Resistance to chemotherapy and hormone therapy in endometrial cancer

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Abstract

Endometrial cancer is the most common gynecological malignancy in developed countries and represents the eighth leading cause of cancer related death in women. The growing incidence of endometrial cancer leads scientists and oncologists to identify effective preventive measures and also molecular markers for diagnosis and prognosis. Chemotherapy and hormone therapy is the mainstay treatment option for advanced and recurrent endometrial cancer and response to therapy is one of the most important factor which favors prognosis and overall survival. In recent years, there have been major advances in the treatment of patients with endometrial cancer. Despite advances made in the treatment of this cancer, the overall survival of patients has not significantly improved because considerable number of patients harbor tumor refractory to these therapies and the majority of the initially responsive tumors become refractory to treatments. Therefore, determination of sensitivity/resistance is becoming increasingly important for individualization of endometrial cancer therapy. The aim of this review is to present the existing knowledge about the molecular markers that could play a crucial role in determining resistance to chemo- and hormone therapy. Extensive literature search for the cell signaling pathways and factors responsible for chemoresistance have been performed and reviewed. Several recent studies suggest that deregulations in the apoptotic pathways (such as p53, Fas/FasL, Bcl-2 family proteins, inhibitor of apoptosis proteins), survival pathways (PI3K/AKT, MAPK), hormone receptor signaling pathways (progesterone receptor), Cyclooxygenase-2 and Her-2 are considered as key factors involved in the onset and maintenance of therapeutic resistance, suggesting that resistance is a multi-factorial phenomenon.

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Introduction

Endometrial cancer represents the most common gynecological malignancy in the western world. While early-stage endometrial cancer has favorable prognosis, advanced or recurrent disease presents a major hurdle. Chemotherapy and hormonal therapy play a major role in the management of these advanced and recurrent endometrial cancers. The majority of patients with advanced endometrial cancers relapse despite state-of-the-art first-line surgery, hormone therapy, and chemotherapy because a proportion of primary tumors are intrinsically refractory to treatment. Moreover, initially responsive tumors become refractory to treatment, due to the emergence of

resistant tumor cells during treatment. This topic has been the subject of intense research, and previous studies on chemoresistance in gynecological cancers that have investigated potential involvement of molecules involved in drug transport, apoptosis, DNA repair, and detoxification pathways (Aebi *et al.* 1996, Coukos & Rubin 1998, Montgomery *et al.* 2000, Agarwal & Kaye 2003, Vasey 2003, Li *et al.* 2007). This review first outlines some of the major regulatory pathways implicated in control of cell fate and the importance of individual molecules in these pathways in the phenomenon of therapeutic resistance in endometrial cancer. The endometrium is a hormone-responsive tissue so it is important to consider hormone

therapy and further acquired resistance in this process. We then elucidated some of the recently established deregulations in steroid receptor signaling pathway and how they are important in hormone therapy. Finally, we explore some potential avenues for future work in this area. Although several review papers have been written in the area of endometrial cancer (Pagel & Bock 1984, Leo *et al.* 1997, Kaaks *et al.* 2002, Decruze & Green 2007, Voskuil *et al.* 2007) yet there are very few studies that have set out to study the biology of chemotherapy and hormone therapy in endometrial cancer, and those that exist are still inconclusive. In this manuscript, we will review the existing knowledge about the biomarkers for the prediction of response to therapy in endometrial cancer. Early identification of the primary chemoresistant patients could lead to treatment with other experimental therapeutics because standard treatment affords them little benefit.

Endometrial cancer

Epidemiology and treatment

Endometrial cancer is the cancer of the uterus that begins in the epithelial glandular lining of the uterus (endometrium) and accounts for about 90% of uterine cancers (adenocarcinoma, type 1). The non-endometrioid (type 2) endometrial cancers are less frequent and have papillary serous or clear-cell histology with a very aggressive clinical outcome. Unopposed estrogen exposure has been associated with type 1 endometrioid cancers and the presence of estrogens is often responsible for initiation of premalignant phase of the disease. Overall, endometrial cancer is the most common gynecologic cancer and ranks as the fourth most common neoplasm and the eighth leading cause of death from cancer in females (Jemal *et al.* 2005). This cancer usually develops after menopause with a peak incidence around 65–75 years and the risk continues to climb with each successive decade. Risk factors for endometrial cancer include age at menarche, age at menopause, history of infertility, obesity, diabetes, polycystic ovarian syndrome, and prior pelvic radiation therapy. People with a family history of this disease, and people in families with some types of inherited colon cancer or breast cancer may have an increased risk (Decruze & Green 2007). However, the most important risk factor for endometrial cancer is prolonged exposure to endogenous or exogenous estrogens (Kitchener 2006). Basically, high levels of estrogen compared with progesterone (PG) in the

body increase the risk of endometrial cancer. This hormonal imbalance causes the lining of the uterus to get thicker and thicker. If the lining builds up and stays that way, then cancer cells can start to grow. