

Redefining the origin and evolution of ovarian cancer: a hormonal connection

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Abstract

Ovarian cancer has the highest mortality of all female reproductive cancers. Late diagnosis, tumour heterogeneity and the development of chemoresistance contribute to this statistic and work against patient survival. Current studies have revealed novel concepts that impact our view on how ovarian cancer develops. The greatest impact is on our understanding that, as a disease, ovarian cancer has multiple cellular origins and that these malignant precursors are mostly derived from outside of the ovaries. In this review, we propose a new concept of a step-wise developmental process that may underwrite ovarian tumorigenesis and progression: (1) migration/recruitment to the ovaries; (2) seeding and establishment in the ovaries; (3) induction of a dormant cancer stage; and (4) expansion and tumor progression. We will discuss the relationship of each step with the changing ovarian function and milieu during the reproductive age and the subsequent occurrence of menopause. The realization that ovarian cancer development and progression occurs in distinct steps is critical for the search of adequate markers for early detection that will offer personalized strategies for prevention and therapy.

Key Words

- ▶ ovarian cancer
- ▶ ovulation
- ▶ menopause
- ▶ gonadotrophin hypothesis

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Introduction

Ovarian cancer is the second most common gynaecologic malignancy and the most common cause of gynaecologic cancer death in the United States. The American Cancer Society estimates that, in 2014, 21,980 cases of ovarian cancer were diagnosed and 14,270 women died from the disease in the United States alone (Siegel *et al.* 2014). As in many cancers, early detection is critical for survival; if detected during stage I, or even during stage II, patient survival rate is high. However, the majority of ovarian cancers are detected at stages III and IV, with a poor 10-year survival rate of 10–20% (Auersperg *et al.* 2001). Early detection of ovarian cancer is difficult partly due to its nonspecific symptoms. Furthermore, the lack of specific

and sensitive early detection markers makes a screening test for the general population practically impossible. A considerable amount of research has gone into finding markers for early diagnosis of ovarian cancer, but all have failed in clinical trials (Barbolina *et al.* 2010, Bai *et al.* 2013). This failure and the poor outcome observed with current standard of care prompt the question of whether we really understand the aetiology and pathobiology of this disease.

Despite numerous studies, the original lesion that gives rise to ovarian cancer has thus far not been identified. The long-standing dogma is that ovarian cancer originates from the surface epithelium layer of the ovary.

The epithelial cells are thought to involute inside the ovary and form cysts. Subsequently, due to accumulation of genetic mutations, the cells turn cancerous and a tumour is formed (Cancer Genome Atlas Research Network 2011, Barker 2014). However, this theory cannot explain the histopathological heterogeneity of the disease. Recent studies including work from our laboratory have shown that ovarian cancer is a complex disease that develops from multiple sites, which are, interestingly, mostly outside of the ovaries (Kurman & Shih Ie 2010, Nowak-Markwitz & Spaczynski 2012, Nezhat et al. 2014, 2015).

In this review, we will discuss the current understanding of ovarian cancer tumorigenesis based on recent studies that have ascribed various cells of origin to each histopathological subtype of ovarian cancer and how these cells, mostly from extra-ovarian origin, can access the ovaries and establish ovarian tumours. More importantly, we propose the concept of a step-wise developmental process that may underwrite ovarian tumorigenesis and progression. The process involves four stages: (1) migration/recruitment to the ovaries; (2) seeding and establishment in the ovaries; (3) induction of a dormant cancer stage; and (4) expansion and tumor progression. Stages 1 to 3 occur during the reproductive age, whereas stage 4 occurs during the peri- and postmenopausal age associated with ovarian atrophy (Fig. 1).

Ovarian cancer histopathological subtypes and different cells of origin

Until recent years, the prevalent theory is that ovarian cancer originates from the single layer of epithelial cells that comprise the ovarian surface epithelium (OSE). It is thought that the process of cyclic ovulation, which

leads to the repeated rupture and subsequent repair of the OSE, contributes to the accumulation of somatic mutations, such as mutations to *TP53*, and eventually to malignant transformation of OSE cells (Fathalla 1971). The process involves the formation within the ovarian cortex of cortical inclusion cysts made up of OSE cells. These cells, which are at this point located at an ectopic site, are thought to be exposed to the ovarian stromal microenvironment which, in conjunction with the cyclic occurrence of ovulation-related inflammation, promotes its malignant transformation (Piek et al. 2001, Burdette et al. 2006). Indeed, p53 overexpression with or without *TP53* mutations in morphologically normal OSE cells were documented next to dysplasias and *in situ* carcinomas (Pothuri et al. 2010). The metaplastic transformation of OSE cells to Mullerian duct-derived epithelia has been ascribed to lead to the different subtypes of ovarian carcinomas (Scully 1995, Auersperg 2013).

