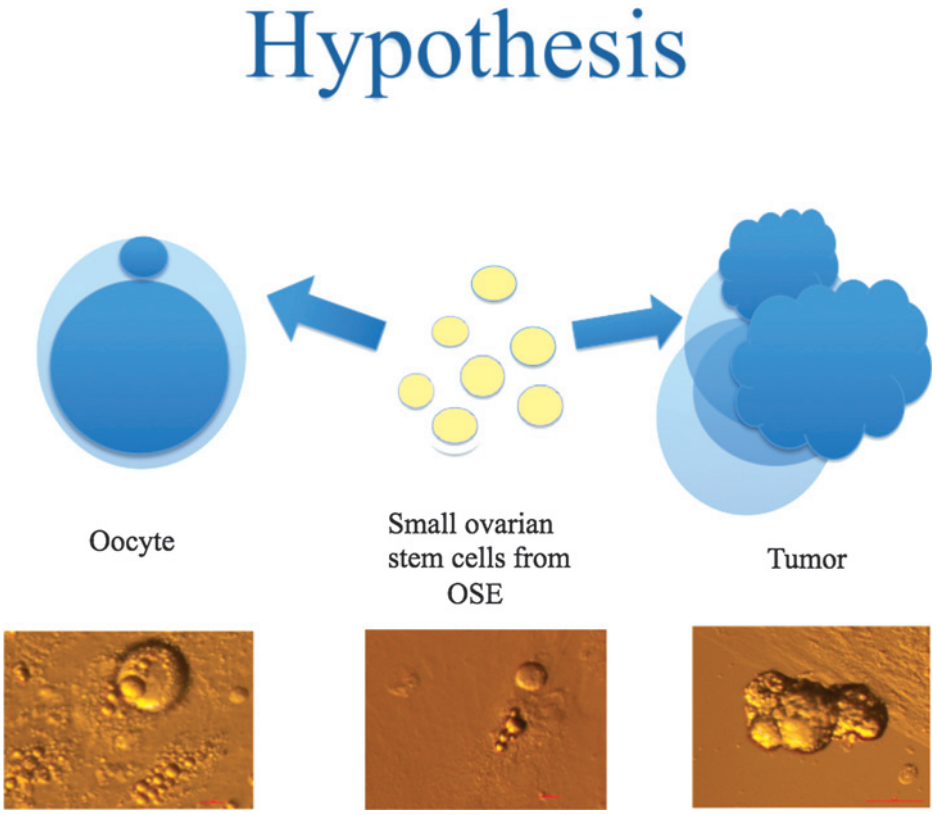

Figure 1. A model representing two different possible options of the development of small stem cells derived from adult human OSE.

which can be very aggressive. Based on the presented data, ovarian small stem cells, like VSELs from other tissues, represent a very possible candidate to be involved in the development of such aggressive ovarian cancer.

Ovarian cortex

Besides OSE, stem cells were isolated also from other compartments of mammalian ovary. It was demonstrated that in ovarian cell cultures derived from the ovarian cortex, different populations of stem cells expressing markers of pluripotency and multipotency can be found, but as explained further in detail, probably different populations of stem cells were described [149–152]. A population of cells, which formed cell colonies, was derived from the human ovarian cortex, and the cells were positive for pluripotent stem cell markers (alkaline phosphatase, SSEA-4, OCT4, SOX-2, NANOG, LIN28, STELLA). The second population of cells shared more similarities with mesenchymal stem cells, since they expressed CD105, CD44, CD90, M-CAM, CD73, and VCAM1. The third population of cells consisted of small cells, which were similar to the previously described VSELs [149, 150]. These different populations of stem cells were able to differentiate *in vitro* into various types of somatic cells of all three germ layers, but they were not capable to form teratoma when injected into immunodeficient mice. Although it was observed that some cells in these cell cultures were VASA-positive, the germinal origin of these cells can probably be excluded, since these cells were very rare and not part of the typical cell colonies [149]. Gong et al. [151] demonstrated that ovarian cell cultures derived from ovarian stroma of mice can form teratomas and colonies, resembling typical embryonic stem cell colonies. They are also able to express markers of pluripotency (*Oct-4, Nanog, Rex- 1, Cripto, Dnmt3b, Tert, Lif RC, Stat3, Bmp4, Fgf4, Foxd3, Sox2, CD9, Gdf3*) but do not express germ cell markers, Fragilis and MVH (mouse Vasa-homologue), or tissue specific stem cell markers – Sca-1, CD44, CD34, and CD45. By using a transgenic mouse model (GFP expressed under OCT-4 promoter), Pacchiarotti et al. [152] were able to generate multipotent germ line stem cells from neonatal and adult mouse ovaries. FACS isolated GFP-Oct-4 positive cells were positive for germ cell markers, VASA and c-Kit, positive for pluripotent stem cell markers, Nanog and GFR-a1, but were negative for CD133, although a distinct population of CD133 positive (but not of Oct-4) cells were observed. In contrast to Gong et al. [151], these cells did not form teratomas when transplanted subcutaneously, intramuscularly, or in ovaries of SCID mice.

Ovarian follicles and follicular fluid

In follicles the oocyte development is supported by granulosa and theca cells. In recent years it was demonstrated that a subpopulation of these cells can exhibit stem cell properties. Kossowska-Tomaszczuk et al. [153] isolated a subpopulation of luteinizing granulosa cells from aspirated ovarian follicular fluid (derived from infertile patients).

The subpopulation of luteinizing granulosa cells were able to survive long term *in vitro*. These cells expressed some specific mesenchymal stem cell markers, such as CD90, CD105, CD44, CD29, CD117, and even the pluripotent stem cell marker POU5F1, but they did not express Nanog, Stellar, or Vasa. Even more, these cells were able to differentiate into neurons, chondrocytes, and osteoblasts. A similar population of aspirated follicular cells was compared to bone marrow-derived mesenchymal stem cells [154]. Gene expression analysis of 84 genes, related to mesenchymal stem cells, revealed a similar gene expression pattern between analyzed populations of cells, though some genes were differentially expressed. These genes were specific mesenchymal stem cell markers and connected to ovarian function. Additionally, these cells were able to differentiate into osteogenic, adipogenic, and pancreatic-like cells. These studies indicate that granulosa cells, which represent the majority of cells derived from aspirated ovarian follicular fluid, have interesting differentiation potential, yet it is unclear if their plasticity is reflected through transdifferentiation or differentiation of stem cells [155]. While the data about granulosa stem cells are growing, still little is known about thecal stem cells. There are in fact only two publications, which describe the isolation of thecal stem cells from mammalian ovaries [156, 157]. Honda et al. [156] were first able to isolate putative thecal stem cells from mouse ovaries, which exhibit typical characteristics of adult stem cells. Isolated cells were able to self-renew and to differentiate *in vivo* and *in vitro*. After exposure to multiple step differentiation protocol these cells differentiated *in vitro* in cells expressing genes typical for thecal cells and secreted androstenedione, which is androgen produced by mature thecal cells. It is known that the presence of androgens in ovaries is necessary for normal folliculogenesis [158–161]. Additionally, the putative thecal stem cells isolated by Honda et al. [156] localized in the ovarian interstitium and thecal layer of follicles as differentiated theca cells after transplantation into the mouse ovaries. The second publication reporting isolation of thecal stem cells used porcine model [157]. The authors named these cells ovarian theca-derived multipotent stem cells. These cells expressed tested mesenchymal stem cell surface markers (CD29, CD44, and CD90) and pluripotent stem cell marker *SOX-2*, but did not express pluripotent stem cell markers *OCT4* and *NANOG*. *In vitro* differentiation tests revealed the differentiation into osteocytes and adipocytes and even more, the differentiation into oocyte-like cells, which expressed some pluripotent stem cell markers (*OCT4, NANOG* and *SOX2*) and oocyte-specific markers (*GDF9B, C-MOS, DAZL, VASA, ZPC, SCP3* and *STELLA*). These two studies show interesting differentiation potential of thecal stem cells, but more studies on these cells should be conducted to isolate them also from human ovaries and to more precisely determine their characteristics.

