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Ovarian cancer: emerging concept on cancer stem cells
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Abstract
Emerging evidence suggests that the capacity of a tumor to grow and propagate is dependent on a small subset of cells within a tumor, termed cancer stem cells. In fact, cancer cells, like stem cells, can proliferate indefinitely through a dysregulated cellular self-renewal capacity. Cancer stem cells may originate due to the distribution into self-renewal and differentiation pathways occurring in multi-potential stem cells, tissue-specific stem cells, progenitor cells and cancer cells. Recent studies have shown that ovarian cancer also contains stem cells or tumor-initiating cells. Moreover, ovarian serous adenocarcinomas were disaggregated and subjected to growth conditions to select for self-renewing, non-adherent spheroids previously shown to be derived from tissue stem cells. A recent study showed that epithelial ovarian cancer was derived from a sub population of CD44+, CD117+ and CD133+ cells. The existence of cancer stem cells would explain why only a small minority of cancer cells is capable of extensive proliferation of the tumor. In this review, we have discussed the studies on ovarian cancer stem cells along with the molecular pathways that could be involved in these cancer stem cells.

Introduction
Ovarian cancer is the fifth leading cause of cancer deaths and has the highest mortality rate among gynecologic cancers. It is the most lethal malignancy of the female reproductive system, at the initial stage the five-year survival rate is nearly 45%, which declines to 30% for patients with an advanced disease [1,2]. Greater than 90% of ovarian cancers arise from the surface epithelium [3], and tumorigenesis has been associated with ovulation-associated wound repair and/or inflammation, possibly leading to abnormal stem cell expansion [3,4]. Over the last several years, it has been increasingly evident that a small population (less than 5%) of cancer cells, referred to as "cancer stem cells (CSCs)", is responsible for the aggressiveness of the disease, metastasis and resistance to therapy [5-7]. Cancer stem cells, like somatic stem cells, are thought to be capable of self-renewal or unlimited proliferation [7]. The recent discovery that CSCs express certain 'stem cell-specific' markers has renewed interest and provided a rise in the idea that CSCs may arise from somatic stem/progenitor cells. Considerable research efforts have been directed toward the identification of cancer stem cell markers in ovarian cancer.

Stem cells, as classically defined, are cells with a capacity for self-renewal and generation of daughter cells that can differentiate into all the way down different cell lineages found in the mature tissue [8]. Stem cells always undergo asymmetric cell divisions, with each cell generating two cells; one that is identical to itself in stemness and another which is committed to a certain lineage. The daughter cell with stem cell like properties maintains its own compart-
normal and malignant stem/progenitor cells in tissue. More recent studies have shown the functions of typical heterogeneity of the parent tumor [28]. Further- tumorigenic cancer cells, thus recreating the full phenotypes; and (iii) Tumors grown from tumorigenic cellscontain phenotypically mixed populations of tumorigenic and non-tumorigenic cancer cells, thus recreating the full phenotypic heterogeneity of the parent tumor [28]. Furthermore, recent studies have shown the functions of normal and malignant stem/progenitor cells in tissue regeneration, cancer progression and targeting therapies [29,30]. In this review we aim to provide insight into the evaluation of the evidence that supports the existence of cancer stem cells and the characterization studies that have tried to identify ovarian cancer stem cells. We also discuss how taking this subpopulation of cells into account may affect the way we treat ovarian cancers in the future.

Cancer stem cells
The identification of a reservoir of stem cells within many adult tissues raises the interesting possibility that all adult tissues have stem cells. Stem cell populations within normal tissues are defined by certain common characteristics: self-renewal to maintain the stem cell pool over time; regulation of stem cell number through a strict balance between cell proliferation, cell differentiation and cell death; and the ability to give rise to a broad range of differentiated cells [31,32]. It is observed that like stem cells, cancer cells are widely thought to be able to proliferate indefinitely through a deregulated self-renewal capacity. In fact, cancer stem cells can thus only be defined experimentally by their ability to generate continuously growing tumors. CSCs have the capacity to self-renew, undergoing divisions that allow the generation of more CSCs and ultimately some of them differentiate into the various cell types that compose the tumor mass. To date, the practical translation of this definition, and the gold standard to define the 'stemness' of cancer cells, has been their ability to generate a phenocopy of the original malignancy in immuno-compromised mice [7].

