Steroid Hormones and Ovarian Cancer

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1. Introduction

Globally, ovarian cancer is the 6th most common malignancy in developed countries, responsible for 100,300 new cases and 64,500 deaths annually (Jemal et al., 2011). Approximately 90% of ovarian cancers arise within the ovarian surface epithelium (OSE) or the fallopian tube surface epithelium; the remainder of ovarian malignancies develops from other ovarian tissues (sex cord-stromal, germ cell, or mixed cell tumors). The overall prognosis for epithelial ovarian carcinoma (EOC) is poor: Diagnosis is typically late-stage due to the lack of effective screening methods and vague presenting symptoms, with 5-year survival at 40% for stage III and 20% for stage IV patients (Heintz et al., 2006). Despite excellent initial activity, the standard treatment consisting of cytoreductive surgery followed by platinum- and taxane-based chemotherapy often fails with a recurrence rate of over 80% in stage III and IV disease. Therefore, novel therapeutic approaches are needed to improve the outcomes in this population.

Research efforts have yielded insight into the etiology, signaling mechanisms, and progression of ovarian cancer, yet much remains poorly understood. Physiologically, steroid hormones are intimately involved in ovulation, reproduction, and function of normal OSE cells. There is growing evidence that estrogen, progesterone, and other hormones also play a role in the development and progression of ovarian cancer (Leung & Choi, 2007). “Incessant ovulation” with repetitive injury and repair of OSE and subsequent cumulative DNA damage is one of the hypothesized risk factors for ovarian cancer (Fathalla, 1971), yet this does not explain the occurrence of the majority of ovarian carcinomas well after the reproductive years (Berek & Hacker, 2010). Interestingly, although oral contraceptives (OCs), increasing parity, and prolonged breastfeeding all decrease cumulative risk (Gwinn et al., 1990; Risch et al., 1994), progestin-only contraceptives offer just as much protective benefit as estrogen-containing OCs without prohibiting ovulation (Risch, 1998). Another hypothesis for the development of ovarian cancer, the “gonadotropin hypothesis,” stipulates that gonadotropins contribute to ovarian carcinogenesis through follicle stimulating hormone (FSH)- and luteinizing hormone (LH)-mediated excess stimulation of ovarian tissue. This hypothesis is consistent with the protective effect of OCs, and the observation that the majority of cases of epithelial ovarian cancer develop postmenopausally after a surge in gonadotropin levels. In vitro, gonadotropins such as FSH activate mitogenic pathways and stimulate ovarian epithelial cell proliferation (Choi et al., 2002). In addition to gonadotropins, excess androgens and estrogen have also been linked to the progression and possibly development of ovarian cancer. In vivo treatment of mice with
estrogen significantly increases tumor growth (Armaiz-Pena et al., 2009). These observations suggest that in addition to regulation of the menstrual cycle, certain steroid hormones may promote the progression—and possibly development—of ovarian cancer, while others offer a protective benefit. The objectives of this chapter are to summarize the signaling mechanisms involved in normal human OSE and its neoplastic counterparts, to highlight the effects of these steroid hormones on ovarian cancer cell growth, and to discuss the current clinical trials utilizing anti-hormonal approaches in ovarian cancer patients.

2. Steroid hormone signaling in normal ovarian surface epithelium and ovarian cancer

2.1 Steroid signaling in normal ovarian surface epithelium

Human ovarian surface epithelium is formed by a single layer of squamous to cuboidal cells covering the outermost layer of the ovary. While the exact function of OSE is unclear, with each ovulatory cycle, the OSE undergoes damage and repair. This damage incites an inflammatory response, the sequelae of which have been implicated in neoplastic transformation to tumor cells (Murdoch & Martinchick, 2004; Ness et al., 2000). Although steroid synthesis takes place elsewhere in the ovary, both normal and malignant OSE display estrogen, progesterone, and androgen receptors (Karlan et al., 1995; Lau et al., 1999; Li et al., 2003), and take part in steroid signaling. OSE also have FSH and LH receptors, though in vitro studies suggest these stimulate cellular growth and proliferation rather than steroidogenesis in OSE (Choi et al., 2004; Ji et al., 2004).

2.1.1 Estrogen and progesterone signaling in normal ovarian surface epithelium

Though the purpose of steroid signaling within the OSE remains uncertain, the effects of these steroids have been characterized in vivo and in vitro. The estrogen receptor (ER) and progesterone receptor (PR) are intracellular nuclear hormone receptors, which upon localization to the nucleus prompt transcriptional activity. Furthermore, the estrogen receptor participates in pathway crosstalk with known mitogenic pathways which have been associated with tumor progression and drug resistance, including transforming growth factor-Beta (TGF-β), human epidermal growth factor receptor 2 (HER-2/neu), and the insulin-like growth factor (IGF) receptors (Arpino et al., 2008; Band & Laiho, 2011; Fagan & Yee, 2008). In OSE, low doses of estrogen cause proliferation of ovarian surface epithelial cells in vitro, which involves the interleukin-6 (IL-6)/signal transducer and activator of transcription 3 (STAT-3) pathway (Syed et al., 2002). Others, however, have documented no effect at low doses of estrogen (Choi et al., 2001b; Karlan et al., 1995), or an inhibitory effect at high doses in OSE (Wright et al., 2005). In vivo, estrogen exposure causes rabbit OSE cell proliferation and an increase in the number of papillae but does not result in spontaneous development of tumors (Bai et al., 2000). In comparing normal OSE to ovarian carcinoma cell lines, Lau et al. (1999) found loss of ERα, PR, and androgen receptor (AR) mRNA in neoplastic cells compared to OSE, suggesting this loss may contribute to neoplastic transformation. Though the role of ERβ in Lau’s work was uncertain, others assert that the ERβ receptor subtype promotes apoptosis in OSE (Bardin et al., 2004).

While estrogen has an unclear effect on cellular proliferation in OSE, progestins have a consistent effect of inhibiting cell growth and inflammation, and promoting apoptosis (Ivarsson et al., 2001; Karlan et al., 1995; Rae et al., 2004a). In vivo, macaques treated with progestin exhibited upregulation of apoptosis in OSE cells (Rodriguez et al., 1998). Though
the exact mechanism by which progesterone exhibits growth-inhibitory effects is not fully-understood, Syed and Ho (2003) demonstrated involvement of the caspase 8 Fas/FasL pathway in progesterone-mediated apoptosis in OSE. Culturing OSE in vitro with the inflammatory mediator IL-1 results in upregulation of several inflammation-associated genes, specifically HSD11B1, which plays a role in conversion of cortisone to cortisol (Rae et al., 2004b). With well-documented anti-inflammatory effects, cortisol prohibits downstream inflammatory signaling and may therefore exhibit a protective effect on the OSE. Together with the anti-inflammatory and growth-inhibitory effects of progesterone, glucocorticoids and progestin may serve to protect the OSE from inflammatory damage resulting from ovulation (Rae & Hillier, 2005).