Oogonial stem cells

Some other studies used a different approach in isolating stem cells from ovaries, since the selection of cells based on VASA (DDX-4) (human) or MVH (mouse) expression was used [162, 163]. These studies focused on the isolation of germinal stem cells, which were propagated *in vitro* and served as precursor cells for derivation of oocytes. Some uncertainty is related with this data, because Zhang et al. [164, 165] could not find any evidence that Ddx4-expressing cells from postnatal mouse ovaries enter mitosis, nor could he find any DDX4-expressing functional oogonial stem cells in adult human and mouse ovaries.

Fallopian tube as a source of stem cells

To confirm the above hypothesis about the tumorigenic potential of small stem cells resembling VSELs, it would be necessary to explore if these cells are present also in epithelium of fallopian tube, since there is evidence that the epithelial ovarian cancer can originate also from there [166–170], but to date such a study has not been performed yet. Even so other populations of cells with stem cell characteristics were isolated from fallopian tube. Toiurin et al. [171] located CD44+/ Ki67+ cells at distal fallopian tube, and furthermore, the quiescence of these cells was induced with tubal ligation. With the help of BrdU, Snegovskikh et al. [172] located putative stem cells at the base of the fallopian tube villi and estimated that 0.5% of all nucleated cells showed these characteristics. Paik et el. [173] isolated a subpopulation of cells, which were not expressing markers of ciliated (β-tubulin) or secretory (PAX8) differentiated cells. These cells expressed stem cell-specific markers CD44, a epithelial cell adhesion molecule, and integrin α6 along with an increased capability of clonal growth and self-renewal. Indumathi et al. [174] performed a wide-range evaluation of an expression profile of surface markers. Results showed the cells derived from the fallopian tube contain cells positive for cell adhesion molecules related to stem cells (CD29, CD44, CD106, CD54, CD13); contain cells positive for hematopoietic markers (CD34, CD45, CD133, CD117); contain cells positive for markers of mesenchymal stem cells (CD73, CD90, CD105, CD146, nestin); and even contain cells positive for pluripotent stem cell markers (SSEA-4, ABCG2, OCT3/4, SOX2).

To conclude, ovarian cancer is a complex and challenging disease. It is still not completely clear from which cells the ovarian tumors originate and which mechanisms are responsible to drive these cells to such behavior. Based on the presented data, we are suggesting that the ovarian small stem cells resembling VSELs are very likely to be involved in the development of aggressive ovarian tumors if their endogenous control system gets disrupted. Despite poor prognosis, chemo-resistance and recurrence of such tumors, the latest discoveries offer some hope that ovarian cancer will be more curable someday. Additionally, exploring different populations of cancer cells from

ovarian tumors, based on the expression of established cancer stem cell markers, opened a wide range of new possibilities as to how to study this complex disease – from the aspect of mechanisms that help us to understand the occurrence of ovarian cancer, to detailed studies of target-directed treatments. The knowledge of stem cells in healthy ovaries can definitely improve the understanding of ovarian cancer stem cells and the manifestation of ovarian cancer.

CONFLICT OF INTEREST

None of the authors has any conflict of interest.

REFERENCES

[1] Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015;136:E359–86.

[2] Wikborn C, Pettersson F, Moberg PJ. Delay in diagnosis of epithelial ovarian cancer. Int J Gynaecol Obstet 1996;52:263–7.

[3] Olson SH, Mignone L, Nakraseive C, Caputo TA, Barakat RR, Harlap S. Symptoms of ovarian cancer. Obstet Gynecol 2001;98:212–7.

[4] Ahmed N, Abubaker K, Findlay J, Quinn M. Cancerous ovarian stem cells: Obscure targets for therapy but relevant to chemoresistance. J Cell Biochem 2013;114:21–34.

[5] Karst AM, Drapkin R. Ovarian cancer pathogenesis: a model in evolution. J Oncol 2010;2010:932371.

[6] Prat J. Ovarian carcinomas: five distinct diseases with different origins, genetic alterations, and clinicopathological features. Virchows Arch 2012;460:237–49.

[7] Tavassoli FA, Devilee P. Pathology and Genetics of Tumours of the Breast and Female Genital Organs. Lyon, France: International Agency for Research on Cancer, and World Health Organization, IAPS Press; 2003.

[8] Shih IeM, Kurman RJ. Ovarian tumorigenesis: a proposed model based on morphological and molecular genetic analysis. Am J Pathol 2004;164:1511–8.

[9] Prahm KP, Karlsen MA, Hłgdall E, Scheller NM, Lundvall L, Nedergaard L, Christensen IJ, Hłgdall C. The prognostic value of dividing epithelial ovarian cancer into type I and type II tumors based on pathologic characteristics. Gynecol Oncol 2015;136:205–11.

[10] Skirnisdottir I, Seidal T, Åkerud H. Differences in clinical and biological features between Type I and Type II tumors in FIGO stages I-II epithelial ovarian carcinoma. Int J Gynecol Cancer 2015;25:1239–47.

[11] Previs R, Leath CA 3rd, Coleman RL, Herzog TJ, Krivak TC, Brower SL, Tian C, Secord AA. Evaluation of in vitro chemoresponse profiles in women with Type I and Type II epithelial ovarian cancers: An observational study ancillary analysis. Gynecol Oncol 2015;138:267–71.

[12] Despierre E, Yesilyurt BT, Lambrechts S, Johnson N, Verheijen R, van der Burg M, Casado A, Rustin G, Berns E, Leunen K, Amant F, Moerman P, Lambrechts D, Vergote I ; EORTC GCG and EORTC GCG Translational Research Group. Epithelial ovarian cancer: rationale for changing the one-fits-all standard treatment regimen to subtype-specific treatment. Int J Gynecol Cancer 2014;24:468–77.

[13] Ahmed AA, Etemadmoghadam D, Temple J, Lynch AG, Riad M, Sharma R, Stewart C, Fereday S, Caldas C, Defazio A, Bowtell D, Brenton JD. Driver mutations in TP53 are ubiquitous in high grade serous carcinoma of the ovary. J Pathol 2010;221:49–56.