Evidence for the existence of cancer stem cells
To assay the cancer stem cells, a xenograft model for breast cancer was developed that allowed specific cancer tumors isolated directly from a patient to be passaged reliably in vivo. In this model, only a subset of cancer cells had the ability to form new tumors [5]. The cancer stem cells isolated from tumors are mostly isolated by flow cytometry as the CD44+ CD24- lineage cell population [5]. Furthermore, dilution assays demonstrated that as few as 100 tumorigenic cancer cells were able to form tumors, while tens of thousands of the other (non-CSCs) populations of cancer cells failed to form tumors in nude mice. These tumorigenic cells have been serially generated in new tumors containing additional CD44+ CD24-low lineage tumor cells as well as the phenotypically mixed population of non-tumorigenic cancer cells [5,7]. In addition, when cultured cells were isolated based on the expression of CD133, a marker expressed by normal CNS stem cells [33], only the CD133+ fraction of cells was capable of forming spheres. These studies suggest that CNS tumors of neural origin contain a stem cell population. Li et al. reported that a highly tumorigenic subpopulation of pancreatic cancer cells expresses the cell surface markers CD44, CD24 and epithelial-specific antigen (ESA) [18]. Table 1 summarizes the studies which have
described the direct isolation of populations containing cancer stem cells in various malignancies. Another phenotype used to distinguish these cells is their presence within the Side Population fraction as determined by their ability to exclude the Hoechst dye [34].

**Figure 1**

*Origin of cancer stem cells. Self-renewal and differentiation potentials are the features of stem cells.* Progenitor cells, the product of stem cells that lose the activity of self-renewal, could differentiate into mature cells, which have the feature of differentiation. The hypothesis is that cancer stem cells are caused by transforming mutations occurring in multi-potential stem cells, tissue-specific stem cells, progenitor cells, mature cells, and cancer cells.

**Therapeutic targets for cancer stem cells**

The field of stem cell research has given new hope for the treatment and even a cure for incurable diseases in human. Particularly, the identification of a rare population of adult stem cells in most tissues/organs in humans...
has emerged as an attractive source of multiple stem/progenitor cells for cell replacement-based therapies and tissue engineering in regenerative medicine. Our recent review discussed that cancer stem/progenitor cell research also offers the possibility of targeting these undifferentiated and malignant cells that provide critical function in cancer initiation and relapse for treating patients diagnosed with advanced and metastatic cancer [30,35,36]. Various strategies consisting of molecular targeting of distinct oncogenic signaling elements activated in the cancer progenitor cells and their local microenvironment during cancer progression can be explored [37]. Furthermore, overcoming the intrinsic and acquired resistance of cancer stem/progenitor cells to current clinical treatments represents a major challenge in treating and curing the most aggressive and metastatic cancers [38]. In addition, hematopoietic stem cells are the most characterized stem cells and it has been used for the therapy to cure cancer [11]. In this review we also described that the molecular mechanisms involved in the intrinsic and acquired resistance of cancer cells to current cancer therapies [38].