2.1.2 Gonadotropin signaling in normal ovarian surface epithelium
The gonadotropins FSH and LH are members of a glycoprotein hormone family which also include thyroid stimulating hormone (TSH) and human placental chorionic gonadotropin (hCG). Gonadotropin receptors belong to the G protein-coupled receptor family (GPCR), which harbor seven transmembrane domains and upon their activation convert guanosine diphosphate (GDP) to guanosine triphosphate (GTP). This subsequently results in downstream activation of the phosphatidylinositol-3-kinase (PI3K) pathway; a pathway whose involvement in oncogenesis has been well-characterized. The type I isoform of gonadotropin-releasing hormone (GnRH) and its receptor are found on OSE, and interestingly, GnRH analogs appear to inhibit growth of OSE and ovarian cancer cells in vitro (Kang et al., 2000). The GnRH type II isoform also has growth inhibitory effects in vitro (Choi et al., 2001a). While the exact mechanism of growth inhibition is not fully-understood, it is discussed in more detail later in the chapter.

Synthesized in the anterior pituitary, the gonadotropins FSH and LH regulate the menstrual cycle: FSH stimulates follicular growth and the FSH receptor (FSHR) is expressed mainly in granulosa cells, while LH triggers ovulation and the LH receptor (LHR) is expressed mostly in theca but also by granulosa cells. FSHRs and LHRs are found in both OSE and ovarian cancer cells (Minegishi et al., 2000; Parrott et al., 2001). Leung and Choi (2007) characterized the signaling pathway of FSHR in preneoplastic immortalized OSE, and found that Extracellular-Related Signaling Kinase 1/2 (ERK 1/2), c-myc, and HER2/neu were upregulated, and cell growth was accelerated in response to FSHR overexpression. Epidermal growth factor receptor (EGFR) is also upregulated via the ERK 1/2 and PI3K/Akt pathways in immortalized OSE treated with gonadotropins (Choi et al., 2005). Others have similarly confirmed increased proliferation in OSE in response to FSH and LH administration (Choi et al., 2002; Syed et al., 2001). In vitro, treatment with FSH results in upregulation oncogenic genes and downregulation of tumor suppressor genes RB1, BRCA1, and BS69 (Ji et al., 2004).

2.2 Steroid signaling in ovarian cancer
Steroid hormone signaling in ovarian cancer involves complex pathways which are not yet fully understood. Figure 1 depicts some of the established routes of signaling in EOC.

2.2.1 The role of estrogen in ovarian cancer
Although the influence of exogenous estrogen in OSE is ambiguous, it most certainly triggers proliferation and cellular growth in ovarian cancer cells (Choi et al., 2001b). This is
accomplished in part through activation of the PI3K/Akt pathway and the transcription factor c-myc (Chien et al., 1994), and via estrogen receptor crosstalk with other pathways such as IGF-1, TGF-α, and EGFR (Simpson et al., 1998; Wimalasena et al., 1993). Estrogen also inhibits apoptosis via bcl-2, an anti-apoptotic protein (Choi et al., 2001b), and increases invasive capacity via upregulation of ezrin (Song et al., 2005), fibulin-1 (Galtier-Dereure et al., 1992), cathepsin D (Galtier-Dereure et al., 1992), and kallikreins (Yousef et al., 2003). In ovarian cancer cell lines treated with 17-B-estradiol, pro-angiogenic hypoxia-inducible factor-1 (HIF-1) expression was increased, the effect of which was abrogated by the Akt inhibitor snf and via Akt short-interfering RNA (siRNA) (Hua et al., 2009).

Both ER subtypes α and β are expressed in EOC. It has been suggested that ERα is mainly responsible for proliferation in the ovary, while ERβ modulates differentiation (Britt & Findlay, 2002). This is supported by the fact that ERβ is abundant in OSE and benign tumors, and ERα is mainly found in malignant ovarian tumors (Brandenberger et al., 1998; Hillier et al., 1998). An increased ERα:ERβ ratio has also been observed in ovarian cancer (Cunat et al., 2004), with an increase in ERα and inverse decrease in ERβ expression throughout tumor progression (Brandenberger et al., 1998). Overexpression of ERβ in an ovarian cancer cell line decreased proliferation by 50%.

In vivo studies support the role of 17-β-estradiol in accelerating tumor growth. Using a transgenic mouse model, Laviolette et al. (2010) showed that treatment of tumors with 17-β-estradiol resulted in the earlier onset of tumors, decreased survival time, and papillary histology, while treatment with progesterone resulted in no difference. Armaiz-Pena et al. (2009) similarly showed that treatment with 17-β-estradiol increased ovarian tumor growth, and inoculation with tumor cells during the proestrus when estrogen levels are high significantly increased tumor burden, compared to inoculation during the estrus phase. Furthermore, treatment with 17-β-estradiol resulted in increased vascular endothelial growth factor (VEGF), increased cell adhesion in ER positive cells, increased migratory potential, and mitogen-activated protein kinase (MAPK) upregulation.
Correspondingly, the estrogen antagonist tamoxifen abrogates the estrogen effect in epithelial ovarian cancer cell lines, and is surprisingly effective in both ER-positive and ER-negative and platinum-resistant ovarian carcinomas (Mabuchi et al., 2004; Markman et al., 1996). In ER-negative ovarian carcinomas, tamoxifen functions independently of estrogen via ERK, c-Jun N-terminal protein kinase (JNK), and p38 (Mabuchi et al., 2004).

2.2.2 The role of progesterone in ovarian cancer

Progesterone decreases cellular proliferation in vitro via multiple pathways. Blumenthal et al. (2003) demonstrated that progesterone activated the cyclin-dependent (CDK) pathway and promoted a more differentiated cell type. In the ovarian cancer cell line SKOV3, progesterone inhibited invasion and suppressed urokinase plasminogen activator (UPA), thereby decreasing the metastatic potential of cells (McDonnel & Murdoch, 2001). As in OSE cells, progesterone induces apoptosis via caspases and the FasL pathway (Syed & Ho, 2003), and enhances tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced cell death (Syed et al., 2007). Recent evidence supports that progesterone also causes apoptosis by upregulating the proapoptotic gene expression of p53 and BAX, and decreasing antiapoptotic gene expression of BCL-2 in ovarian cancer cells, thereby preventing oxidative damage (Nguyen & Syed, 2010).

In vivo, treatment of athymic mice with progesterone suppressed tumorigenesis after inoculation with the ovarian cancer cell line SKOV3 and increased survival (McDonnel et al., 2005). Furthermore, progesterone treatment in athymic nude mice inoculated with platinum-resistant SKOV3 enhanced platinum activity and sensitivity (Murdoch et al., 2008). However, the actions of progesterone in ovarian carcinomas is complex, and clinical trials utilizing progesterone have not proved as promising as in vivo and in vitro observations. In part, this may be due to the action of progesterone receptor membrane component 1 (PGRMC1), which recently in vitro was found to promote ovarian tumor cell proliferation, while its depletion slows cellular growth (Peluso et al., 2008). In vivo, PGRMC1-depleted mice had fewer and smaller tumors, again supporting the pro-oncogenic nature of PGRMC1 (Peluso et al., 2009).

As is the case with estrogen receptors, different progesterone receptor isoforms exist, including isoforms A and B. Akahira et al. examined the expression of PRA and PRB in the normal ovary, and in benign, borderline, and malignant ovarian tumors (2002). Using immunohistochemistry and RT-PCR in normal and malignant tissue and cell lines, they found that PRA receptor expression declined (p<0.05) during the transition from benign to borderline to malignant tissues, whereas there was no significant difference in PRB expression. They suggest that downregulation of PRA is associated with the development of ovarian cancer.