However, the absence of such precursor lesions in patients diagnosed at an early stage suggests that another possible cellular origin could be derived from outside of the ovary itself. Such evidence for this is the observation of pre-neoplastic 'p53 signature'-containing lesions termed 'serous tubal intraepithelial carcinomas' (STICs) in the fimbria of fallopian tubes obtained from patients with *BRCA1* and *BRCA2* mutations, whose ovary histology was normal (Piek et al. 2001, Kurman & Shih Ie 2010). The p53 signature is localized predominantly in the fimbriated end of the fallopian tubes, which is a preferred site for *BRCA+* and sporadic tubal carcinomas. It should be noted, however, that it is equally common in non-neoplastic tubes from women with and without *BRCA* mutations, suggesting that the p53 signature occurs independently of *BRCA* status (Lee et al. 2007).

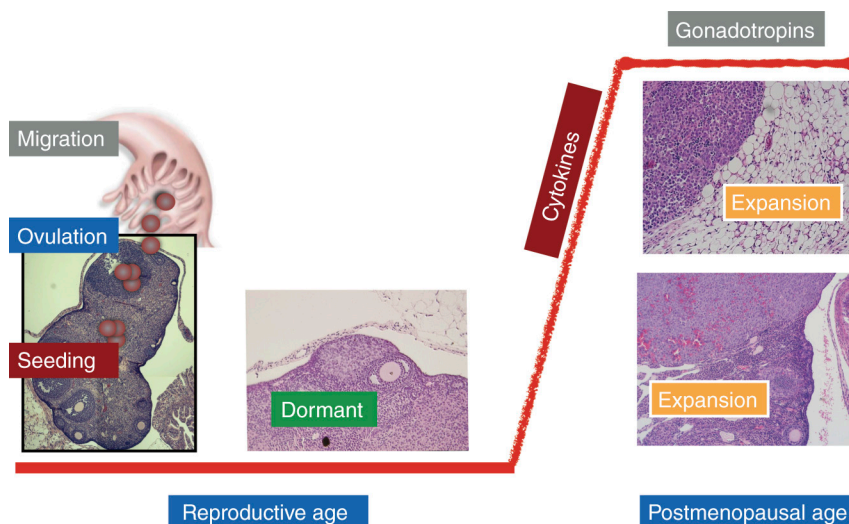


Figure 1

Proposed stages of ovarian tumorigenesis and progression. We propose a step-wise developmental process that may underwrite ovarian tumorigenesis and progression: (1) migration/recruitment to the ovaries; (2) seeding and establishment in the ovaries; (3) induction of a dormant cancer stage and (4) expansion and tumour progression. Histological sections illustrate: (1–2) normal ovarian tissue; (3) HGSC in ovarian stroma; (4) HGSC invading the omentum and ovarian stroma.

Regardless of being anatomically separated, the ovary and fimbria are, in fact, contiguous at a narrow isthmus and their epithelia share common epithelial and mesenchymal markers as well as relevant receptors for cell proliferation such as FSH and LH (Hess *et al.* 1999, Auersperg *et al.* 2001, Wright *et al.* 2011, Flesken-Nikitin *et al.* 2013, Ng *et al.* 2014). Although part of the fimbrial epithelium, STICs could be composed of slow-growing and noninvasive cells. However, they can be induced to rapidly proliferate and acquire invasive potential once they are embedded within the ovarian microenvironment (Flesken-Nikitin *et al.* 2013). Currently, the OSE cells and epithelial cells lining the fimbriated end of the fallopian tubes are thought to be the cellular origin of either high-grade or low-grade serous ovarian cancer (Li *et al.* 2011, Karst *et al.* 2011, Flesken-Nikitin *et al.* 2013, Perets *et al.* 2013, Ng *et al.* 2014).

In addition to possibly providing the cellular origin, the fallopian tubes can also provide the gateway for other cells from within the peritoneal cavity to access the ovaries. This is thought to be similar to the way retrograde menstruation can promote endometriosis (Nap *et al.* 2004). Such cells can include those from the gastrointestinal tract or cervix that are thought to give rise to mucinous ovarian tumours (Lee & Nucci 2003, Khunamornpong *et al.* 2006). The presentation of tumours with mucinous histopathology in an organ that do not contain any mucin-secreting goblet cells highlights the possibility of an extra-ovarian origin of some ovarian cancers. This is exemplified especially in ovarian mucinous adenocarcinoma, which has two distinctive types: intestinal and endocervical. Recent studies demonstrate that the mRNA expression profile of ovarian mucinous adenocarcinoma is more similar to colonic epithelium and mucinous colorectal carcinoma than to other types of ovarian carcinoma (Heinzelmann-Schwarz *et al.* 2006). Similarly, the endocervical type of ovarian mucinous carcinoma has been linked with endometriosis (Moriya *et al.* 2003). In addition, cells from the endometrium are thought to be the origin of clear cell and endometrioid ovarian cancers, as studies showed that these ovarian cancer subtypes share the morphology and genetic profile of normal endometrial cells (Wang *et al.* 2015). Taken together, these studies provide evidence of an extra-ovarian origin of specific ovarian cancers. Furthermore, the role of the fallopian tube as the access point for these cells to reach the ovaries is supported by epidemiologic studies that demonstrate that tubal ligation can reduce the risk of ovarian clear cell and endometrioid carcinoma (Gaitskell *et al.* 2016).

Overall, these studies provide evidence that ovarian cancer can initiate from cells originating from various sites outside of the ovaries. Whether they originate from normal epithelial stem cells, mesenchymal stem cells, or already transformed cancer stem cells remains to be elucidated. It is quite possible though that ovarian cancers can originate from any of these cell types, which is definitely reflected in the ensuing histopathological subtype of the cancer that is formed. Nevertheless, such normal stem cells or cancer stem cells are phenotypically different, which explains the diverse phenotype of ovarian cancer stem cells that has been described thus far (Ng & Barker 2015) and also highlights the complexity in finding unique markers for early detection of ovarian cancer.

This complexity in terms of cellular origin may explain the failure in developing early detection markers based on the concept of a single disease and a single marker able to identify all the different types of ovarian cancer (Visintin *et al.* 2008). Consequently, there is a need to redefine the different stages in the evolution of the disease, which will also help in the design of appropriate combination of markers that will detect not only what we define as ovarian cancer, but also a wide range of peritoneal and gynaecologic cancers (Mor *et al.* 2005).

We next discuss how malignant cells access the ovaries and the complex interplay between them and the ovarian microenvironment during the process of ovarian tumorigenesis and progression. As mentioned above, we propose four stages and these are discussed below.

Stages of ovarian tumorigenesis and progression

Recruitment/migration of cells from extra-ovarian origin to the ovaries: role of ovulation

How do these malignant precursors originating from outside of the ovaries access the organ and establish ovarian cancer? It can be inferred that the process is initiated by the migration and homing of these cells to the ovaries. It is known that, during ovulation, the ovarian follicles rupture to release the oocyte. The rupture and repair process, which repeatedly occur every menstrual cycle, creates a local inflammatory microenvironment at the site of the ovulatory wound, in which cytokines and growth factors are produced (Richards *et al.* 2002), thus making the ovary a plausible source of chemotactic factors that can recruit extra-ovarian premalignant and/or malignant cells.

Using a nude mouse model, we delivered mCherry-positive (mCherry+) ovarian tumor-initiating cells (mCherry-OTICs) (Alvero *et al.* 2009) through intrauterine (i.u.) or intra-peritoneal (i.p.) injection (Yang-Hartwich *et al.* 2014) that resulted in the formation of extensive carcinomatosis, mostly in areas rich in fat, such as the omentum, diaphragm and peritoneum. More importantly, 38% of the mice that were injected with mCherry-OTICs developed tumours in the ovaries. To determine whether the process of ovulation may explain the presence of ovarian tumours, mice were superovulated before the i.p. injection of mCherry-OTICs. All superovulated mice (100%) were found to bear ovarian tumours compared with 38% in mice that were not superovulated. These findings demonstrate that ovulation can enhance the homing and establishment of malignant cells in the ovaries (Yang-Hartwich *et al.* 2014).

Since ovulation is characterized by the production of cytokines and chemokines, which play a role in follicle rupture and remodelling, immune cell infiltration and angiogenesis (Machelon & Emilie 1997), it is conceivable that these factors may also act as chemoattractants to malignant cells.

Indeed, we demonstrated the role of the SDF-1/CXCR4 pathway in the recruitment of ovarian cancer stem cells to the ovaries (Yang-Hartwich *et al.* 2014) (Fig. 2). SDF-1, one of the chemokines upregulated in the ovaries during ovulation, is known to regulate a variety of physiological processes including chemotaxis of spermatozoa, trafficking of specific stem/progenitor cells to other tissue/organs and the migration of embryonic germ cells to the gonads

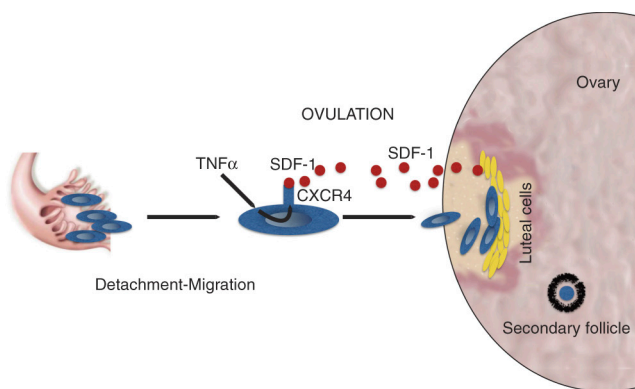


Figure 2

Recruitment and migration of extra-ovarian malignant precursors to the ovaries. During the process of ovulation, the high levels of TNF α and SDF-1 cooperate to recruit extra-ovarian malignant precursors to the ovaries. TNF α is able to induce the expression of CXCR4 in ovarian cancer cells. SDF-1, the ligand for CXCR4, plays a role in the recruitment and attraction of these CXCR4-positive cells.