[14] Kurman RJ, Shih IeM. Molecular pathogenesis and extraovarian origin of epithelial ovarian cancer shifting the paradigm. Hum Pathol 2011;42:918–31.

[15] Hershkovitz D, Vlodavsky E, Simon E, Ben-Izhak O. KRAS mutation positive mucinous adenocarcinoma originating in mature ovarian teratoma: case report and review of literature. Pathol Int 2013;63:611–4.

[16] Nogales FF, Musto ML, Sáez AI, Robledo M, Palacios J, Aneiros J. Multifocal intrafollicular granulosa cell tumor of the ovary associated with an unusual germline p53 mutation. Mod Pathol 2004;17:868–73.

[17] Rajesh NG, Rekha K, Krishna B. Significance of p53 expression in ovarian tumors and its correlation to the morphological differentiation. Indian J Pathol Microbiol 2007;50:284–7.

[18] Kinross KM, Montgomery KG, Kleinschmidt M, Waring P, Ivetac I, Tikoo A, Saad M, Hare L, Roh V, Mantamadiotis T, Sheppard KE, Ryland GL, Campbell IG, Gorringe KL, Christensen JG, Cullinane C, Hicks RJ, Pearson RB, Johnstone RW, McArthur GA, Phillips WA. An activating Pik3ca mutation coupled with Pten loss is sufficient to initiate ovarian tumorigenesis in mice. J Clin Invest 2012;122:553–7.

[19] Chen VW, Ruiz B, Killeen JL, Coté TR, Wu XC, Correa CN. Pathology and classification of ovarian tumors. Cancer 2003; 97:2631–42.

[20] Brown J, Friedlander M, Backes FJ, Harter P, O'Connor DM, de la Motte Rouge T, Lorusso D, Maenpaa J, Kim JW, Tenney ME, Seckl MJ. Gynecologic Cancer Intergroup (GCIG) consensus review for ovarian germ cell tumors. Int J Gynecol Cancer 2014; 24:S48–54.

[21] Haroon S, Zia A, Idrees R, Memon A, Fatima S, Kayani N. Clinicopathological spectrum of ovarian sex cord-stromal tumors; 20 years' retrospective study in a developing country. J Ovarian Res 2013;6:87.

[22] Kurman RJ, Carcangiu ML, Herrington CS, Young RH. WHO Classification of Tumours. 4th ed. Vol. 6. Lyon: IARC; 2014. Classification of tumours of the ovary; pp. 44–56.

[23] Patrono MG, Minig L, Diaz-Padilla I, Romero N, Rodriguez Moreno JF, Garcia-Donas J. Borderline tumours of the ovary, current controversies regarding their diagnosis and treatment. Ecancermedicalscience 2013;7:379.

[24] Dasari S, Tchounwou PB. Cisplatin in cancer therapy: molecular mechanisms of action. Eur J Pharmacol 2014;740: 364–78.

[25] Siddik ZH. Cisplatin: mode of cytotoxic action and molecular basis of resistance. Oncogene 2003;22:7265–79.

[26] Santabarbara G, Maione P, Rossi A, Gridelli C. Pharmacotherapeutic options for treating adverse effects of Cisplatin chemotherapy. Expert Opin Pharmacother 2016;17:561–70.

[27] du Bois A, Lück HJ, Meier W, Adams HP, Möbus V, Costa S, Bauknecht T, Richter B, Warm M, Schröder W, Olbricht S, Nitz U, Jackisch C, Emons G, Wagner U, Kuhn W, Pfisterer J; Arbeitsgemeinschaft Gynäkologische Onkologie Ovarian Cancer Study Group. A randomized clinical trial of cisplatin/paclitaxel versus carboplatin/paclitaxel as first-line treatment of ovarian cancer. J Natl Cancer Inst 2003;95:1320–9.

[28] Ozols RF, Bundy BN, Greer BE, Fowler JM, Clarke-Pearson D, Burger RA, Mannel RS, DeGeest K, Hartenbach EM, Baergen R; Gynecologic Oncology Group. Phase III trial of carboplatin and paclitaxel compared with cisplatin and paclitaxel in patients with optimally resected stage III ovarian cancer: a Gynecologic Oncology Group study. J Clin Oncol 2003;21:3194–200.

[29] Sousa GF, Wlodarczyk SR, Monteiro G. Carboplatin: molecular mechanisms of action associated with chemoresistance. Braz J Pharm Sci 2014;50:693–701.

[30] Muggia FM, Braly PS, Brady MF, Sutton G, Niemann TH, Lentz SL, Alvarez RD, Kucera PR, Small JM. Phase III randomized study of cisplatin versus paclitaxel versus cisplatin and paclitaxel in patients with suboptimal stage III or IV ovarian cancer: a gynecologic oncology group study. J Clin Oncol 2000;18: 106–15.

[31] Jordan MA. Mechanism of action of antitumor drugs that interact with microtubules and tubulin. Curr Med Chem Anticancer Agents 2002;2:1–17.

[32] Gligorov J, Lotz JP. Preclinical pharmacology of the taxanes: implications of the differences. Oncologist 2004;9:3–8.

[33] Galluzzi L, Senovilla L, Vitale I, Michels J, Martins I, Kepp O, Castedo M, Kroemer G. Molecular mechanisms of cisplatin resistance. Oncogene 2012;31:1869–83.

[34] Parekh H, Wiesen K, Simpkins H. Acquisition of taxol resistance via P-glycoprotein- and non-P-glycoprotein-mediated mechanisms in human ovarian carcinoma cells. Biochem Pharmacol 1997;53:461–70.

[35] Orr GA, Verdier-Pinard P, McDaid H, Horwitz SB. Mechanisms of Taxol resistance related to microtubules. Oncogene 2003;22:7280–95.

[36] Du J, Li B, Fang Y, Liu Y, Wang Y, Li J, Zhou W, Wang X. Overexpression of Class III β-tubulin, Sox2, and nuclear Survivin is predictive of taxane resistance in patients with stage III ovarian epithelial cancer. BMC Cancer 2015;15:536.

[37] Chen M, Huang H, He H, Ying W, Liu X, Dai Z, Yin J, Mao N, Qian X, Pan L. Quantitative proteomic analysis of mitochondria from human ovarian cancer cells and their paclitaxel-resistant sublines. Cancer Sci 2015;106:1075–83.

[38] Borley J, Brown R. Epigenetic mechanisms and therapeutic targets of chemotherapy resistance in epithelial ovarian cancer. Ann Med 2015;47:359–69.

[39] Bapat SA, Mali AM, Koppikar CB, Kurrey NK. Stem and progenitor-like cells contribute to the aggressive behavior of human epithelial ovarian cancer. Cancer Res 2005;65:3025–9.

[40] Alvero AB, Chen R, Fu HH, Montagna M, Schwartz PE, Rutherford T, Silasi DA, Steffensen KD, Waldstrom M, Visintin I, Mor G. Molecular phenotyping of human ovarian cancer stem cells unravels the mechanisms for repair and chemoresistance. Cell Cycle 2009;8:158–66.