2.2.3 The role of gonadotropins in ovarian cancer

An alternative theory to the incessant ovulation hypothesis is that excessive gonadotropin stimulation leads to the development of ovarian cancer. Early animal models simulated hypogonadism, which prevented development of ovarian tumors in mice (Marchant, 1961). Oral contraceptives, which are proven to reduce the risk of EOC, also provide negative feedback on the gonadotropin axis and reduce circulating gonadotropins, which further supports this hypothesis. Additionally, the peak incidence of ovarian cancer occurs postmenopausally, with a temporal relationship to an increase in circulating gonadotropins (Choi et al., 2007).
Similar to estrogen, FSH promotes ovarian cancer cell growth in vitro. One way this occurs is through inhibition of apoptosis through activation of survivin via the PI3K/Akt pathway, and downregulation of the programmed cell death gene 6 (PCD6) and death receptor 5 (DR5) (Huang et al., 2010). Promotion of cell growth through FSH also occurs through activation of the MAPK, PI3K/Akt pathways (Choi et al., 2002). Overexpression of the FSH receptor promotes well-established oncogenic signaling pathways including upregulation of EGFR (Choi et al., 2004), and angiogenesis promotion via VEGF upregulation (Schiffenbauer et al., 1997; Wang et al., 2002). In ovarian cancer cell lines, both FSH and LH exploit the PI3K/Akt pathway to upregulate cyclooxygenase (COX)-1 and COX-2, resulting in increased cell motility and invasion (Lau et al., 2010). The FSH receptor (FSHR) is also prominent in ovarian carcinomas—Ji et al reported higher levels of the FSHR in cancerous ovarian tissue compared to normal controls (2004). The role of LH is controversial; in one study, LHR mRNA expression decreased in the transition from benign to malignant ovarian lesions (Lu et al., 2000). LH also appears to abrogate the proliferative effects of FSH (Zheng et al., 2000), but also has been reported to stimulate growth of OSE and contribute to tumor progression (Tashiro et al., 2003).

In vivo, treatment of rats with gonadotropins increased OSE proliferation (Stewart et al., 2004); similarly, mice treated with gonadotropins experienced increased ovarian cellular proliferation and decreased apoptosis (Burquette et al., 2006). Mice displaying hypergonadotropism from overexpression of FSH develop multicystic hemorrhagic ovaries, while overexpression of LH resulted in the formation of granulosa cell tumors (Kumar et al., 1999; Risma et al., 1995). However, it is important to note that these tumors formed in stromal or granulosa cells—not epithelial cells—and to date, no evidence exists establishing that gonadotropins can initiate malignant transformation of the ovarian epithelium.

2.2.4 The role of androgens in ovarian cancer

The role of androgens in promoting growth, tumorigenesis, and tumor progression in prostate cancer is well-established. In ovarian cancer cell lines, androgens 5α-dihydrotestosterone (DHT) and testosterone also cause cell proliferation which is mediated by upregulation of interleukin-6 (IL-6) (Syed et al., 2001; Syed et al., 2002). Levine and Boyd (2001) found that androgen receptor allele length influenced age at diagnosis with ovarian cancer; those patients with a shorter AR allele length were diagnosed 7 years earlier than those with normal allele length. In vitro, androgens decrease TGF-β receptors levels, which provides a mechanism for ovarian cancer cells to evade the growth inhibitory effects of TGF-β (Evangelou et al., 2000). Shi et al. (2011) examined the role of DHT, testosterone, and dehydroepiandrosterone (DHEA) in breast and ovarian cancer cell lines, and found that DHT but not the other androgens induced the degradation of the tumor suppressor p27 in both cell lines. Furthermore, in ovarian carcinoma cell lines, testosterone and androstenedione were found to increase cell viability, and induce telomerase activity which was blocked by PI3K inhibitors (Nourbakhsh et al., 2010). Sheach et al. (2009) found that the ovarian cancer cell lines OVCAR3 and OSEC2 expressed the androgen receptor, and treatment with androgen upregulated 121 genes, including G-protein-related genes Rab25 and Rab35. Level of gene expression correlated with tumor grade as well.

2.2.5 The role of GnRH in ovarian cancer

Two of twelve vertebrate GnRH isoforms exist in humans: GnRHI and GnRHIi. GnRHI and its receptor are present in 80% of biopsies of ovarian carcinomas (Emons et al., 1989). While
GnRH functions in a paracrine manner systemically, there is evidence that GnRH functions in an autocrine manner in epithelial ovarian cancer, with GnRHI and GnRHIi mRNA identified in both ovarian carcinoma and OSE (Choi et al., 2001a; Kang et al., 2000). Although the traditional gonadotropin GnRH signaling pathway involves G \(_{aqp}\) protein kinase C, and MAP kinases, the autocrine mechanism of GnRH-related signaling in ovarian carcinomas differs (So et al., 2008). Interestingly, it appears that GnRH receptors in ovarian malignancy function via the pertussis toxin-sensitive protein G \(_{ai}\) and activate protein phosphatase (Imai et al., 2006). This alternate pathway is responsible for the antiproliferative effects of GnRH in ovarian carcinomas; however, the antiapoptotic function of GnRHI remains controversial. GnRHI and its agonists exert an inhibitory effect in ovarian cancer in vitro and in vivo, reducing cell growth and inducing cell cycle arrest in G\(_0\)/G\(_1\) (Kim et al., 1999). However, GnRH agonists are in fact protective against apoptosis in ovarian cancer cell lines treated with doxorubicin (Sugiyama et al., 2005). Somewhat paradoxically, there is also emerging evidence that GnRH promotes tumor cell migration, metastasis, and invasion. In vitro, GnRHI enhances Akt pathway activation, and increased nuclear beta-catenin accumulation. This was reversed with siRNA targeting the GnRH receptor, and with treatment of cells with a PI3K/Akt inhibitor. GnRH treatment also increases type I matrix metalloproteinase (MT-1MMP) levels, thereby increasing cells’ invasiveness and metastatic potential which increases cell migration and invasion during tumor progression through activation of Rho GTPases and accumulation of p120 catenin, with reversible effects upon inhibition of p120 (Cheung et al., 2010). In vivo, mice treated with a GnRH agonist displayed increased tumor weight (Romero et al., 2009). Thus, while GnRH and its agonists work via G\(_{ai}\) to decrease cellular growth and proliferation, it also enhances other pathways which promote cellular migration and invasion, essentially increasing metastatic potential.

3. Endogenous steroid hormones and ovarian cancer

3.1 Endogenous hormones
Population-based studies have supported the in vitro findings that progestins harbor protective effects against ovarian cancer. Conditions in which excess progesterone is present decrease cumulative risk of developing EOC, including multiple gestations, multiparity with an additional 10% decrease in risk with each birth, and breastfeeding (Lambe et al., 1999; Risch et al., 1994). While these findings support the incessant ovulation hypothesis by sparing the epithelium from monthly ovulations and repetitive damage, there is also evidence that progesterone independently increases the magnitude of benefit by promoting cellular apoptosis (Rodriguez et al., 1998).