Pathways of self-renewal and carcinogenesis
Since the cancer stem cells share common properties with normal stem cells, it is reasonable to think that they have overlapping regulatory mechanisms. Indeed, one of the most outstanding questions concerning the biology of stem cells is: how do multi-potent stem cells select a particular differentiation pathway and start to differentiate? Another question is how do stem cells decide to maintain particular differentiation pathway and start to differentiate? Stem cells is: how do multi-potent stem cells select a particular differentiation pathway and start to differentiate? Among these, the Sonic Hedgehog (Shh), Notch and Wnt signaling transduction pathways play a major role in the self-renewal of stem cells [39-41]. Recent advances in the understanding of the role of Wnt, Hedgehog, Shh, and Notch signaling pathways in regulating stem cell self-renewal have shed new light on carcinogenesis [Figure 2] [7,42,43]. The next obvious question is the possible connection between tumors and the (Hedgehog) Hh and Wnt pathways and how the activation of these pathways leads, in some cases, to such highly efficient tumorigenesis. Recent genetic evidence suggests that somatic stem cells are the producers of CSCs; that the Wnt and Hh pathways function in the normal regulation of stem-cell number in at least some tissues; and that expansion of the somatic stem-cell population may be the first step in the formation of at least some types of cancers [44-46]. Numerous arguments support a stem-cell origin for human cancer. Foremost is the observation that stem cells possess many of the features that characterize the malignant phenotype, including self-renewal and unlimited replicative potential [47]. Also, the mutations that initiate tumor formation seem to accumulate in cells that persist throughout life, as suggested by the exponential increase of cancer incidence with age. This is thought to reflect a requirement for four to seven mutations in a single cell to effect malignant transformation [47]. Although similar signaling pathways may regulate self-renewal in normal stem cells and cancer stem cells, there are mechanistic differences in some cancers. Interestingly, the mechanistic differences in self-renewal between normal stem cells and cancer stem cells can thus be targeted to deplete cancer stem cells without damaging normal stem cells.

Ovarian tumors
The ovaries contain three main types of cells germ cells, stromal cells and epithelial cells which give rise to germ cell, stromal and epithelial ovarian tumors, respectively. Epithelial ovarian cancers (EOC) were the most common type of ovarian cancers. Comprising nearly 90% of all ovarian cancers EOCs are derived from relatively pluripotent cells of the celomic epithelium or “modified mesothelium”. These cells originate from the primitive mesoderm and can undergo metaplasia. Approximately 10% to 20% of epithelial ovarian neoplasms are borderline or low malignant potential tumors and are characterized by a high degree of cellular proliferation in the absence of stromal invasion. Of the invasive epithelial ovarian cancers, about 55–60% are serous, 15% endome-
trioid, 5–10% clear cell and <5% mucinous [48] (Figure 3). The various histological subtypes of ovarian carcinoma have identifiable precursor lesions and multiple early genetic alterations. Figure 3 explains the various histological subtypes of ovarian cancer and their associated specific mutations. Mutations may be one of the major factors contributing to the origin of ovarian cancer stem cells. Many of the histological subtypes resemble the epithelial component of the lower genital tract, including papillary serous tumors that have an appearance resembling the glandular epithelium lining the fallopian tube. Mucinous tumors, on the other hand, contain cells resembling endocervical glands, and endometrioid tumors contain cells resembling the endometrium. Non-epithelial types of ovarian cancer include sex cord-stromal tumors (6% of ovarian cancers) and germ cell tumors (3% of all malignant ovarian neoplasms) [49-51]. The histological subtypes of ovarian carcinoma have identifiable precursor lesions and early genetic alterations. Figure 3 explains the histological subtypes and its specific mutations in ovarian carcinoma. Mutations are one of the major alteration factors for the origin of cancer ovarian stem cells.

Markers and their roles in ovarian tumors
In general, tumor markers can be used for one of four purposes: (i) screening a healthy population or a high risk population for the presence of cancer; (ii) making a diagnosis of cancer or of a specific type of cancer; (iii) deter-
mining the prognosis of a patient; and (iv) monitoring the course in a patient in remission or while receiving surgery, radiation, or chemotherapy.