[41] Clarke MF, Dick JE, Dirks PB, Eaves CJ, Jamieson CH, Jones DL, Visvader J, Weissman IL, Wahl GM. Cancer stem cells–perspectives on current status and future directions: AACR Workshop on cancer stem cells. Cancer Res 2006;66: 9339–44.

[42] Moore N, Houghton J, Lyle S. Slow-cycling therapy-resistant cancer cells. Stem Cells Dev 2012;21:1822–30.

[43] Bomken S, Fiser K, Heidenreich O, Vormoor J. Understanding the cancer stem cell. Br J Cancer 2010;103:439–45.

[44] Visvader JE. Cells of origin in cancer. Nature 2011;469:314–22.

[45] Tang DG. Understanding cancer stem cell heterogeneity and plasticity. Cell Res 2012;22:457–72.

[46] Greaves M, Maley CC. Clonal evolution in cancer. Nature 2012;481:306–13.

[47] Anderson K, Lutz C, van Delft FW, Bateman CM, Guo Y, Colman SM, Kempski H, Moorman AV, Titley I, Swansbury J, Kearney L, Enver T, Greaves M. Genetic variegation of clonal architecture and propagating cells in leukaemia. Nature 2011;469:356–61.

[48] Notta F, Mullighan CG, Wang JC, Poeppl A, Doulatov S, Phillips LA, Ma J, Minden MD, Downing JR, Dick JE. Evolution of human BCR-ABL1 lymphoblastic leukaemia-initiating cells. Nature 2011;469:362–7.

[49] Wang W, Quan Y, Fu Q, Liu Y, Liang Y, Wu J, Yang G, Luo C, Ouyang Q, Wang Y. Dynamics between cancer cell subpopulations reveals a model coordinating with both hierarchical and stochastic concepts. PLoS One 2014;9:e84654.

[50] Schatton T, Murphy GF, Frank NY, Yamaura K, Waaga-Gasser AM, Gasser M, Zhan Q, Jordan S, Duncan LM, Weishaupt C, Fuhlbrigge RC, Kupper TS, Sayegh MH, Frank MH. Identification of cells initiating human melanomas. Nature 2008; 451:345–9.

[51] Boiko AD, Razorenova OV, van de Rijn M, Swetter SM, Johnson DL, Ly DP, Butler PD, Yang GP, Joshua B, Kaplan MJ, Longaker MT, Weissman IL. Human melanoma-initiating cells express neural crest nerve growth factor receptor CD271. Nature 2010;466:133–7

[52] Kelly PN, Dakic A, Adams JM, Nutt SL, Strasser A. Tumor growth need not be driven by rare cancer stem cells. Science 2007;317:337.

[53] Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med 1997;3:730–7.

[54] Lapidot T, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Cortes J, Minden M, Paterson B, Caligiuri MA, Dick JE. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature 1994;367:645–8.

[55] Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, Henkelman RM, Cusimano MD, Dirks PB. Identification of human brain tumour initiating cells. Nature 2004;432: 396–401.

[56] Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, Dewhirst MW, Bigner DD, Rich JN. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature 2006;444:756–60.

[57] Ferrandina G, Bonanno G, Pierelli L, Perillo A, Procoli A, Mariotti A, Corallo M, Martinelli E, Rutella S, Paglia A, Zannoni G, Mancuso S, Scambia G. Expression of CD133–1 and CD133–2 in ovarian cancer. Int J Gynecol Cancer 2008;18:506–14.

[58] Baba T, Convery PA, Matsumura N, Whitaker RS, Kondoh E, Perry T, Huang Z, Bentley RC, Mori S, Fujii S, Marks JR, Berchuck A, Murphy SK. Epigenetic regulation of CD133 and tumorigenicity of CD133+ ovarian cancer cells. Oncogene 2009;28:209–18.

[59] Ricci-Vitiani L, Lombardi DG, Pilozzi E, Biffoni M, Todaro M, Peschle C, De Maria R. Identification and expansion of human colon-cancer-initiating cells. Nature 2007;445:111–5.

[60] O'Brien CA, Pollett A, Gallinger S, Dick JE. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. Nature 2007;445:106–10.

[61] Shmelkov SV, Butler JM, Hooper AT, Hormigo A, Kushner J, Milde T, St Clair R, Baljevic M, White I, Jin DK, Chadburn A, Murphy AJ, Valenzuela DM, Gale NW, Thurston G, Yancopoulos GD, D'Angelica M, Kemeny N, Lyden D, Rafii S. CD133 expression is not restricted to stem cells, and both CD133+ and CD133− metastatic colon cancer cells initiate tumors. J Clin Invest 2008;118:2111–20.

[62] Quintana E, Shackleton M, Foster HR, Fullen DR, Sabel MS, Johnson TM, Morrison SJ. Phenotypic heterogeneity among tumorigenic melanoma cells from patients that is reversible and not hierarchically organized. Cancer Cell 2010;18: 510–23.

[63] Giancotti FG. Mechanisms governing metastatic dormancy and reactivation. Cell 2013;155:750–64.

[64] Sosa MS, Bragado P, Aguirre-Ghiso JA. Mechanisms of disseminated cancer cell dormancy: an awakening field. Nat Rev Cancer 2014;14:611–22.

[65] Zhou BB, Zhang H, Damelin M, Geles KG, Grindley JC, Dirks PB. Tumour-initiating cells: challenges and opportunities for anticancer drug discovery. Nat Rev Drug Discov 2009;8: 806–23.

[66] Saito Y, Uchida N, Tanaka S, Suzuki N, Tomizawa-Murasawa M, Sone A, Najima Y, Takagi S, Aoki Y, Wake A, Taniguchi S, Shultz LD, Ishikawa F. Induction of cell cycle entry eliminates human leukemia stem cells in a mouse model of AML. Nat Biotechnol 2010;28:275–80.

[67] Borah A, Raveendran S, Rochani A, Maekawa T, Kumar DS. Targeting self-renewal pathways in cancer stem cells: clinical implications for cancer therapy. Oncogenesis 2015;4: e177.

[68] Moitra K. Overcoming multidrug resistance in cancer stem cells. Biomed Res Int 2015;2015:635745.

[69] Pan Y, Jiao J, Zhou C, Cheng Q, Hu Y, Chen H. Nanog is highly expressed in ovarian serous cystadenocarcinoma and correlated with clinical stage and pathological grade. Pathobiology 2010;77:283–8.

[70] Lee M, Nam EJ, Kim SW, Kim S, Kim JH, Kim YT. Prognostic impact of the cancer stem cell-related marker NANOG in ovarian serous carcinoma. Int J Gynecol Cancer 2012;22: 1489–96.

[71] Siu MK, Wong ES, Kong DS, Chan HY, Jiang L, Wong OG, Lam EW, Chan KK, Ngan HY, Le XF, Cheung AN. Stem cell transcription factor NANOG controls cell migration and invasion via dysregulation of E-cadherin and FoxJ1 and contributes to adverse clinical outcome in ovarian cancers. Oncogene 2013;32:3500–9.