3.2 Hormone receptors
Much effort has been directed at correlating hormone receptor status and outcomes in ovarian cancer. Geisler et al. (1996) examined estrogen and progesterone receptor status as prognostic indicators in patients with optimally cytoreduced stage IIIIC serous carcinoma of ovary. Out of 96 patients, those with an estrogen receptor level <10 fmol/mg had better mean survival (41 vs 34 mos) than patients with higher levels of ER, whereas there was no correlation between PR status and survival. Others have found opposing results: One study examined ER mRNA expression in 35 stage III-IV ovarian carcinoma patients receiving neoadjuvant chemotherapy, and in multivariate analysis found that elevated baseline ER mRNA levels predicted prolonged progression-free survival (p=0.041) and overall survival.
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(p=0.01), independently of pathological grade and age (Zamagni et al., 2009). Another group examined hormone receptor status in older and younger patients with advanced papillary serous ovarian carcinoma (Liu et al., 2009). They reported a higher percentage of ER-positivity and PR-negativity in older patients, while both groups were largely Her2/neu receptor-negative. Although there was no significant association between receptor status and survival, for the younger cohort, those who expressed both ER and PR receptor types had better overall survival (OS) (p=0.056) at 51 months, compared to 35 months in those not expressing both receptors. In a large cohort of Danish patients with ovarian cancer, Hogdall et al (2007) examined the prognostic value of ER and PR status via immunohistochemistry and microarray analysis in 582 women with EOC and 191 patients with serous borderline ovarian tumors. They noted that ER positivity increased with stage (p=0.0003), PR positivity increased with increasing grade (p=0.0006), and tissue ER and/or PR expression greater than 10% pointed to a more favorable prognostic outcome.

Estrogen receptor subtypes have also received attention in predicting ovarian cancer outcome. Halon et al. (2011b) examined ERβ via immunohisto- and immunocytochemistry in 43 patients pre-chemotherapy and 30 patients post-chemotherapy with stage III ovarian cancer. They determined that patients with higher initial ERβ expression (>30% of cells) enjoyed longer OS and progression-free survival (PFS) (p=0.0016, p=0.032, respectively). Similarly, Halon et al. (2011a) looked at ERα expression in the same group of patients and found that loss of ERα expression predicted significantly shorter OS and PFS.

Another group (Yue et al., 2010) also examined steroid receptors as possible prognostic markers in EOC. They described the steroid and xenobiotic receptor (SXR, also known as the pregnane X receptor), which regulates gene transcription and triggers proliferation of ovarian cancer cells in vitro and in vivo and induces drug resistance (Gupta et al., 2008). In 141 cases of EOC, SXR immunostaining was correlated with older patient age, clear cell histology, higher grade, and ER- and PR-positive tissues. SXR expression correlated with a higher likelihood of recurrence, and worse disease-free and overall survival. However, ER and PR status were not associated with disease-free survival (DFS) or OS.

### 3.3 Circulating hormones

In general, there is no documented consistent association between circulating hormone levels and the risk of developing EOC. Studies have addressed serum FSH and LH, and have not found any relationship between serum levels and ovarian cancer incidence (Akhmedkhanov et al., 2001; Arslan et al., 2003). Although there is no known association between the development of EOC and LH levels, high LH levels have been observed in BRCA1 mutation carriers compared to controls without BRCA1 mutations, which may comprise part of the BRCA1 phenotype (Jernstrom et al., 2005). While serum circulating levels of gonadotropins are nonpredictive, high levels of gonadotropins in ovarian cysts and peritoneal fluid are associated with malignancy. In comparing patients with and without malignant ovarian neoplasms, there was no difference in serum levels of LH or FSH, but ovarian cyst fluid levels of LH and FSH were elevated in serous ovarian carcinomas compared to benign tumors (Kramer et al., 1998). Similarly, peritoneal fluid aspirates contained elevated levels of FSH and LH in ovarian carcinoma versus benign tumors (Chudecka-Glaz et al., 2004; Halperin et al., 2003). One recent study actually found that in 67 patients with ovarian cancer versus controls, increased circulating levels of FSH were correlated with a reduced prediagnostic risk of EOC, which argues against the excess gonadotropin hypothesis in ovarian carcinogenesis (McSorley et al., 2009).
In a large European study, 192 ovarian cancer cases and 346 matched controls were compared for serum levels of testosterone, androstenedione, dehydroepiandrosterone, and sex hormone binding globulin (SHBG) (Rinaldi et al., 2007). Free testosterone levels were inversely related to risk of EOC (p=0.01) in postmenopausal women, whereas free testosterone was positively associated with EOC risk in women under age 55, though this was not statistically significant. Other circulating androgens were not associated with risk.

In a nested case-control study of 31 cases of ovarian cancer and 63 controls, FSH and LH were measured (Helzlsouer et al., 1995). Mean FSH was lower in the cases (p=0.04) compared to controls, and LH was also lower but the difference was not statistically significant. The risk of ovarian cancer increased with higher androstenedione levels (p=0.03) and higher DHEA levels (p=0.02). Lukanova et al. (2003) followed by evaluating pre-diagnostic levels of serum testosterone, DHEA-sulfate, estrone, and SHBG in a case-control study nested within three cohorts, including 132 patients with primary EOC matched with 2 controls per case. There was no apparent association between any of the five hormone levels and ovarian carcinoma risk. Increased levels of circulating androstenedione in premenopausal patients did increase overall risk, but the low number of subjects in that subgroup precluded a definitive association.

In nonepithelial ovarian cancer (NEOC), circulating levels of steroid hormones may provide information about risk of NEOC development (Chen et al., 2010). In a case-control study within the Finnish Maternity Cohort, serum specimens were obtained from women with a singleton pregnancy that preceded their diagnosis of NEOC: 41 women had sex cord stromal tumors (SCST), and 21 had germ cell tumors (GCT). Doubling of testosterone, androstenedione, and 17-hydroxyprogesterone were associated with a two-fold increased risk of SCST compared to matched controls, a trend which remained after exclusion of women with a 2-, 4-, or 6-year time lag between blood donation and SCST diagnosis.

4. Exogenous hormones and ovarian cancer

4.1 Infertility treatment and risk of ovarian cancer

The relationship between infertility and steroid hormones is a controversial one. Many initially speculated that exogenous treatment with FSH and LH in infertility patients would lead to subsequent epithelial proliferation and tumorigenesis. In a case-control study, Whittemore et al. (1992) concluded that infertility patients who used infertility medications were at an increased risk of EOC compared to fertile women not using infertility medications. Since that time, however, other studies have emerged refuting that theory, and in fact it seems that infertility itself rather than gonadotropin use is an inherent risk for ovarian cancer (Ness et al., 2000; Tworoger et al., 2007). Brinton et al. reported on the relationship between ovarian cancer and infertility in a retrospective cohort study of 12,193 subjects (2004a). There were 45 identified cases of ovarian carcinoma, and infertility patients had a significantly elevated risk when compared to the general population, with a higher rate for primary versus secondary infertility, and the highest rate for those patients with endometriosis (Relative Risk or Hazard Ratio: RR=2.72 for patients with primary infertility and endometriosis). Examining the same patient cohort, they also reported on the risk of ovarian cancer with ovulation-stimulating drugs (Brinton et al., 2004b). They concluded a “slight but insignificant elevation in risk associated with drug usage among certain subgroups,” including those using clomiphene citrate, which warrants continued surveillance.
Hormone replacement therapy

The topic of hormone replacement therapy (HRT) use and ovarian cancer risk has sparked much debate and controversy. Several large cohort studies deserve mention.

In a large study as part of the Cancer Prevention Study II Nutrition Cohort, a group of 54,436 postmenopausal women were followed starting in 1992 (Hildebrand et al., 2010). Over 15 years of follow-up, 297 incident cases of EOC were identified. Relative to never users, estrogen-only HRT was associated with a twofold higher relative risk (RR=2.07) of ovarian cancer, each 5-year increment of use was associated with a 25% higher risk, and greater than or equal to 20 years with a threefold higher risk (RR=2.89). Neither current nor former combination estrogen and progesterone use was associated with an increased ovarian cancer risk.