(Zuccarello *et al.* 2011, Ratajczak *et al.* 2012, Greenbaum *et al.* 2013). The receptor for SDF-1, CXCR4, is expressed in ovarian tumours and ovarian cancer cell lines and has been associated with ovarian cancer progression (Barbolina *et al.* 2010, Popple *et al.* 2012). We showed that corpus-luteum-derived SDF-1 is able to attract ovarian cancer stem cells (Yang-Hartwich *et al.* 2014). Moreover, we showed that TNF α , which is another pro-inflammatory cytokine that is associated with ovulation (Hunt 1993), can enhance the levels of CXCR4 mRNA in the ovarian cancer cells (Fig. 2). As such, it was not surprising that in our mouse model, we found that ovarian cancer stem cells injected intravaginally are able to reach the ovaries and concentrate in close proximity to the corpus luteum (Yang-Hartwich *et al.* 2014). The ovulatory hormones may further enhance the homing process by increasing the secretion of chemokines and cytokines by ovarian cells. We will further discuss the role of hormones, specifically gonadotrophins, in the 'Expansion and tumour progression: role of the hormonal milieu during the peri- and post-menopausal age' section below.

Taken together, these findings support the epidemiological correlation between increased ovulation and ovarian cancer and may explain the findings that conditions that inhibit ovulation, such as multiple pregnancies, breastfeeding, late menarche, and the use of oral contraceptives, can reduce ovarian cancer risk (Purdie *et al.* 2003, Tung *et al.* 2005).

Seeding and establishment in the ovaries: role of the ovarian stroma

During ovulation, in addition to the secretion of chemokines/cytokines, another key event that occurs is the rupture of the OSE and exposure of the underlying ovarian stroma. We observed that ovarian cancer cells adhere better to stromal cells than to epithelial cells (Yang-Hartwich *et al.* 2014). As such, using ovarian organ cultures from superovulated mice, we demonstrated that ovarian cancer cells preferentially attach to areas of the ovaries where ovulation has occurred (i.e. corresponding to the location of ruptured OSE). Further studies revealed that a key difference between OSE cells and ovarian stromal cells is the specific type of proteins that compose their extra-cellular matrix. Stromal cells isolated from mouse ovaries express higher levels of collagen IV and tenascin C compared with epithelial cells from the same organ preparation. Quantification of known receptors for these proteins in the ovarian cancer cells showed high level of expression of integrins

$\alpha 1\beta 1$ and $\alpha 2\beta 1$ as well as integrin $\alpha V\beta 6$, the receptors for collagen IV and tenascin C, respectively (Fig. 3). Similar to other mucosal surfaces, the normal surface epithelium plays a critical role in protecting the ovaries from external factors, including microorganisms and malignant cells. Damage to the surface epithelium has been described as ‘opening the door’ for these external factors. As such, ovulation is a risk factor that damages the protective epithelium and exposes the environment of the ovarian stroma and corpus luteum (discussed below), both of which are important in the capture and maintenance of the seeded cells, respectively. Taken together, these findings highlight two ovulation-related processes that can impact ovarian tumour formation, particularly the subtypes that originate from outside the ovaries: (1) the release of chemotactic factors that can recruit malignant precursors and (2) the resulting breakage in the epithelial barrier as a result of oocyte release, which consequently exposes the underlying stroma that provides the optimal scaffold for the recruited malignant precursor cells.

Besides releasing chemokines/cytokines, the existing pro-inflammatory microenvironment within the post-ovulatory ovary can also provide factors that function as a ‘niche’ that promotes the survival of the seeded cells. The corpus luteum and its follicular fluid is a natural niche for the seeded cells that can support their survival. If these cells have an already overtly malignant phenotype, such microenvironment can regulate their phenotype, growth and differentiation. Indeed, cytokines present in the

post-ovulatory ovaries such as IL-6, IL-8, CCL2/MCP-1 and CCL5/RANTES have been demonstrated to modulate ovarian cancer (Freedman *et al.* 2004). These factors have been shown to induce *in vitro* growth of ovarian cancer cell lines, induce expression of angiogenic factors such as VEGF and inversely correlate with patient survival (Kolomeyevskaya *et al.* 2015, So *et al.* 2015, Eichten *et al.* 2016).

In addition to promoting survival, the repeated exposure to this pro-inflammatory microenvironment can also enhance the chance for replicative DNA errors in the seeded cells, which can lead to perpetuation of DNA damage and contribute to further malignant transformation (Fathalla 1971). This suggests that, if the seeded cells are not overtly malignant (i.e. mesenchymal stem cells from the endometrium), insults resulting from the recurring pro-inflammatory microenvironment, which occurs every ovulatory cycle, can aid in the eventual malignant transformation of the seeded cells that were initially benign.