[72] Liu S, Sun J, Cai B, Xi X, Yang L, Zhang Z, Feng Y, Sun Y. NANOG regulates epithelial-mesenchymal transition and chemoresistance through activation of the STAT3 pathway in epithelial ovarian cancer. Tumour Biol 2016;Jan 22. Epub ahead of print

[73] Virant-Klun I, Kenda-Suster N, Smrkolj S. Small putative NANOG, SOX2, and SSEA-4-positive stem cells resembling very small embryonic-like stem cells in sections of ovarian tissue in patients with ovarian cancer. J Ovarian Res 2016; 9:12.

[74] He QZ, Luo XZ, Wang K, Zhou Q, Ao H, Yang Y, Li SX, Li Y, Zhu HT, Duan T. Isolation and characterization of cancer stem cells from high-grade serous ovarian carcinomas. Cell Physiol Biochem 2014;33:173–84.

[75] Di J, Duiveman-de Boer T, Zusterzeel PL, Figdor CG, Massuger LF, Torensma R. The stem cell markers Oct4A, Nanog and c-Myc are expressed in ascites cells and tumor tissue of ovarian cancer patients. Cell Oncol (Dordr) 2013;36:363–74.

[76] Du Y, Ma C, Wang Z, Liu Z, Liu H, Wang T. Nanog, a novel prognostic marker for lung cancer. Surg Oncol 2013;22: 224–9.

[77] Luo W, Li S, Peng B, Ye Y, Deng X, Yao K. Embryonic stem cells markers SOX2, OCT4 and Nanog expression and their correlations with epithelial-mesenchymal transition in nasopharyngeal carcinoma. PLoS One 2013;8:e56324.

[78] Meng HM, Zheng P, Wang XY, Liu C, Sui HM, Wu SJ, Zhou J, Ding YQ, Li J. Over-expression of Nanog predicts tumor progression and poor prognosis in colorectal cancer. Cancer Biol Ther 2010;9:295–302.

[79] Gu TT, Liu SY, Zheng PS. Cytoplasmic NANOG-positive stromal cells promote human cervical cancer progression. Am J Pathol 2012;181:652–61.

[80] Goodison S, Urquidi V, Tarin D. CD44 cell adhesion molecules. Mol Pathol 1999;52:189–96.

[81] Cannistra SA, Kansas GS, Niloff J, DeFranzo B, Kim Y, Ottensmeier C. Binding of ovarian cancer cells to peritoneal mesothelium in vitro is partly mediated by CD44H. Cancer Res 1993;53:3830–8.

[82] Berner HS, Davidson B, Berner A, Risberg B, Kristensen GB, Trope CG, Van de Putte G, Nesland JM. Expression of CD44 in effusions of patients diagnosed with serous ovarian carcinoma–diagnostic and prognostic implications. Clin Exp Metastasis 2000;18:197–202.

[83] Cannistra SA, DeFranzo B, Niloff J, Ottensmeir C. Functional heterogeneity of CD44 molecules in ovarian cancer cell lines. Clin Cancer Res 1995;1:333–42.

[84] Sliutz G, Tempfer C, Winkler S, Kohlberger P, Reinthaller A, Kainz C. Immunohistochemical and serological evaluation of CD44 splice variants in human ovarian cancer. Br J Cancer 1995;72:1494–7.

[85] Rodríguez-Rodríguez L, Sancho-Torres I, Mesonero C, Gibbon DG, Shih WJ, Zotalis G. The CD44 receptor is a molecular predictor of survival in ovarian cancer. Med Oncol 2003;20:255–63.

[86] Shi J, Zhou Z, Di W, Li N. Correlation of CD44v6 expression with ovarian cancer progression and recurrence. BMC Cancer 2013;13:182.

[87] Saegusa M, Machida D, Hashimura M, Okayasu I. CD44 expression in benign, premalignant, and malignant ovarian neoplasms: relation to tumour development and progression. J Pathol 1999;189:326–37.

[88] Ross JS, Sheehan CE, Williams SS, Malfetano JH, Szyfelbein WM, Kallakury BV. Decreased CD44 standard form expression correlates with prognostic variables in ovarian carcinomas. Am J Clin Pathol 2001;116:122–8.

[89] Zhang S, Balch C, Chan MW, Lai HC, Matei D, Schilder JM, Yan PS, Huang TH, Nephew KP. Identification and characterization of ovarian cancer-initiating cells from primary human tumors. Cancer Res 2008;68:4311–20.

[90] Cheng W, Liu T, Wan X, Gao Y, Wang H. MicroRNA-199a targets CD44 to suppress the tumorigenicity and multidrug resistance of ovarian cancer-initiating cells. FEBS J 2012;279:2047–59.

[91] Alvero AB, Chen R, Fu HH, Montagna M, Schwartz PE, Rutherford T, Silasi DA, Steffensen KD, Waldstrom M, Visintin I, Mor G. Molecular phenotyping of human ovarian cancer stem cells unravels the mechanisms for repair and chemoresistance. Cell Cycle 2009;8:158–66.

[92] Craveiro V, Yang-Hartwich Y, Holmberg JC, Sumi NJ, Pizzonia J, Griffin B, Gill SK, Silasi DA, Azodi M, Rutherford T, Alvero AB, Mor G. Phenotypic modifications in ovarian cancer stem cells following Paclitaxel treatment. Cancer Med 2013;2: 751–62.

[93] Chen J, Wang J, Chen D, Yang J, Yang C, Zhang Y, Zhang H, Dou J. Evaluation of characteristics of CD44+CD117+ ovarian cancer stem cells in three dimensional basement membrane extract scaffold versus two dimensional monocultures. BMC Cell Biol 2013;14:7.

[94] Yin AH, Miraglia S, Zanjani ED, Almeida-Porada G, Ogawa M, Leary AG, Olweus J, Kearney J, Buck DW. AC133, a novel marker for human hematopoietic stem and progenitor cells. Blood 1997;90:5002–12.

[95] Miraglia S, Godfrey W, Yin AH, Atkins K, Warnke R, Holden JT, Bray RA, Waller EK, Buck DW. A novel five-transmembrane hematopoietic stem cell antigen: isolation, characterization, and molecular cloning. Blood 1997;90:5013–21.

[96] Balaji AB, Jamil K, Ram GM, Raju GS. Pluripotent lineage of CD133 stem cells isolated from human skin samples. Indian J Exp Biol 2013;51:107–15.

[97] Marlicz W, Zuba-Surma E, Kucia M, Blogowski W, Starzynska T, Ratajczak MZ. Various types of stem cells, including a population of very small embryonic-like stem cells, are mobilized into peripheral blood in patients with Crohn's disease. Inflamm Bowel Dis 2012;18:1711–22.

[98] New SE, Alvarez-Gonzalez C, Vagaska B, Gomez SG, Bulstrode NW, Madrigal A, Ferretti P. A matter of identity - Phenotype and differentiation potential of human somatic stem cells. Stem Cell Res 2015;15:1–13.