Furthermore, recent studies have identified different prognostic and diagnostic surface markers for ovarian cancer [52] and these markers need to be analyzed for their role in ovarian cancer. One of the well-known tumor antigens is the epithelial cell mucin MUC1, a transmembrane glycoprotein that is differentially expressed on tumor cells compared with normal epithelial cells [53,54]. MUC1 is expressed either not at all or in small amounts on various normal epithelia but aberrantly or neoexpressed at high levels on the majority of adenocarcinomas. Tumor-associated alterations of MUC1 are characterized by hypoglycosylation, increased sialylation, and altered carbohydrate core-type expression [53]. Engelmann et al reported that MUC1 molecule is not only expressed on mature cancer cells, but also on tumor cells that have multiple characteristics of stem and progenitor cells [55]. This study demonstrates MUC1 expressed breast cancer cell line MCF7 as a source of a minor population of cells with characteristics of tumor stem/progenitor cells to show for the first time that these cells also express the hypoglycosylated (tumor) form of MUC1, previously described only on mature MCF7 cells and other tumors and tumor cell lines. Moreover, these cells give rise to MUC1+ tumors in vivo and that these tumors maintain a small population of MUC1+ cells with the stem/progenitor characteristics [55]. Our recent finding demonstrated the tumor-specific expression of Tumor Associated Glycoprotein-72 (TAG-72) in ovarian cancer and its association with disease stage may serve as a potential marker for effective disease management [56]. In addition, surface marker mucins are overexpressed in many epithelial malignancies including ovarian cancer, suggesting a possible role in the pathogenesis of these cancers. Other studies from our laboratory have provided experimental evidence that the MUC4 mucin interacts with HER2 potentiates its downstream signaling and enhances the motility of ovarian cancer cells. Our findings provide experimental support for the hypothesis that MUC4 mucin expression is associated with a higher metastatic potential and thereby a poor prognosis in ovarian cancer [57]. The future direction of these studies will be to explore the roles of MUC4 and TAG-72 in ovarian cancer stem/progenitor cells.

**Ovarian cancer stem cells**

A recent study describes that ovarian cancer cell lines were shown to possess "side population" (SP) cells that have been described as cancer stem cells due to their stem-like characteristics including the ability to differentiate into tumors with different histologies. These putative cancer stem cells reflect the various histological subtypes observed in ovarian carcinoma. They also provide a model of cancer metastasis in which these cells are able to
colony, expand, and differentiate into heterogeneous
tumor phenotypes similar to primary tumors. In such a
model, both the primary tumors and metastasis would
display similar genetic and expression profiles because
both populations are supposedly derived from the same
lineage of cancer stem cells [58]. Ovarian cancer stem
cells, like somatic stem cells, are shown to be capable of
unlimited self-renewal and proliferation. In general,
multipotent cancer stem cells may account for the histo-
logical heterogeneity often found in tumors [25,27,59].
Moreover, ovarian somatic stem cells would be expected
to divide asymmetrically, yielding both a daughter cell
that proceeds to terminal differentiation, and an undiffer-
entiated copy capable of self-renewal. Repeated asymmet-
ric self-renewal of somatic stem cells or their immediate
progenitor’s stem cells lead to the accrual of mutations over time, which might ultimately lead to their
transformation into cancer stem cells and malignant pro-
gression.

Furthermore, another study describes that two mouse
ovarian cancer cell lines such as MOVCAR7 and 4306 con-
tain candidate cancer stem cells [25]. These two murine
ovarian cancer cells have large SP, making them suitable
to study ovarian cancer stem cell biology. A similar, albeit
very small, SP was also identified in the human ovarian
cancer stem cell lines ICROV-1, SKOV-3 and OVCAR-3
and also in cells claimed from patient ascetic fluid [25].
Further, a study proved that isolated and characterized
ovarian cancer-initiating cells (OCICs) are fully capable of
reestablishing their original tumor hierarchy in vivo. These
cells are very organized self-renewing, anchorage-inde-
dependent spheres and were reproducibly dividable using
antibodies against both CD44 and CD117 [27]. These
OCICs were capable of intraperitoneal tumorigenesis and
could serially propagate tumors in animals. Conse-
quently, this study fulfills all currently accepted criteria for
the existence of a subpopulation of tumor-initiating cells
[27], and their specific detection and targeting could be
highly valuable for therapy of recurrent, chemo-resistant
disease. Whereas advanced ovarian cancer is generally ini-
tially responsive to standard chemotherapies (cisplatin and paclitaxel), that responsive almost inevitably fol-
lowed by drug resistant phenotype [2,60]. One accepted
hypothesis about chemoresistance is standard therapies
failed to target tumor progenitors, which are have like
normal stem cells because of expression of membrane
efflux transporters. Zhang et al showed that OCICs, under
stem cell selective conditions, over express ABCG2 and
are more resistant to cisplatin and paclitaxel, suggesting a
possible role for these cells in ovarian cancer chemoresis-
tance [27].