Induction of a dormant cancer stage: role of the ovarian microenvironment during the reproductive age

If the seeding of malignant precursor cells to the ovaries occurs during the reproductive age, why is it that majority of women diagnosed with ovarian cancer are 50–60 years old, which is the peri- or post-menopausal period? This gap in time, which can be more than 30 years, makes it hard to reconcile the hypothesis that seeding of malignant precursors occurs during the time of ovulation (i.e. reproductive age) with the known time-frame of ovarian cancer clinical presentation. It is plausible that the seeded cells, whether they are already overtly malignant at the time of seeding or benign and induced to transform by the microenvironment, can be promoted to enter a dormant stage by the factors produced by the natural niche established within the ovarian stroma. The entry into dormancy and the subsequent reactivation are not only triggered by intrinsic cellular programmes, but also are dependent on specialized microenvironmental niches (Gao *et al.* 2012). The ‘ovarian niche’, which is rich in factors maintaining cellular dormancy (Ng & Barker 2015), will provide these malignant cells with the advantage for survival, immunological escape and maintenance of a stem cell phenotype (Fig. 3).

The concept of tumour dormancy or tumour latency has been used to explain how cancer patients without any clinically measurable disease at the conclusion of treatment develop recurrence years later. Recurrence

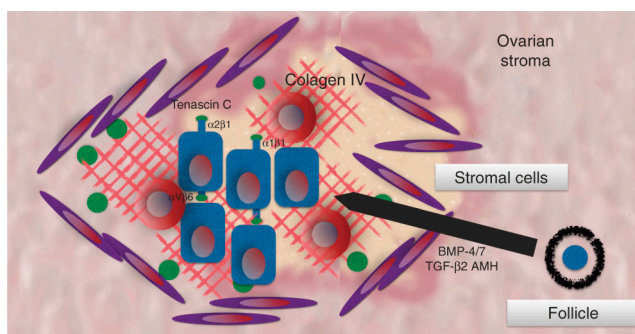


Figure 3

Seeding and induction of tumour dormancy by the ovarian microenvironment. Malignant precursors expressing a specific repertoire of integrins are able to bind collagen IV and Tenascin C, which are expressed by the ovarian stromal cells that are exposed underneath the ovulation site. TGF- $\beta 2$, BMP-4 and -7 and AMH are present in the ovaries during the reproductive age and primarily function to induce follicle growth. These factors, however, have been shown to have an opposite effect in cancer cells. They are able to stimulate tumour cell dormancy by inducing cell cycle arrest.

occurs mostly in metastatic sites years after the removal of the primary tumour and conclusion of treatment suggesting that: (1) seeding has occurred before the initiation of treatment and (2) that these disseminated cells were able to survive in the circulation, establish in the distant site, and enter a latent or dormant state for years (Lyu *et al.* 2013, Telleria 2013). Studies that looked into dormant metastatic cells in organs, such as the liver and bone marrow, have questioned how such organs with a strong proliferative and regenerative microenvironment can have a growth-inhibitory effect on the disseminated tumour cells. As discussed in detail below, this is not the case with the ovaries and its microenvironment.

The ovaries contain the ovarian follicles, which are the basic units of reproductive biology. Each follicle is composed of a single oocyte surrounded by a layer of granulosa and theca cells (Bhide & Homburg 2016, Terauchi *et al.* 2016). The growth of the follicles is strictly controlled. Only a handful of follicles initiate growth and only one follicle is allowed to undergo full maturation. For the remaining thousands of follicles, growth is arrested and they remain quiescent for months to years (Roy & Greenwald 1996). There is much to be unravelled regarding the exact mechanisms and signalling pathways that control follicular growth and maturation. Nevertheless, some of the factors that are known to be present in the ovaries during the reproductive age have been described to activate signalling pathways that lead to cellular dormancy.

TGF β is one of the locally produced cytokines in the ovaries and TGF β 2 particularly is upregulated during ovulation (Pangas *et al.* 2006, Yu *et al.* 2016). TGF β has been shown to be involved in the control of follicle growth by stimulating FSH and LH receptor expression (Dunkel *et al.* 1994, Moriya *et al.* 2003), amplifying FSH-induced aromatase activity, and inducing the production of inhibin and progesterone. On the contrary, studies have shown the ability of TGF β 2 to induce tumour cell dormancy. By activating the MAPK p38 α / β signalling pathway, TGF β 2 can induce DEC2/SHARP1 and p27 expression as well as cyclin-dependent kinase 4 (CDK4) downregulation in disseminated tumour cells (Bragado *et al.* 2013).