[99] Sallustio F, Serino G, Schena FP. Potential Reparative Role of Resident Adult Renal Stem/Progenitor Cells in Acute Kidney Injury. Biores Open Access 2015;4:326–33.

[100] Zhang Z, He Q, Deng W, Chen Q, Hu X, Gong A, Cao X, Yu J, Xu X. Nasal ectomesenchymal stem cells: multi-lineage differentiation and transformation effects on fibrin gels. Biomaterials 2015;49:57–67.

[101] Ustiashvili M, Kordzaia D, Mamaladze M, Jangavadze M, Sanodze L. Investigation of functional activity human dental pulp stem cells at acute and chronic pulpitis. Georgian Med News 2014;234:19–24.

[102] Lu C, Xu F, Gu J, Yuan Y, Zhao G, Yu X, Ge D. Clinical and biological significance of stem-like CD133(+)CXCR4(+) cells in esophageal squamous cell carcinoma. J Thorac Cardiovasc Surg 2015;150:386–95.

[103] Pal SK, He M, Wilson T, Liu X, Zhang K, Carmichael C, Torres A, Hernandez S, Lau C, Agarwal N, Kawachi M, Yen Y, Jones JO. Detection and phenotyping of circulating tumor cells in high-risk localized prostate cancer. Clin Genitourin Cancer 2015;13:130–6.

[104] Cantile M, Collina F, D'Aiuto M, Rinaldo M, Pirozzi G, Borsellino C, Franco R, Botti G, Di Bonito M. Nuclear localization of cancer stem cell marker CD133 in triple-negative breast cancer: a case report. Tumori 2013;99:e245–50.

[105] Sibin MK, Lavanya CH, Bhat DI, Rao N, Geethashree N, Vibhuti W, Chetan GK. CD133 and BMI1 expressions and its prognostic role in primary glioblastoma. J Genet 2015;94:689–96.

[106] Jing F, Kim HJ, Kim CH, Kim YJ, Lee JH, Kim HR. Colon cancer stem cell markers CD44 and CD133 in patients with colorectal cancer and synchronous hepatic metastases. Int J Oncol 2015;46:1582–8.

[107] Virant-Klun I, Stimpfel M, Cvjeticanin B, Vrtacnik-Bokal E, Skutella T. Small SSEA-4-positive cells from human ovarian cell cultures: related to embryonic stem cells and germinal lineage? J Ovarian Res 2013;6:24.

[108] Curley MD, Therrien VA, Cummings CL, Sergent PA, Koulouris CR, Friel AM, Roberts DJ, Seiden MV, Scadden DT, Rueda BR, Foster R. CD133 expression defines a tumor initiating cell population in primary human ovarian cancer. Stem Cells 2009;27:2875–83.

[109] Liu BL, Liu SJ, Baskys A, Cheng H, Han Y, Xie C, Song H, Li J, Xin XY. Platinum sensitivity and CD133 expression as risk and prognostic predictors of central nervous system metastases in patients with epithelial ovarian cancer. BMC Cancer 2014;14:829.

[110] Stewart JM, Shaw PA, Gedye C, Bernardini MQ, Neel BG, Ailles LE. Phenotypic heterogeneity and instability of human ovarian tumor-initiating cells. Proc Natl Acad Sci U S A 2011;108:6468–73.

[111] Ferrandina G, Martinelli E, Petrillo M, Prisco MG, Zannoni G, Sioletic S, Scambia G. CD133 antigen expression in ovarian cancer. BMC Cancer 2009;9:221.

[112] Ricci F, Bernasconi S, Porcu L, Erba E, Panini N, Fruscio R, Sina F, Torri V, Broggini M, Damia G. ALDH enzymatic activity and CD133 positivity and response to chemotherapy in ovarian cancer patients. Am J Cancer Res 2013;3:221–9.

[113] Zhou Q, Chen A, Song H, Tao J, Yang H, Zuo M. Prognostic value of cancer stem cell marker CD133 in ovarian cancer: a meta-analysis. Int J Clin Exp Med 2015;8:3080–8.

[114] Marchitti SA, Brocker C, Stagos D, Vasiliou V. Non-P450 aldehyde oxidizing enzymes: the aldehyde dehydrogenase superfamily. Expert Opin Drug Metab Toxicol 2008;4:697–720.

[115] Marcato P, Dean CA, Giacomantonio CA, Lee PW. Aldehyde dehydrogenase: its role as a cancer stem cell marker comes down to the specific isoform. Cell Cycle 2011;10:1378–84.

[116] Kryczek I, Liu S, Roh M, Vatan L, Szeliga W, Wei S, Banerjee M, Mao Y, Kotarski J, Wicha MS, Liu R, Zou W. Expression of aldehyde dehydrogenase and CD133 defines ovarian cancer stem cells. Int J Cancer 2012;130:29–39.

[117] Yasuda K, Torigoe T, Morita R, Kuroda T, Takahashi A, Matsuzaki J, Kochin V, Asanuma H, Hasegawa T, Saito T, Hirohashi Y, Sato N. Ovarian cancer stem cells are enriched in side population and aldehyde dehydrogenase bright overlapping population. PLoS One 2013;8:e68187.

[118] Meng E, Mitra A, Tripathi K, Finan MA, Scalici J, McClellan S, Madeira da Silva L, Reed E, Shevde LA, Palle K, Rocconi RP. ALDH1A1 maintains ovarian cancer stem cell-like properties by altered regulation of cell cycle checkpoint and DNA repair network signaling. PLoS One 2014;9:e107142.

[119] Mizuno T, Suzuki N, Makino H, Furui T, Morii E, Aoki H, Kunisada T, Yano M, Kuji S, Hirashima Y, Arakawa A, Nishio S, Ushijima K, Ito K, Itani Y, Morishige K. Cancer stem-like cells of ovarian clear cell carcinoma are enriched in the ALDH-high population associated with an accelerated scavenging system in reactive oxygen species. Gynecol Oncol 2015; 137:299–305.

[120] Silva IA, Bai S, McLean K, Yang K, Griffith K, Thomas D, Ginestier C, Johnston C, Kueck A, Reynolds RK, Wicha MS, Buckanovich RJ. Aldehyde dehydrogenase in combination with CD133 defines angiogenic ovarian cancer stem cells that portend poor patient survival. Cancer Res 2011;71:3991–4001.

[121] Deng S, Yang X, Lassus H, Liang S, Kaur S, Ye Q, Li C, Wang LP, Roby KF, Orsulic S, Connolly DC, Zhang Y, Montone K, Bützow R, Coukos G, Zhang L. Distinct expression levels and patterns of stem cell marker, aldehyde dehydrogenase isoform 1 (ALDH1), in human epithelial cancers. PLoS One 2010;5: e10277.

[122] Liu S, Liu C, Min X, Ji Y, Wang N, Liu D, Cai J, Li K. Prognostic value of cancer stem cell marker aldehyde dehydrogenase in ovarian cancer: a meta-analysis. PLoS One 2013;8:e81050.

[123] Kristiansen G, Denkert C, Schlüns K, Dahl E, Pilarsky C, Hauptmann S. CD24 is expressed in ovarian cancer and is a new independent prognostic marker of patient survival. Am J Pathol 2002;161:1215–21.