The UK Million Women Study enrolled 948,576 women and followed them for an average of 5.3 years for incident ovarian cancer (Beral et al., 2007). They identified 2273 cases of ovarian cancer, with 1591 associated deaths, and reported that current users of HRT were significantly more likely to develop (RR=1.2, p=0.0002) and die (RR=1.23, p=0.0006) from EOC versus never users. As in other studies, risk increased with duration of use, but interestingly did not differ by type of HRT used, and past users were not at an increased risk.

In a large prospective study, Rodriguez et al. (2001) examined the effects of postmenopausal estrogen use and risk of ovarian cancer. Of 211,581 women prospectively enrolled in the American Cancer Society’s Prevention Study II, 944 ovarian cancer deaths were recorded over 14 years. Ovarian cancer mortality in women using estrogen replacement therapy (ERT) was higher than never users (RR=1.51), and risk was slightly but not significantly increased in former ERT users (RR=1.16). They noted that duration of use was correlated to risk, baseline current use for ≥10 years resulted in an RR of 2.20, versus former use for ≥10 years which resulted in a decreased but still elevated relative risk of 1.59. Risk did, however, decrease with increasing time since last use. The increased risk of ovarian cancer mortality persisted for up to 29 years.

Another large prospective cohort involved 329 women who developed ovarian cancer from a pool of 44,241 women in the Breast Cancer Detection Demonstration Project (Lacey et al., 2002). After adjustment for age and OC use, ever use of ERT was the only form of HRT associated with ovarian cancer (RR=1.6), in a dose dependent manner with RR for 10-19 years at 1.8 and more than 20 years at 3.2 (p<0.001). The addition of progesterone in an estrogen-progestosterone regimen after estrogen-only use was still associated with an elevated risk (RR=1.5), but was not significant for estrogen-progestosterone use only (RR=1.1). Women who used estrogen-only therapy for greater than 10 years were at a significantly increased risk of ovarian cancer, whereas estrogen-progestin only users were not at an increased risk. Data from the Women’s Health Initiative also merits mention (Anderson et al., 2003). This was a randomized, double-blind, placebo-controlled trial with 16,608 postmenopausal women given a daily tablet of estrogen-progestosterone versus placebo. They identified 32 cases of ovarian cancer, and the hazard ratio for HRT use was 1.58, suggesting that continuous combination therapy may increase EOC risk.

In the clear cell and endometrioid non-serous subtypes of epithelial ovarian cancer, endometriosis is a known precursor lesion to the development of these malignancies. Correspondingly, there have been reports that HRT use is more strongly associated with clear cell and endometrioid histologic subtypes than serous EOC (Riman, 2003; Risch, 2002).
In light of these large cohort studies and the lack of definitive molecular evidence that steroid hormones initiate carcinogenesis, it is most likely that exogenous hormones hasten and perhaps fuel pre-existing lesions. As Risch points out, given the long latency between HRT use and development of these neoplasms, it is unlikely that these agents cause malignant transformation (2002).

4.3 Oral contraceptives and BRCA carriers

Whereas hormone replacement therapy may increase risk of ovarian carcinoma, oral contraceptive use diminishes the risk of EOC. Use over five years decreases risk by up to 50% lasting 10 to 15 years (Gwinn, 1985; Gwinn et al., 1990). While the exact protective mechanism is unknown, it is hypothesized that the apoptotic effects of progesterone are responsible for this effect. In a case-control study of 546 women with ovarian cancer and 4228 controls, women who had used OCs had a 40% reduction in risk compared to those who had never used them, with a protective effect lasting for 15 years (The Centers for Disease Control, 1987). BRCA mutation carriers harbor an especially high lifetime risk of development of EOC, ranging from 40 to 45% in BRCA1 carriers and 15-20% amongst BRCA2 mutation carriers (Narod et al., 2002). Studies support the use of OCs in high-risk women. Narod et al. particularly addressed this subgroup, and conducted a cohort study on 207 women with hereditary ovarian cancer, with 161 of their sisters serving as controls (1998). Lifetime history of oral contraceptive use was obtained, and the investigators found a decreased odds ratio of 0.5 for ovarian cancer associated with oral contraceptive use, with adjustment for age and parity. As in prior studies, the risk decreased with increasing duration of use, with a 60% reduction in risk with use for 6 or more years. In specifically examining BRCA mutation status, OR for OC use and development of EOC for BRCA1 carriers was 0.5, and was 0.4 for BRCA2 mutation carriers. A subsequent study in 1311 matched pairs of women with BRCA1 and BRCA2 mutations, however, revealed a modest increase in breast cancers in BRCA1 mutation carriers (OR=1.2) compared to controls, whereas there was no increased risk for BRCA2 mutation carriers (OR=0.94) (Narod et al., 2002). While oral contraceptives undoubtedly provide benefit in ovarian cancer prevention and are recommended in young BRCA mutation carriers, there is a minimal increased risk of breast cancer which must be weighed against the benefits.

5. Clinical trials with hormonally-targeted therapeutics in ovarian cancer

Several clinical trials in ovarian cancer have examined the utility of hormonal therapy in ovarian carcinoma, including antiestrogens (tamoxifen, fulvestrant), aromatase inhibitors (letrozole), progestins and progesterone receptor antagonists (medroxyprogesterone acetate, megestrol acetate, mifepristone), GnRH agonists (leuprolide acetate, triptorelin, goserelin), and antiandrogens (flutamide). Virtually all the trials examine these agents in the recurrent setting or in combination with other therapies; rarely are they studied as a component of up-front therapy. Given the overall low response rate to chemotherapy in recurrent ovarian cancer, most hormonal agents have a modest effect, with tamoxifen used the most frequently. Hormone-directed therapy is particularly popular in the setting of “chemical recurrence,” in other words a rising CA-125 without clinical or radiographic evidence of disease. In this situation, clinicians are reluctant to initiate cytotoxic chemotherapy as early initiation does not prolong OS (Rustin et al., 2010), while patients often experience anxiety waiting for full return of their disease. With much more acceptable side effects than cytotoxic chemotherapy,
hormonal therapeutics offer some activity and are generally well-tolerated. Not surprisingly, levels of endocrine receptor expression correlate positively with response; however, even patients without high receptor expression may enjoy clinical benefit.

5.1 Estrogen antagonists
5.1.1 Selective estrogen receptor modulators
Tamoxifen is a selective estrogen receptor modulator (SERM) with mixed estrogen agonist and antagonist activity. In the endometrium, it acts in an agonistic manner and has been associated with endometrial carcinoma (Bland et al., 2009); in the breast it is an antagonist. Use in ovarian carcinoma is generally restricted to the setting of recurrence or maintenance, with modest effects. Nonetheless, it is an attractive option for patients with biochemical recurrence and a rising CA-125 in the absence of obvious radiologic disease or symptoms. In this situation, early treatment offers no advantage and unnecessary toxicity, yet watchful waiting often causes patient anxiety. In this regard, tamoxifen may offer some activity while maintaining a favorable side effect profile.

Markman et al. explored this particular patient group in a retrospective review of patients with recurrent small-volume disease who received tamoxifen prior to initiation of cytotoxic chemotherapy (2004). Of 56 patients, the median duration of treatment was 3 months, but 42% of patients remained on tamoxifen for over 6 months, and 19% were still on tamoxifen at 9 months. They concluded that while tamoxifen is a reasonable treatment option, it is unknown whether the delay in chemotherapy resulted from the tamoxifen itself or the natural history of the patients’ disease.