**Conclusion and perspective**

The aforementioned studies showed that a so-called ovari-
an cancer stem cell, with high proliferative capacity, self-
renewal properties and multi-lineage potential, could be
responsible for tumor development and the differentia-
tion of more mature epithelial ovarian cells contributing
to tumorigenesis. There are important consequences for
cancer treatment if the growth of tumors is at least in part,
dependent on a cancer stem cell population. The cancer
stem cell hypothesis posits that cancer stem cells are a
minor population of self-renewing cancer cells that fuel
tumor growth and remain in patients after conventional
therapy has been completed. The hypothesis predicts that
effective tumor eradication will require obtaining agents
that can target cancer stem cells while sparing normal
stem cells. Experimental evidence suggests that ovarian
cancer stem cells are relatively resistant to conventional
chemotherapeutic agents. Current cancer therapies often
engender severe toxicity because of their general effects on
all rapidly dividing cells. Identification of candidate tar-
gets for more specific mechanism-based cancer therapy
using techniques such as gene chips could reveal signature
patterns of transcriptional output which are characteristic
of activated self-renewal pathways.

Emerging evidence suggests that these pathways also con-
tral patterning and growth in self-renewing adult tissues
by regulating the stem-cell compartment. Thus, pharma-
cological inhibition of these pathways in the worst case
might result in severe toxicity due to a loss of normal
stem-cell compartments. Further research will be needed
to determine whether continuous pathway activity is
required in normal and tumor tissues, and whether these
requirements differ sufficiently as to allow therapeutic
intervention. Even if pathway inhibition is prohibited by
normal physiological requirements, other mechanism-
based approaches that exploit aberrant pathway activa-
tion might be feasible. It has been proposed that mali-
gnancy is determined in all tissues by mis-regulation of a
common set of genes that control growth by affecting cell
proliferation, apoptosis, invasion and angiogenesis. This
hypothesis is supported by the demonstration that multi-
ple types of normal human cells can be made tumorigenic
by the expression of a defined set of viral and cellular pro-
teins. Therapeutic agents for the treatment of such tumors
might target not only self-renewal pathway components,
but also other critical transcriptional targets of the self-
renewal pathways, or proteins that co-operate with them
to deregulate growth.

It is important that agents directed against cancer stem
cells discriminate between cancer stem cells and normal
stem cells. This will require the identification of realistic
drug targets unique to cancer stem cells. The identification
of such targets and the development of anti-cancer agents
will require a deeper understanding of normal stem cell biology as well as cancer biology. More importantly, identification of the ovarian cancer stem cell would provide a critical step in advancing the development of novel therapeutic strategies in the management of ovarian cancer. Furthermore, characterizations of such progenitor or cancer stem cells in drug resistant (Cispaltin, Paclitaxel and etc) manner for ovarian cancer will likely lead to a greater understanding of early events leading to the genesis of this elusive disease, in addition to providing new therapeutics targets aimed at the cells directly responsible for its propagation.

Abbreviations
CSC: Cancer Stem Cell; CNS: Central Nervous System; ESA: Epithelial-Specific Antigen; Shh: Sonic Hedgehog; Hh: Hedgehog; EOC: Epithelial ovarian Cancer; OCIC: Ovarian Cancer Initiating Cells; SP: Side Population

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
PPM participated in drafting the full manuscript and creating figures. SKB participated in substantial contribution to conception and revising it critically for important intellectual content.

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