[124] Choi YL, Kim SH, Shin YK, Hong YC, Lee SJ, Kang SY, Ahn G. Cytoplasmic CD24 expression in advanced ovarian serous borderline tumors. Gynecol Oncol 2005;97:379–86.

[125] Surowiak P, Materna V, Kaplenko I, Spaczyn´ski M, Dietel M, Kristiansen G, Lage H, Zabel M. Unfavorable prognostic value of CD24 expression in sections from primary and relapsed ovarian cancer tissue. Int J Gynecol Cancer 2006;16:515–21.

[126] Gao MQ, Choi YP, Kang S, Youn JH, Cho NH. CD24+ cells from hierarchically organized ovarian cancer are enriched in cancer stem cells. Oncogene 2010;29:2672–80.

[127] Schmandt RE, Broaddus R, Lu KH, Shvartsman H, Thornton A, Malpica A, Sun C, Bodurka DC, Gershenson DM. Expression of

c-ABL, c-KIT, and platelet-derived growth factor receptor-beta in ovarian serous carcinoma and normal ovarian surface epithelium. Cancer 2003;98:758–64.

[128] Tonary AM, Macdonald EA, Faught W, Senterman MK, Vanderhyden BC. Lack of expression of c-KIT in ovarian cancers is associated with poor prognosis. Int J Cancer 2000; 89:242–50.

[129] Khalifeh I, Munkarah AR, Schimp V, Morris R, Lawrence WD, Ali-Fehmi R. The impact of c-kit and ki-67 expression on patients prognosis in advanced ovarian serous carcinoma. Int J Gynecol Pathol 2005;24:228–34.

[130] Raspollini MR, Amunni G, Villanucci A, Baroni G, Taddei A, Taddei GL. c-KIT expression and correlation with chemotherapy resistance in ovarian carcinoma: an immunocytochemical study. Ann Oncol 2004;15:594–7.

[131] Chau WK, Ip CK, Mak AS, Lai HC, Wong AS. c-Kit mediates chemoresistance and tumor-initiating capacity of ovarian cancer cells through activation of Wnt/β-catenin-ATP-binding cassette G2 signaling. Oncogene 2013;32:2767–81.

[132] Talbot P, Martin GG, Ashby H. Formation of the rupture site in preovulatory hamster and mouse follicles: loss of the surface epithelium. Gamete Res 1987;17:287–302.

[133] Kruk PA, Auersperg N. Human ovarian surface epithelial cells are capable of physically restructuring extracellular matrix. Am J Obstet Gynecol 1992;167:1437–43.

[134] Bowen NJ, Walker LD, Matyunina LV, Logani S, Totten KA, Benigno BB, McDonald JF. Gene expression profiling supports the hypothesis that human ovarian surface epithelia are multipotent and capable of serving as ovarian cancer initiating cells. BMC Med Genomics 2009;2:71.

[135] Virant-Klun I, Zech N, Rozman P, Vogler A, Cvjeticanin B, Klemenc P, Malicev E, Meden-Vrtovec H. Putative stem cells with an embryonic character isolated from the ovarian surface epithelium of women with no naturally present follicles and oocytes. Differentiation 2008;76:843–56.

[136] Virant-Klun I, Rozman P, Cvjeticanin B, Vrtacnik-Bokal E, Novakovic S, Rülicke T, Dovc P, Meden-Vrtovec H. Parthenogenetic embryo-like structures in the human ovarian surface epithelium cell culture in postmenopausal women with no naturally present follicles and oocytes. Stem Cells Dev 2009;18:137–49.

[137] Virant-Klun I, Skutella T, Stimpfel M, Sinkovec J. Ovarian surface epithelium in patients with severe ovarian infertility: a potential source of cells expressing markers of pluripotent/multipotent stem cells. J Biomed Biotechnol 2011;2011:381928

[138] Virant-Klun I, Skutella T, Hren M, Gruden K, Cvjeticanin B, Vogler A, Sinkovec J. Isolation of small SSEA-4-positive putative stem cells from the ovarian surface epithelium of adult human ovaries by two different methods. Biomed Res Int 2013;2013:690415.

[139] Virant-Klun I, Skutella T, Kubista M, Vogler A, Sinkovec J, Meden-Vrtovec H. Expression of pluripotency and oocyte-related genes in single putative stem cells from human adult ovarian surface epithelium cultured in vitro in the presence of follicular fluid. Biomed Res Int 2013;2013:861460.

[140] Parte S, Bhartiya D, Telang J, Daithankar V, Salvi V, Zaveri K, Hinduja I. Detection, characterization, and spontaneous differentiation in vitro of very small embryonic-like putative stem cells in adult mammalian ovary. Stem Cells Dev 2011;20: 1451–64.

[141] Bui HT, Van Thuan N, Kwon DN, Choi YJ, Kang MH, Han JW, Kim T, Kim JH. Identification and characterization of putative stem cells in the adult pig ovary. Development 2014;141: 2235–44.

[142] Sriraman K, Bhartiya D, Anand S, Bhutda S. Mouse ovarian very small embryonic-like stem cells resist chemotherapy and retain ability to initiate oocyte-specific differentiation. Reprod Sci 2015;22:884–903.

[143] Shin DM, Zuba-Surma EK, Wu W, Ratajczak J, Wysoczynski M, Ratajczak MZ, Kucia M. Novel epigenetic mechanisms that control pluripotency and quiescence of adult bone marrow-derived Oct4(+) very small embryonic-like stem cells. Leukemia 2009;23:2042–51.

[144] Kucia M, Reca R, Campbell FR, Zuba-Surma E, Majka M, Ratajczak J, Ratajczak MZ. A population of very small embryonic-like (VSEL) CXCR4(+)SSEA-1(+)Oct-4+ stem cells identified in adult bone marrow. Leukemia 2006;20: 857–69.

[145] Ratajczak MZ, Zuba-Surma EK, Shin DM, Ratajczak J, Kucia M. Very small embryonic-like (VSEL) stem cells in adult organs and their potential role in rejuvenation of tissues and longevity. Exp Gerontol 2008;43:1009–17.

[146] Paczkowska E, Kucia M, Koziarska D, Halasa M, Safranow K, Masiuk M, Karbicka A, Nowik M, Nowacki P, Ratajczak MZ, Machalinski B. Clinical evidence that very small embryonic-like stem cells are mobilized into peripheral blood in patients after stroke. Stroke 2009;40:1237–44.

[147] Chang YJ, Tien KE, Wen CH, Hsieh TB, Hwang SM. Recovery of CD45(−)/Lin(−)/SSEA-4(+) very small embryonic-like stem cells by cord blood bank standard operating procedures. Cytotherapy 2014;16:560–5.

[148] Shin DM, Liu R, Klich I, Wu W, Ratajczak J, Kucia M, Ratajczak MZ. Molecular signature of adult bone marrow-purified very small embryonic-like stem cells supports their developmental epiblast/germ line origin. Leukemia 2010;24: 1450–61.