Bone morphogenetic proteins (BMPs) are another cellular factor, which belongs to the TGF β family of proteins and are secreted by granulosa and theca cells during ovarian folliculogenesis and thus are present in the ovaries during the reproductive age. BMPs have also been shown to induce cancer cell dormancy. The family

member BMP7, which is secreted by bone stromal cells, has been shown to induce dormancy in prostate cancer stem-like cells by activating p38 kinase and increasing expression of the cell cycle inhibitor, p21 (Kobayashi *et al.* 2011). The ability of BMPs to induce cancer cell dormancy is further validated by studies showing that the BMP4 antagonist, COCO, is able to induce dormant breast cancer cells to undergo reactivation in the lung (Gao *et al.* 2012).

Finally, another factor that may promote dormancy or at least curtail the incessant growth of malignant precursors that may have seeded in the ovaries is the anti-mullerian hormone (AMH). AMH is also a member of the TGF β family of proteins and is continuously secreted during the reproductive age. In the ovaries, AMH functions to inhibit ovarian folliculogenesis and granulosa cell division (Ueno *et al.* 1988, Kim *et al.* 1992, Durlinger *et al.* 1999). Indeed, studies using ovarian cancer cells have shown that AMH is able to inhibit colony formation as well as cancer cell growth (Masiakos *et al.* 1999).

Taken together, these findings provide the rationale for our hypothesis that the malignant precursors that were able to reach and establish in the ovaries during the time of ovulation, and hence during the reproductive age, may be induced to enter a dormant state by signals that are active in the microenvironment created by the ovarian niche (Fig. 3).

Expansion and tumour progression: role of the hormonal milieu during the peri- and post-menopausal age

If malignant precursors do enter dormancy and remain in that state during the reproductive age, it is plausible that ovarian atrophy associated with menopause can lead to the loss of the ovarian niche, and consequently, the exit of malignant cells from dormancy. Furthermore, the hormonal milieu associated with the peri- or post-menopausal age may be the driving force in the progression of ovarian cancer.

During the process of menopause, follicles become atrophic, leading to the decline of oestrogen, which consequently promotes the secretion of the gonadotrophin follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary gland (Chakravarti *et al.* 1976). High levels of these gonadotrophins have been associated with ovarian cancer, specifically related to cell growth and EMT, as detailed below (Nishizuka *et al.* 1979, Cramer & Welch 1983). Indeed, according to the 'gonadotrophin hypothesis', chronically high levels of FSH may promote the malignant transformation of OSE

cells and hence generation of ovarian cancer (Lu *et al.* 2000, Zheng *et al.* 2000). Interestingly, the expression of gonadotrophin receptors is higher in the early stages of ovarian cancer than in more advanced stages (Lu *et al.* 2000). This further suggests that if gonadotrophins have a role in ovarian cancer, it may be during the beginning of its expansion.

Numerous epidemiologic studies provided information that supports the gonadotrophin hypothesis. These include the well-documented protective effects of oral contraceptives and multiparity against ovarian cancer, both of which suppress gonadotrophin secretion by the pituitary gland (Riman *et al.* 1998). Furthermore, women with polycystic ovary syndrome, which is characterized by high levels of LH, have a greater risk of developing ovarian cancer (Schildkraut *et al.* 1996). In addition, numerous studies have also shown that the use of oral contraceptives reduces the risk of ovarian cancer by 30–50% (Cancer and Steroid Hormone Study 1987, Beral *et al.* 2008). Risk reduction increases with the time of use and the protective effect lasts for more than 10–20 years after the last use (Risch *et al.* 1983). It is thought that the resulting persistent levels of circulating oestrogen due to the use of oral contraceptive may decrease the gonadotrophin levels, especially FSH via negative feedback regulation (Syed *et al.* 2001, Tung *et al.* 2005, Gharwan *et al.* 2015). Similarly, multiple case-control studies demonstrate that parity can decrease the risk of ovarian cancer; one pregnancy lowers the risk by as much as one-third and the reduction in risk increases with each additional pregnancy. Moreover, pregnancy before the age of 25 is twice as protective against ovarian cancer as a pregnancy after the age of 35 (Joly *et al.* 1974, Cramer *et al.* 1983, Risch *et al.* 1983, Whittemore *et al.* 1992). Interestingly, hCG, which signals through the LH/CGR, has been shown to suppress the growth of benign, borderline and malignant ovarian cells. This suggests that the protective effect of parity is not only by reducing the frequency/amount of ovulation, but also by inhibiting the growth of the cells via the LH pathway (Toungeman *et al.* 2002).