In a prospective trial, the Gynecologic Oncology Group (GOG) examined 105 patients with stage III or IV epithelial ovarian cancer whose disease recurred or persisted after surgery and were treated with tamoxifen 20 mg twice daily (Hatch et al., 1991). They reported an 18% response rate: 10% of patients had a complete response (CR) with a median duration of 7.4 months, while 8% experienced a partial response (PR), and 38% of patients had short-term disease stabilization. Median duration for PR or stable disease (SD) was 3 months. Of those with a complete response, 89% had elevated ER levels, versus 59% in SD or PR groups. Markman et al. (1996) followed with an ancillary report on the group, examining those patients with platinum-refractory disease. They reported an objective response rate of 13% in patients with platinum-resistant ovarian cancer, and a median duration of response of 4.4 months. The Mid-Atlantic Oncology Program (MAOP) conducted three separate phase II trials in patients with refractory ovarian carcinoma, and treated patients with either high-dose megestrol acetate, high-dose tamoxifen, or aminoglutethimide (Ahlgren et al., 1993). Of 30 patients who received high-dose megestrol acetate (800 mg/day for 30 days, then 400 mg/day thereafter), there were no identified responses. Among 29 patients treated with tamoxifen (80 mg/day for 30 days, then 40 mg/day thereafter), 17% responded, and two of those responses exceeded five years. Finally, aminoglutethimide was administered at a dose of 1g/day to 15 patients, and no responses were observed.

A more recent trial explored the role of tamoxifen versus thalidomide and its effects on VEGF expression in a randomized phase III trial in 138 women with stage III or IV EOC, primary peritoneal cancer, or fallopian tube carcinoma who were disease-free following first line chemotherapy and experienced a “biochemical” recurrence only as defined by rising CA-125 (Hurteau et al., 2010). Thalidomide was not superior to tamoxifen on interim analysis, with a similar risk of progression, higher toxicity, and an increased risk of death, and the trial was closed. VEGF expression was also not a prognostic factor in determining response.
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Tamoxifen has also been explored in combination with cytotoxic agents. In a phase II trial, 50 patients with recurrent or progressive ovarian cancer after platinum-based chemotherapy received either 100 mg/m² cisplatin or 400 mg/m² carboplatin q3 weeks with tamoxifen 80 mg/day for 30 days followed by 40 mg/day thereafter (Benedetti Panici et al., 2001). Overall response rate was 50% with a 30% CR and 20% PR, with a higher response rate (64%) in the platinum-sensitive group compared to platinum-resistant cases (39%). Toxicity included nausea and vomiting, neuropathy, nephrotoxicity, and bone marrow suppression. While encouraging, it is difficult to interpret the effects of tamoxifen without a comparison group of platinum-alone, or platinum plus paclitaxel as is often used for recurrent platinum-sensitive disease.

In combination with the EGFR inhibitor gefitinib, tamoxifen was administered to 56 patients with platinum- and taxane-refractory EOC, peritoneal cancer, or fallopian tube cancer (Wagner et al., 2007). Sixteen patients had stable disease, although there were no tumor responses and in 10% the medications were discontinued secondary to adverse events, the most common of which were rash and diarrhea. Median time to progression was 58 days, with a median survival of 253 days. The investigators concluded that the drug combination was not efficacious.

5.1.2 Aromatase inhibitors

Letrozole is a non-steroidal aromatase inhibitor. It competitively and reversibly binds to aromatase and thereby prevents conversion of androgens to estrogen. In the setting of relapsed ovarian cancer, aromatase inhibitors can achieve a response in 35.7% of patients and stable disease in 20-42% of patients (Li et al., 2008). In a phase II setting, Smyth et al. (2007) investigated letrozole 2.5 mg orally daily in previously-treated patients with ER+ ovarian carcinoma. Of 42 patients, 17% had CA-125 response (defined as a decrease >50%), and 26% had not progressed (defined as doubling of CA-125). In terms of radiologic response, 9% of patients had a partial response, and 42% had stable disease at 12 weeks. Progression free survival of greater than 6 months was observed in 26% of patients. Response correlated to level of ER expression as defined by immunohistochemistry.

In another phase II trial examining patients with recurrent ovarian cancer, 50 patients received letrozole 2.5 mg daily (Bowman et al., 2002). Primary tumors were assessed for ER, PR, EGFR, erbB2, and HSP27 expression via immunohistochemistry. Though no PR or CR was observed, 10 patients experienced stable disease for at least 12 weeks. Those with stable disease exhibited significantly higher ER and PR levels, implying that endocrine receptor expression may help identify those patients most likely to benefit from treatment.

Walker et al (2007) aimed to explore estrogen-related gene expression and its predictive value in patient response to letrozole. Protein expression was measured via immunohistochemistry in tissue sections of tumors from patients treated with letrozole, and eight genes were significantly differentially expressed amongst patients who responded or had disease stabilization versus those who progressed. They concluded that these results might help identify those patients who would benefit most from endocrine therapy.

In 2010, Pan and Kao (2010) published a case report of two patients with endometrioid type histology. Both patients with advanced ER+ endometrioid ovarian carcinoma were treated with letrozole. The first patient had undergone optimal debulking, followed by completion of carboplatin and paclitaxel, had residual disease on second look surgery, and was subsequently disease-free for 30 months with letrozole treatment. The second patient was on her third recurrence and also experienced a 30 month remission with letrozole.
5.1.3 Estrogen receptor antagonists
Fulvestrant is a pure estrogen receptor antagonist without agonistic effects on other tissues. It competitively binds the ER, blocking estrogen binding and causing degradation and internalization of the estrogen receptor. In a phase II trial of fulvestrant in 26 women with ER positive recurrent ovarian or primary peritoneal carcinoma, patients received 500 mg IM on day 1, 250 mg IM on day 15, and 250 mg IM on day 29 and every 28 days thereafter (Argenta et al., 2009). The group had been heavily pretreated, with a median of 5 chemotherapy regimens prior to enrollment. Half of women experienced disease stabilization, though there was only one complete response and one partial response. Median time to progression was 62 days, and the regimen was well tolerated.

5.2 Progesterone
Given its promising apoptotic effects in vitro and in vivo, clinical trials with progestins have been disappointing. Several trials have used megestrol acetate or medroxyprogesterone acetate in the recurrent setting. Zheng et al. provide a nice summary of 13 trials, with 432 patients total (2007). Complete response was observed in 10 patients (2.3%), with a partial response in 4.9% of patients and stable disease in 47 or 10.9% of patients. They note that of the ten patients, 6 of them were reported in one study which also noted an overall 45% response rate (Geisler, 1985). A higher dose regimen does not appear to provide any additional benefit: In a phase II trial of 800 mg/day for 1 month followed by 400 mg/day thereafter, patients did not experience an overall increased benefit from higher doses, with an overall response rate of 10% (Veenhof et al., 1994) and 3 thromboembolic events. Interestingly, combination therapy with megestrol acetate and ethinyl estradiol yielded a partial response rate of 17% and stable disease in 24% of 25 patients with recurrent EOC, all patients had ER+ tumors (Fromm et al., 1991). Surprisingly, combination of progestins with tamoxifen does not appear to provide any clinical benefit (Jakobsen et al., 1987); nor does combination with chemotherapy. Based on in vitro evidence that megestrol acetate may reverse P-glycoprotein-mediated drug resistance, a phase I trial investigating the combination of megestrol acetate and paclitaxel was initiated (Markman et al., 2000). In 44 patients with paclitaxel-resistant EOC, four patients exhibited a response. However, 32% of patients experienced peripheral neuropathy, four patients developed venous blood clots, and one patient suffered from a stroke. Given the relatively low level of activity and significant toxicity, the authors did not recommend further study of the treatment regimen.

