Emerging evidence for the stem-cell and diverse tissue origins of epithelial ovarian cancer: could this be a step towards new treatment approaches?

Introduction

Ovarian cancer is the fifth leading cause of death among cancer-related mortalities worldwide and the most lethal among all gynaecological conditions in women[1]. Although ovarian cancer can arise from germ and somatic cells in the reproductive tract, the most common cell type it is associated with, in 90% of all cases, is the epithelial layer of cells lining the ovary[2]. The multifactorial basis of epithelial ovarian cancer (EOC) initiation and progression poses a major clinical challenge in screening and diagnosis of the disease with most number of patient diagnoses in the clinically-advanced stages. The current therapeutics such as hormonal treatment, cytoreductive surgery and chemotherapy have mostly been ineffective with high rates of cancer relapses[2]. Therefore, a greater insight into the molecular landscape of ovarian cancer and the different cell types involved in the genesis of this malignancy can give us a better perspective for its early diagnosis and effective management. This essay will explore the existing evidence for a stem cell origin of EOC in animal and human models as well as some recent findings on other possible non-ovarian origins of carcinogenesis.

The Concept of Cancer Stem Cells

EOC is a complex condition which can be subdivided into eight distinct subtypes depending on the histological appearance of the cancerous tissue (Fig.1)[3]. Despite the pressing necessity for improvement in the diagnosis and treatment of EOC, its etiology still remains poorly understood. The conventional hypothesis for EOC origin involves the ovarian surface epithelium (OSE) - a single mesothelial layer of cells surrounding the ovary[4]. Interestingly, during tumorigenesis the OSE is thought to acquire complex epithelial phenotypes resembling Müllerian duct derivatives – oviducts, endometrium and cervix which are not normally present in the ovary[4]. The tumours usually consist of a small population of relatively undifferentiated cells and a larger population of fully differentiated cells along with inflammatory and endothelial cells[5]. This heterogeneity is thought to arise

from the ability of tumour progenitor cells to divide asymmetrically giving rise to exact copies of themselves but also to a more differentiated progeny. Recently, the term cancer stem cell (CSC) has been introduced into the field of oncology to describe the small population of undifferentiated cells which remains persistent in various tumours. These cells possess stem cell-like phenotype and have the ability to initiate and sustain tumour growth[6]. CSCs are thought to possess five defining characteristics: capability of long-term self-renewal, ability to give rise to differentiated offspring, constituting a small fraction of the tumour mass, ability to reproduce the tumour and expression of specific cell surface markers[7].

Type of EOC	Resemblance	Incidence
Serous	Fallopian Tube	70%
Endometrioid	Endometrium	10-15%
Mucinous	Endocervix	3%
Clear Cell	Urogenital Tract	10%
Squamous Cell	Squamous Epithelium	Rare
Transitional (Brenner)	Gastrointestinal Mucosa	Rare
Undifferentiated	Undifferentiated Epithelium	Rare
Mixed epithelial	Mixed Epithelium	Rare

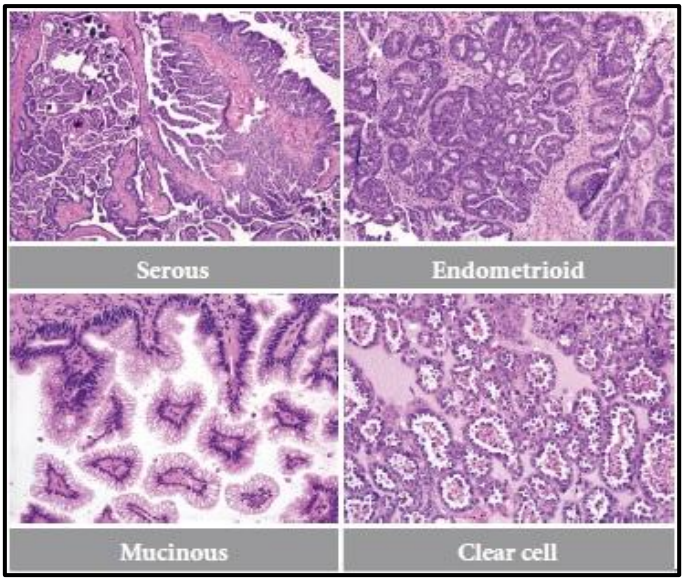

Figure 1 The table above lists the eight distinct subtypes of EOC, the epithelial structures they resemble and their incidence[3]. On the right, there are histological preparations of the four most common subtypes of EOC – serous, endometrioid, mucinous and clear cell cancers depicting their distinct tissue morphology (adapted from Karst and Drapkin, 2010)[8].

Evidence for stem cell origin of EOC

- ### Animal models

Over the last decade animal models such as mice, rats and rabbits have been instrumental in identifying CSC and in testing their tumorigenicity[4]. In a recent study transgenic mice were utilised to show that there is a slow and asymmetrically dividing population of cells within the normal OSE which represented the first candidate population

of stem/progenitor cells found in the mouse ovary[9]. Stem cell-specific markers' gene expression has also been identified in normal OSE through gene expression profiling that further support the evidence for the stem-cell nature of OSE[10]. These studies, however, do not provide evidence for the link between the stem cell nature of the OSE and its malignant potential.

Recently, Szotek *et. al* identified a small side population (SP) of stem cells in genetically engineered mice, capable of excluding Hoechst 33342 (a DNA-binding dye) and expressing surface markers such as BCRP1 (a membrane drug transporter) which has previously been linked to drug resistance in other cancers[5]. Moreover, SP were observed to be more tumorigenic both *in vitro* and *in vivo* compared to non-SP[5]. Interestingly such populations of cells have also been identified in human lines and patients' ascites (tumour formations present in late-stage EOC). This research helped develop a model for chemoresistance - a major problem in more than 70% of cases with EOC - whereby an SP of stem cell-like cells could evade treatment due to cell surface efflux pumps, slow proliferation and various mechanisms for DNA repair[11]. This model is also consistent with previous hypotheses that chemoresistance is due to failure of eradication of such stem cell-like cells. This research group also identified Müllerian Inhibiting Substance (MIS) as a potential therapeutic due to its inhibiting properties on SP cells' proliferation *in vitro*[5]. However, this study alone cannot conclude whether SP consists entirely of CSC and if MIS testing would produce similar results *in vivo*.

- **<u>Human Lines</u>**

One of the earliest evidence for the stem cell nature of EOC in humans comes from studies of the physiological and molecular composition of patients' ascites. Isolated cells from these have shown a stem-cell phenotype due to the presence of stem cell-specific surface markers, self-renewal abilities and multipotency. Furthermore, when introduced into immunosuppressed mice these cells were able to induce the formation of tumours phenotypically similar to human ovarian cancers[12]. Also, their survival was followed through three generations which reflects their *in vivo* capacity for self-renewal[12]. However, as ascites are only present in late stages of tumorigenesis including metastasis, this piece of evidence cannot provide a good indication of the events during early tumour formation. In a different

study, Zhang *et al.* used primary ovarian cancer tissue and identified a population of cells which expressed stem cell markers such as CD44 and CD117. These cells were highly malignant and could induce tumour formation both *in vitro* and *in vivo* which resembled the phenotypic appearance of human ovarian tumours[11]. Both studies used a technique of inoculating identified populations of stem-like cells onto immunosuppressant mice. This approach, however, could give rise to misinterpretations as the potentially tumorigenic cells are essentially placed out of their natural microenvironment.

Evidence for Diverse Tissue Origins

Although most research so far has focused on OSE as a primary origin of EOC, recently new evidence has emerged suggesting that actually EOC might not arise in the ovary itself but elsewhere in the reproductive tract, hence the Müllerian appearance of the tumours (Fig.2).