[149] Stimpfel M, Skutella T, Cvjeticanin B, Meznaric M, Dovc P, Novakovic S, Cerkovnik P, Vrtacnik-Bokal E, Virant-Klun I. Isolation, characterization and differentiation of cells expressing pluripotent/multipotent markers from adult human ovaries. Cell Tissue Res 2013;354:593–607.

[150] Stimpfel M, Cerkovnik P, Novakovic S, Maver A, Virant-Klun I. Putative mesenchymal stem cells isolated from adult human ovaries. J Assist Reprod Genet 2014;31:959–74.

[151] Gong SP, Lee ST, Lee EJ, Kim DY, Lee G, Chi SG, Ryu BK, Lee CH, Yum KE, Lee HJ, Han JY, Tilly JL, Lim JM. Embryonic stem cell-like cells established by culture of adult ovarian cells in mice. Fertil Steril 2010;93:2594–601, 2601.e1–9.

[152] Pacchiarotti J, Maki C, Ramos T, Marh J, Howerton K, Wong J, Pham J, Anorve S, Chow YC, Izadyar F. Differentiation potential of germ line stem cells derived from the postnatal mouse ovary. Differentiation 2010;79:159–70.

[153] Kossowska-Tomaszczuk K, De Geyter C, De Geyter M, Martin I, Holzgreve W, Scherberich A, Zhang H. The multipotency of luteinizing granulosa cells collected from mature ovarian follicles. Stem Cells 2009;27:210–9.

[154] Dzafic E, Stimpfel M, Novakovic S, Cerkovnik P, Virant-Klun I. Expression of mesenchymal stem cells-related genes and plasticity of aspirated follicular cells obtained from infertile women. Biomed Res Int 2014;2014:508216.

[155] Dzafic E, Stimpfel M, Virant-Klun I. Plasticity of granulosa cells: on the crossroad of stemness and transdifferentiation potential. J Assist Reprod Genet 2013;30:1255–61.

[156] Honda A, Hirose M, Hara K, Matoba S, Inoue K, Miki H, Hiura H, Kanatsu-Shinohara M, Kanai Y, Kono T, Shinohara T, Ogura A. Isolation, characterization, and in vitro and in vivo differentiation of putative thecal stem cells. Proc Natl Acad Sci U S A 2007, 104:12389–94.

[157] Lee YM, Kumar BM, Lee JH, Lee WJ, Kim TH, Lee SL, Ock SA, Jeon BG, Park BW, Rho GJ. Characterisation and differentiation of porcine ovarian theca-derived multipotent stem cells. Vet J 2013;197:761–8.

[158] Vendola KA, Zhou J, Adesanya OO, Weil SJ, Bondy CA. Androgens stimulate early stages of follicular growth in the primate ovary. J Clin Invest 1998;101:2622–9.

[159] Murray AA, Gosden RG, Allison V, Spears N. Effect of androgens on the development of mouse follicles growing in vitro. J Reprod Fertil 1998;113:27–33.

[160] Yang MY, Fortune JE. Testosterone stimulates the primary to secondary follicle transition in bovine follicles *in vitro*. Biol Reprod 2006;75:924–32.

[161] Otala M, Makinen S, Tuuri T, Sjoberg J, Pentikainen V, Matikainen T, Dunkel L. Effects of testosterone, dihydrotestosterone, and 17beta-estradiol on human ovarian tissue survival in culture. Fertil Steril 2004;82:1077–85.

[162] Zou K, Yuan Z, Yang Z, Luo H, Sun K, Zhou L, Xiang J, Shi L, Yu Q, Zhang Y, Hou R, Wu J. Production of offspring from a germline stem cell line derived from neonatal ovaries. Nat Cell Biol 2009;11:631–6.

[163] White YA, Woods DC, Takai Y, Ishihara O, Seki H, Tilly JL. Oocyte formation by mitotically active germ cells purified from ovaries of reproductive-age women. Nat Med 2012;18: 413–21.

[164] Zhang H, Zheng W, Shen Y, Adhikari D, Ueno H, Liu K. Experimental evidence showing that no mitotically active female germline progenitors exist in postnatal mouse ovaries. Proc Natl Acad Sci U S A 2012;109:12580–5.

[165] Zhang H, Panula S, Petropoulos S, Edsgärd D, Busayavalasa K, Liu L, Li X, Risal S, Shen Y, Shao J, Liu M, Li S, Zhang D, Zhang X, Gerner RR, Sheikhi M, Damdimopoulou P, Sandberg R, Douagi I, Gustafsson JÅ, Liu L, Lanner F, Hovatta O, Liu K. Adult human and mouse ovaries lack DDX4-expressing functional oogonial stem cells. Nat Med 2015;21: 1116–8.

[166] Lee Y, Miron A, Drapkin R, Nucci MR, Medeiros F, Saleemuddin A, Garber J, Birch C, Mou H, Gordon RW, Cramer DW, McKeon FD, Crum CP. A candidate precursor to serous carcinoma that originates in the distal fallopian tube. J Pathol 2007;211:26–35. Erratum in J Pathol 2007;213:116.

[167] Piek JM, van Diest PJ, Zweemer RP, Jansen JW, Poort-Keesom RJ, Menko FH, Gille JJ, Jongsma AP, Pals G, Kenemans P, Verheijen RH. Dysplastic changes in prophylactically removed Fallopian tubes of women predisposed to developing ovarian cancer. J Pathol 2001;195:451–6.

[168] Kessler M, Fotopoulou C, Meyer T. The molecular fingerprint of high grade serous ovarian cancer reflects its fallopian tube origin. Int J Mol Sci 2013;14:6571–96.

[169] Auersperg N. The stem-cell profile of ovarian surface epithelium is reproduced in the oviductal fimbriae, with increased stem-cell marker density in distal parts of the fimbriae. Int J Gynecol Pathol 2013;32:444–53.

[170] Chene G, Ouellet V, Rahimi K, Barres V, Meunier L, DeLadurantaye M, Provencher D, Mes-Masson AM. Expression of stem cell markers in preinvasive tubal lesions of ovarian carcinoma. Biomed Res Int 2015;2015:808531.

[171] Tiourin E, Velasco VS, Rosales MA, Sullivan PS, Janzen DM, Memarzadeh S. Tubal ligation induces quiescence in the epithelia of the fallopian tube fimbria. Reprod Sci 2015;22: 1262–71.

[172] Snegovskikh V, Mutlu L, Massasa E, Taylor HS. Identification of putative fallopian tube stem cells. Reprod Sci 2014;21:1460–4.

[173] Paik DY, Janzen DM, Schafenacker AM, Velasco VS, Shung MS, Cheng D, Huang J, Witte ON, Memarzadeh S. Stem-like epithelial cells are concentrated in the distal end of the fallopian tube: a site for injury and serous cancer initiation. Stem Cells 2012;30:2487–97.

[174] Indumathi S, Harikrishnan R, Rajkumar JS, Sudarsanam D, Dhanasekaran M. Prospective biomarkers of stem cells of human endometrium and fallopian tube compared with bone marrow. Cell Tissue Res 2013;352:537–49.