Mifepristone is a progesterone antagonist, more commonly known for its abortifacent properties. Rocereto et al. (2000) conducted a phase II study of mifepristone in the treatment of recurrent of persistent EOC, fallopian tube, or primary peritoneal cancer. Patients with persistent or recurrent disease less than 1 year after chemotherapy were eligible, and received mifepristone 200 mg daily for 28 day cycles. Of 44 patients enrolled, 34 were evaluable and 9 (26.5%) of patients experienced a response (9% CR, 17.5% PR). Duration of response was 1 to 3 months, except in one patient who continues to respond after three years. The major cited toxicity was rash.

5.3 GnRH agonists
GnRH agonists, principally leuprolide acetate, triptorelin, and goserelin, have been investigated in recurrent ovarian cancer. Emons & Schulz reported on 245 published phase II trials in patients with recurrent disease, mostly platinum-refractory, treated with GnRH
agonists: 9% had an objective remission and 26% experienced disease stabilization (2000). Compared to estrogen-directed endocrine therapy, responses are minimal and GnRH agonist therapy is generally not used in this setting anymore.

Initial studies with GnRH agonists were small, with 5 to 37 patients, and employed leuprolide acetate in the setting of recurrent EOC. Of 161 combined patients in 7 studies, there were 2 reported CRs, 15 PRs, and a disease stabilization rate of about 24% (So et al., 2008).

Triptorelin was the next generation GnRH agonist examined in EOC, and results were generally disappointing. In a large European Organisation for Research and Treatment of Cancer (EORTC) study, 74 patients with progressive ovarian cancer were treated with the LHRH agonist triptorelin via IM injections of 3.75 mg (on days 1, 8, and 28 followed by monthly injections thereafter) (Duffaud et al., 2001). There were no objective responses, and only 16% of patients experienced stable disease with a median PFS of 5 months for SD. Though the treatment was well-tolerated, the authors concluded that triptorelin exhibits only modest efficacy in this patient cohort. Emens et al. also conducted a large study, with the benefit of a prospective randomized double blind trial design (1996) in 135 patients with Stage III or IV EOC after cytoreduction. Patients received standard platinum-based chemotherapy and were randomized to placebo or triptorelin 3.75 mg IM. There was no difference in survival between the two groups.

Following triptorelin, cetrorelix—a GnRH antagonist—was found in vitro to have better activity in ovarian carcinoma, and was hypothesized to act directly on the GnRH receptors in the tumor as well as centrally (Yano et al., 1994). A phase II study utilized cetrorelix 10 mg subcutaneously daily in 17 patients with platinum-resistant recurrent EOC or mullerian carcinoma. Three patients had a PR of 9, 16, and 17 weeks, there was one grade 4 anaphylactic reaction, and two patients exhibited a 20% increase in cholesterol not requiring treatment. Stable disease was observed in 35% of patients for up to 62 months. They also examined LHRH receptor status, and two of the three responding patients were LHRH positive.

Goserelin, another GnRH agonist, was evaluated in combination with tamoxifen in a phase II trial for patients with recurrent advanced ovarian cancer (Hasan et al., 2005). Patients had received a median of 3 prior chemotherapy regimens, and 17 of 26 patients had platinum-resistant disease. Tamoxifen was prescribed at 20 mg orally daily, and goserelin was provided subcutaneously at 3.6 mg once monthly. The overall response rate was 50%, with one CR, 2 PRs, and 10 with SD. Median PFI was 4 months, while median OS was 13 months. The regimen was well-tolerated. Zidan et al. (2002) treated 15 patients with advanced recurrent disease with 3.6 mg of goserelin monthly; two of these patients had not received initial chemotherapy due to poor performance status. They reported one CR lasting 8 months, one PR lasting 14 months, and disease stabilization in 20% for a median of 7.5 months, and there was no significant toxicity. Most recently, a 2007 study considered the activity of goserelin 3.6 mg subcutaneously monthly and bicalutamide (an oral anti-androgen often used in prostate cancer) 50 mg orally daily in patients in their second or greater complete disease remission (Levine et al.). Of 35 patients, PFS for second disease remission was 11.4 months, and was 11.9 months for patients in their third or fourth disease remission. There was no association between androgen receptor expression and PFS. Toxicities included liver function abnormalities, fatigue, and hot flushes. They concluded that the combination did not prolong PFS in patients with second or greater disease remission.
Emons et al. recently reported on a novel compound, AEZS-108, which is composed of [D-Lys]LHRH linked to doxorubicin (2010). In a phase I dose escalation trial of 17 women with metastatic unresectable EOC, endometrial, or breast cancer and immunohistochemical LHRH positivity, a total of 6 patients exhibited responses, both in the highest dose group. Dose-limiting toxicities included leukopenia and neutropenia.

### 5.4 Antiandrogens in ovarian cancer

Few studies exist assessing the efficacy of antiandrogenic therapy in ovarian cancer. Flutamide, a nonsteroidal antiandrogen, has been studied in the phase II setting (Vassilomanolakis et al., 1997). In 24 patients with relapsed stage III or IV ovarian cancer, flutamide was given at a dose of 100 mg three times daily. There was one partial response lasting 3 months, and two patients with stable disease for 7 and 8 months. Reported toxicity was mild. Another trial utilized flutamide in 68 pretreated patients with EOC (median prior chemotherapy of two regimens), dosed at 750 mg/daily for at least 2 months (Tumolo et al., 1994). Of 68 patients, there was one complete and one partial response lasting 44 and 72 weeks, and 28% of patients experienced stable disease for a median of 24 weeks. Toxicities included nausea and vomiting in 34.5% of patients. The authors concluded that flutamide was ineffective in heavily pretreated patients, in light of the significant percentage of side effects.

### 5.5 Ongoing clinical trials with hormonally-targeted therapeutics in ovarian cancer

While there are many clinical trials open to ovarian carcinoma patients, there are not many currently investigating hormonally-directed agents. There is currently one phase II trial exploring tamoxifen in combination with the EGFR tyrosine kinase inhibitor ZD839 in patients with recurrent ovarian cancer refractory to platinum and taxane-based therapy (NCT00189358). There are a few trials exploring the newer SERMs. One of the newer agents, arzoxifene, is a SERM with higher potency than raloxifene. A current study, NCT00003670, is examining arzoxifene in patients with metastatic refractory EOC or peritoneal cancer in the phase II setting, and aims to correlate response to serum estradiol, FSH, LH, and SHBG. Another SERM, toremifene citrate, has just finished assessment in the recurrent ovarian cancer population, but results have not yet been published (NCT00003865). Endoxifen, a tamoxifen-related compound with higher affinity for the estrogen receptor, is undergoing evaluation in the setting of hormone-receptor positive breast, solid, desmoid, or gynecologic tumors which have not responded to standard chemotherapy (NCT01273168).