The role of gonadotrophins in ovarian carcinogenesis is particularly important given the hypergonadotropic conditions in post-menopausal women, which is the time that most ovarian cancer patients present with advanced-stage disease (Gharwan *et al.* 2015). Approximately, 2–3 years after menopause, the levels of FSH and LH are particularly high, reaching almost 10–20 times (50–100 mIU/mL) the levels observed in women of reproductive age (Chakravarti *et al.* 1976). When compared over a wide range of doses, FSH and

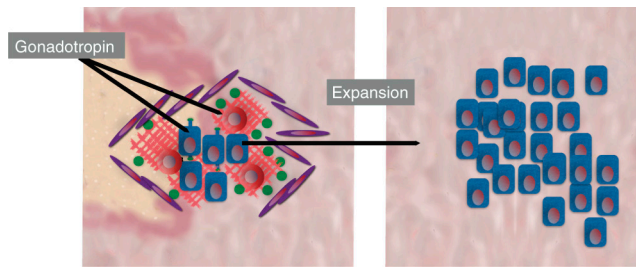
LH were found to be equally potent in stimulating the growth of primary cultures of OSE as well as malignant ovarian cancer cells (Syed *et al.* 2001, Huang *et al.* 2011). Gonadotrophins have been shown to promote cell growth via the IL-6/STAT3 pathway (Huang *et al.* 2008) as well as by activating the oncoprotein gankyrin (Bai *et al.* 2013, Liu *et al.* 2014), leading to uncontrolled cell cycle progression and proliferation mediated by cyclin D1 (Tang *et al.* 2010, Zhang *et al.* 2013).

FSH has also been linked to epithelial–mesenchymal transition (EMT) (Yang *et al.* 2014). This cellular process involves the disruption of epithelial integrity, gain of mesenchymal markers and the progressive acquisition of a motile and invasive phenotype (Islam *et al.* 1996).

Cancer cells with a mesenchymal phenotype migrate away from their primary tumour, interacting with stromal cells, invading adjacent tissues, and lymphatic system and/or blood stream into secondary tumour sites (Onder *et al.* 2008, Nuti *et al.* 2014). Once settled, these mesenchymal cancer cells will have the capacity to interact with the new environment, promoting tumour growth and cancer cell survival. In the case of ovarian cancer, they are able to lose cell-to-cell contact and shed into the peritoneal cavity and with the help of the ascitic fluid, establish metastatic disease (Vergara *et al.* 2010). Moreover, EMT is critical in ovarian cancer as it commands the spreading of the disease without any pathognomonic signs and/or symptoms, leading to late-stage diagnosis and high mortality in ovarian cancer.

When exposed to FSH, epithelial ovarian cancer cells are induced to express the mesenchymal markers vimentin and N-cadherin, and the inhibition of the epithelial marker, E-cadherin (Yang *et al.* 2014). Furthermore, FSH and LH can significantly increase the expression of matrix metalloproteinases (MMP)-2 and MMP-9 in ovarian cancer cell lines, thus enhancing invasiveness (Yang *et al.* 2014).

These data have led to the proposed concept that gonadotrophins have carcinogenic properties (Lee *et al.* 2015). However, contrary to this proposed ‘gonadotrophin hypothesis’, which infers a carcinogenic role for gonadotrophins, we propose that the abrupt and sustained increase in FSH during the peri- or post-menopausal age may play a role instead in the expansion and invasiveness of the seeded malignant precursors (Figs 4 and 5B). This would explain why not every menopausal woman develops cancer. More importantly, this highlights the requirement for the presence of pre-seeded cells in the ensuing effect of the gonadotrophins. Taken together, this underlies the concept of a step-wise process in ovarian tumorigenesis and progression.

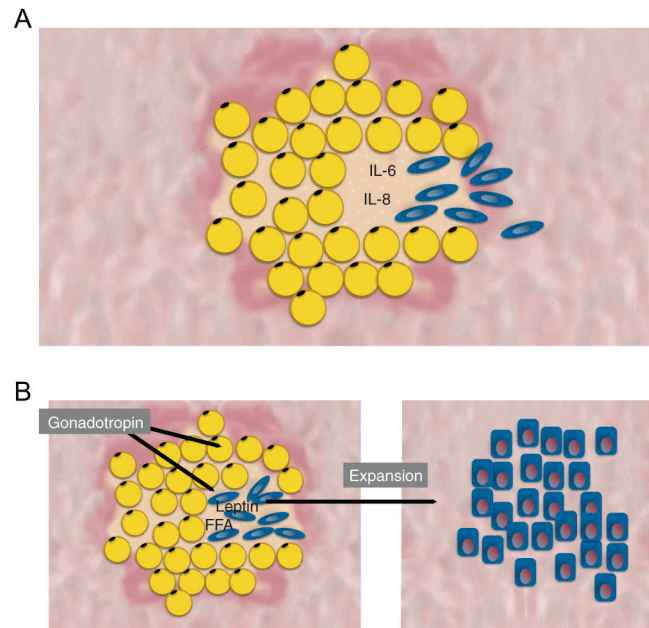
**Figure 4**

Tumour expansion and progression. During menopause, ovaries become atrophic and nonresponsive to gonadotrophins. The lack of negative feedback leads to enhanced levels of gonadotrophins, which are able to activate relevant pathways for tumor growth and expansion.