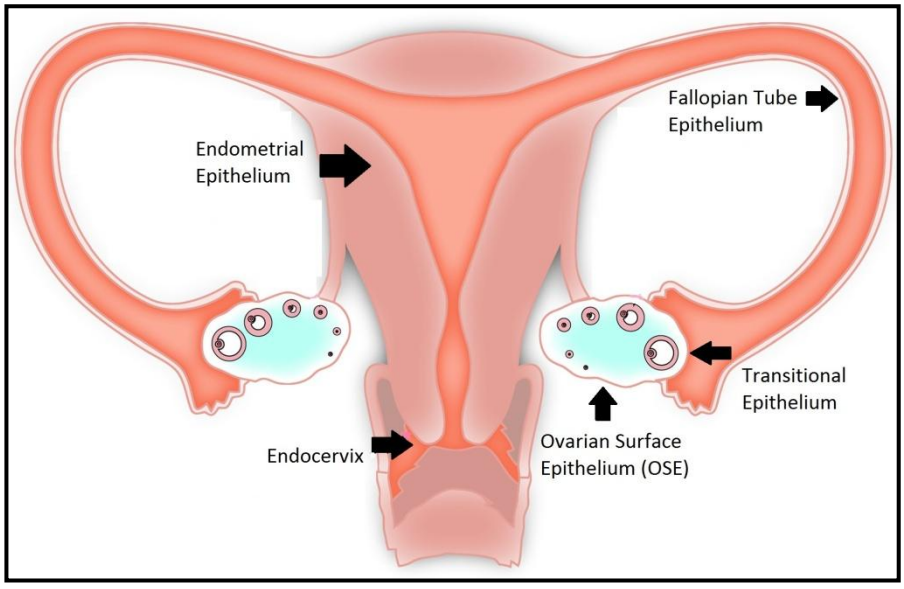

Figure2 A figure summarising possible origins of EOC in the female reproductive tract. The Fallopian tube, endometrium, cervix and OSE have all been derived from the embryonic coelomic epithelium which could explain why several types of epithelium can result in similar tumours (adapted from Lopez et. al, 2013)[13].

- **Fallopian tube origin**

In contrast to the well-established view that ovarian cancers arise in the ovary, primary lesions of EOC were detected in the Fallopian tubes of women with BRCA mutation which confers susceptibility to breast and ovarian cancer[14]. Serous ovarian cancers are most common amongst EOC (Fig.1) with high-grade serous cancer (HGSC) accounting for more than 90% of all cases[7]. It has been proposed that HGSC originates from the fimbriated distal end of the Fallopian tube. In a recent study, the Fallopian tube secretory epithelial cells

(FTSEC) were genetically immortalised and transformed with specific mutations known to be involved in HGSC resulting in the *in vitro* generation of the disease thereby suggesting an FTSEC origin[15]. Kim *et. al* showed the first direct evidence that HGSC can arise from the fallopian tube, spread to the ovary and metastasise to the abdominal cavity. They used the Cre-lox system to induce mutations in tumour-suppressor genes in cells derived from the Fallopian tube but not from the ovary and followed the progress of the disease[3]. Despite the direct evidence, it is still not clear if the same process occurs *in vivo* in humans as the study was performed on transgenic mice which have different physiology and reproductive tract anatomy.

- **Endometrium origin**

The endometrium has also been suggested as a possible tissue of origin of endometrioid and clear cell ovarian cancers (Fig.1). In women with endometriosis, normally a benign condition where the endometrial epithelium is found outside the uterus, an increased risk of developing endometrioid tumours has been reported due to malignant transformation of the endometrial epithelium which eventually metastasises to the Fallopian tube and the ovary[16]. In support to this hypothesis, tubal ligation reduced the risk of endometrioid and clear cell tumours as the endometrial cells could not spread through the reproductive tract[17].

- **Transitional Epithelium origin**

Transitional epithelium – a tissue layer lying on the boundary between the Fallopian tube and the ovary – has recently been associated with mucinous and Brenner tumours (Fig.1)[18]. Using fate-tracing experiments, Nikitin *et. al* have identified a population of stem cells located at the ovarian hilum in mice (the area where blood vessels and nerves enter the ovary) that expresses stem cell markers and give rise to more differentiated offspring such as OSE. Moreover, when tumour-suppressor genes are mutated in these stem cells, they initiate tumours which genetically and phenotypically resemble human ovarian cancers[19]. It is yet to be tested if such regions exist in human ovaries, but this hypothesis is appealing as these regions can represent niches for cancer-prone stem cells[19].

Conclusion

The last decade has seen a tremendous improvement in our understanding of the etiology of EOC. In multiple studies of mouse models and human lines, a stem-cell nature for the origin of this cancer has been suggested. Creating an integrated genetic and molecular profile for ovarian cancer stem cells will allow defining and isolating such populations of cells, previously linked to chemoresistance thereby potentially leading to new therapeutic strategies. Furthermore, a paradigm shift followed after several recent studies indicated that the site of ovarian cancer origin can be distant from the ovary itself and that the different subtypes of EOC might actually have different origins. This discovery underscores the importance of personalised medicine where each specific EOC subtype is treated differently according to its tissue origins. In conclusion, further research in the direction of eliciting the physiological, etiological and molecular basis of EOC can help mitigate mortality and morbidity in cancer patients and aid in more effective management of this malignancy.

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