Some studies with aromatase inhibitors have completed recruitment. One study with combination anastrozole and gefitinib, an EGFR inhibitor, has completed enrollment in a phase II trial for patients with relapsed ovarian cancer (NCT00181688). Exemestane has also been utilized in a phase II trial in patients with recurrent stage II to IV ovarian cancer (NCT00261027). The trial has completed enrollment, and initial results presented at the American Society of Clinical Oncology reported that in 24 patients, 36% experienced stable disease lasting a median duration of 23 weeks (Verma et al., 2006). One patient had stable disease lasting greater than 95 weeks.

Though there are no current trials investigating progesterone-based compounds and active disease, NCT00445887 is studying the use of oral levonorgestrel to prevent ovarian carcinoma in patients at high risk of developing EOC.
6. Granulosa cell tumors of the ovary

As aforementioned, while it is generally agreed upon that gonadotropins contribute to tumor progression in ovarian carcinoma, there is a lack of clear evidence that they can initiate carcinogenesis in EOC. Claims that gonadotropins may cause EOC are derived from in vivo studies, in which rodents treated with excess gonadotropins formed sex cord stromal tumors, specifically granulosa cell tumors. In α-inhibin deficient mice, which results in a lack of negative feedback on the gonadotropin axis and resultant excess gonadotropins, gonadal stromal tumors formed (Matzuk et al., 1992). However, the tumors failed to form in these α-inhibin deficient mice in the absence of gonadotropins (Kumar et al., 1996). Dorward et al. (2007) treated hypogonadotropic immunodeficient mice with grafted ovaries from prepubertal genetically susceptible mice with an LH analog or FSH, and found that LH-treated mice developed granulosa cell tumors, while FSH-treated mice did not.

The role of granulosa cells in the ovary is well-established: they convert androgens to estradiol via aromatase, and are stimulated to do so by FSH. They also produce progesterone during the later stages of the menstrual cycle. Similarly, steroid signaling in granulosa cells has been extensively studied in vivo. In granulosa cells, FSH activates several pathways, including ERK, MAPK, and PI3K (Hunzicker-Dunn & Maizels, 2006). Estrogen protects against FasL-mediated apoptosis in the G1 to S phase transition of the cell cycle (Quirk et al., 2006), and GnRH agonists stimulate apoptosis (Takekida et al., 2003). In malignant granulosa cell tumors (GCTs), ER2 is upregulated (Chu et al., 2000). Nearly all GCTs express progesterone receptors, while about 30% express estrogen receptors (Hardy et al., 2005).

Granulosa cell tumors comprise approximately 5% of all ovarian tumors (Schumer & Cannistra, 2003), and often present with symptoms associated with estrogen excess—such as vaginal bleeding or virilization in postmenopausal women, or precocious puberty in juveniles. Given their relative rarity, there is a paucity of clinical trials, but antihormonal therapy has had activity in recurrent disease. There have been reports of disease stabilization or response after administration of megestrol acetate, goserelin, leuprolide, and anastrozole (Fishman et al., 1996; Freeman & Modesitt, 2006; Malik & Slevin, 1991; Martikainen et al., 1989).

7. Conclusions

In summary, steroid signaling plays an important part in both normal ovarian surface epithelial cells and in malignant epithelial ovarian carcinoma. The pathways that promote growth and inhibit apoptosis in OSE are often exploited and upregulated in EOC, while protective components are downregulated, thereby allowing evasion of apoptosis as well as cellular migration and invasion. While estrogen and FSH promote cellular growth and tumor progression in vitro and in vivo, there is no definitive evidence that they can initiate ovarian carcinogenesis. Progesterone impressively enhances apoptosis, and is valuable in preventive efforts, yet treatment with progestins in ovarian cancer has not yielded equally impressive results in the clinical setting. The reasons for this remain unclear, and warrant further investigation into signaling pathways and receptor crosstalk mechanisms. Perhaps even more interesting, the GnRH signaling pathway appears to operate via dual and opposing mechanisms, inhibiting cell growth through one pathway yet increasing cellular migration and invasion through another. Efforts to exploit the growth inhibitory effects
while curbing the invasive component may generate more effective GnRH analogs. Despite our growing knowledge surrounding steroid hormone signaling in OSE and EOC, much still remains unknown.

While serum levels of steroid hormones are generally not useful as disease markers in determining disease risk or prognosis, ovarian tumors undoubtedly contain elevated levels of gonadotropins. Whether or not these elevated levels are a byproduct of aberrant signaling pathways or a contributor to carcinogenesis remains to be determined. Tumor expression of estrogen and progesterone receptors does appear to correlate to treatment response in patients, though results regarding estrogen receptor expression and prognosis are mixed. Certainly, the shift in ERα: ERβ expression and loss of PRB in tumor progression merits further investigation, and indeed has triggered exploration into targeted therapy. Inhibiting these receptor subtypes may result in more robust, specific responses in disease. While fulvestrant is an ERα antagonist, attention has now turned towards developing ERβ agonists. Benzopyran-derived selective estrogen receptor beta-agonist-1 (SERBA-1) is a selective ERβ receptor agonist which has been studied in mice and prostate hyperplasia, with promising effects (Norman et al., 2006). Monoaryl-substituted salicylaldoximes also show high ERβ affinity and are interesting new compounds (Bertini et al., 2011).

The role of exogenous hormones in ovarian cancer also remains unclear. As Risch suggests, at best HRT may accelerate the proliferation of pre-existing malignancy (Risch, 2002). Despite the completion of multiple large cohort studies, confounding and bias are likely responsible for ambiguous results. Still, the use of HRT is best avoided in women at high risk of developing EOC, such as BRCA mutation carriers, or women with a strong family history of ovarian cancer. Conversely, use of oral contraceptive prophylaxis is recommended for women with a high risk of EOC, to be weighed against a perhaps slightly elevated risk of breast cancer with extended use.

Hormonally-targeted therapeutics offer modest benefit for women with recurrent ovarian cancer, along with a much more tolerable side effect profile when compared to cytotoxic chemotherapy. In all fairness, clinical trials have not included hormone antagonists as first-line agents; they are most frequently studied in recurrent disease when even cytotoxic chemotherapy yields little benefit. It is possible that antihormonals may offer even more activity when used up front in combination with cytotoxic compounds, and this is an area warranting further investigation.

As is the case with emerging targeted therapies and resistance, inhibition of one pathway often results in upregulation of another, requiring combination with other therapeutics. Combination therapy of hormone antagonists with novel targeted agents offers exciting opportunities for overcoming resistance and improving patient outcomes. For example, the estrogen receptor pathway participates in crosstalk with multiple other mitogenic pathways, and concomitant inhibition of these pathways could produce a synergistic effect. Targeting other portions of the hormone receptors themselves has also evoked interest. Small molecule inhibitors target alternative binding sites on the estrogen and androgen receptor, which may result in improved selectivity or novel interactions (Shapiro et al., 2011). Likewise, while estrogen and progesterone receptor expression does correlate with response to antihormonal therapy, those patients without receptor expression also experience some benefit. Thus, better markers for response are needed. Post-translational modification of steroid receptors—particularly the estrogen receptor—include phosphorylation, ubiquitination, sumoylation, methylation, and palmitoylation, and affect receptor stability, localization, and perhaps drug resistance (Le Romancer et al., 2011).
Steroid hormones and their receptors participate in complex signaling which is not yet fully understood. Gaining insight into these interactions and downstream effectors is paramount to developing new targeted therapies and advancing the treatment of ovarian cancer.

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