The omentum and visceral fat as another possible site of ovarian cancer initiation

A major challenge in the treatment of patients with ovarian cancer is the diagnosis at an advanced stage (stage III or IV) with patients presenting with extensive carcinomatosis and omental cake (Lengyel 2010). It is also a recurrent clinical observation that despite periodic medical consultation wherein no signs of cancer are detected, patients with ovarian cancer typically present with advance and very progressive disease a short period of time later. How does a small and undetectable cancer metastasize and progress to overt disease within months? Does ovarian cancer dissemination follow the classical metastatic process observed in other types of solid tumours? Possibly not. Instead, it is possible that during the seeding of malignant cells to the ovaries, as described above, the same malignant precursors also seed in other parts of the abdomen with the same recruitment and supportive microenvironment as the ovaries. If this is true, these sites need to provide the same adequate niche to support the survival of the seeded cells.

The main site of ovarian cancer metastasis is the omentum, such that 80% of patients present with omental metastases (Lengyel 2010). Moreover, both primary and recurrent high-grade serous ovarian carcinomas preferentially metastasize to adipose tissue (Nieman et al. 2011). Are these sites real metastatic sites? Or is it possible that the omentum may also be a possible location of initial seeding and initiation? The omentum and the ovary may provide a similar 'niche microenvironment' necessary for seeding and maintenance of malignant cells. Comparable inflammatory mediators observed during ovulation (IL-6, leptin and TNF α) are present in the omentum and adipocyte tissue (Brannian & Hansen 2002, Yang-Hartwich et al. 2014).

**Figure 5**

The role of the omentum in ovarian tumorigenesis and progression. (A) Adipocytes, specifically in the omentum, secrete a variety of factors such as IL-6 and IL-8, which can also function as chemoattractants and thereby recruit malignant precursors to the omentum. (B) Gonadotropins are also able to increase leptin secretion in adipocytes. Leptin has been shown to affect stemness pathways in cancer cells. Free fatty acids (FFAs) from adipocytes can likewise promote tumor growth.

It has been demonstrated that human omental adipocytes, mostly via the secretion of IL-8, can induce homing, attraction and invasiveness of ovarian cancer cells (Fig. 5A) (Nieman et al. 2011). More importantly, it has recently been reported that the adipocyte microenvironment could induce cancer stem cell pathways (Kato et al. 2015). Adipocyte-derived leptin promotes changes in the breast cancer cells that result in enhanced tumorigenicity and metastasis (Strong et al. 2015). Moreover, adipocyte-secreted IL-6 and leptin have pro-EMT activity, as well as promoting self-renewal and cancer stem cell signalling in breast cancer by activating the STAT pathway (Wolfson et al. 2015). Leptin has also been studied in ovarian cancer where it is found to enhance stemness by upregulating CD44 and EMT by promoting Vimentin and E-Cadherin (Kato et al. 2015). Interestingly, changes on circulating leptin were reported as a marker of early detection of ovarian cancer (Mor et al. 2005, Visintin et al. 2008). Thus, in addition to the known pro-growth effects of adipocyte cultures in ovarian cancer cells (Lengyel 2010), these results suggest that the adipocyte microenvironment can actively contribute to

maintaining and supporting cancer stem cells (Fig. 5B). Our unpublished data uncovered initial evidence that, in addition, adipocyte cultures are able to increase factors conferring chemoresistance (Cardenas C, Mor G, Montagna M, Pitruzzello M, Lima E and Alvero A, unpublished observations). Taken together, these findings suggest that organs rich in adipocytes, such as the omentum, may not only be sites of metastasis, but also possible sites of origin, i.e. the site of initial seeding and transformation of malignant precursor cells that originate from other parts of the abdomen. Thus, we propose that the seeding of malignant precursors, which occurs during the reproductive age, may take place both in the ovaries and in the adipocyte-rich organs, and tumour expansion occurs simultaneously in these sites. If seeding has already occurred in multiple sites early on, this may explain why seemingly cancer-free women are diagnosed with advanced-stage disease within months.

Conclusion

To summarize, we discuss recent studies that support the extra-ovarian origin of ovarian cancer. These include studies that identified the different cell types that can serve as malignant precursors, as well as pathways that can induce its specific recruitment and migration to the ovaries. More importantly, we propose four stages of ovarian cancer initiation and progression, based on a novel hypothesis that upon seeding the malignant precursors can be actively regulated by the ovarian and fat microenvironment. We describe how the distinct cytokine/chemokine and hormonal milieu during reproductive and menopausal age can differentially control the phenotype of these malignant precursors thereby promoting and regulating a specific stage in the described process.

Although much progress has been made, specific studies looking into each of the proposed stages of ovarian cancer tumorigenesis and progression may hold the clue to more relevant biomarkers for the early detection and treatment of ovarian cancer.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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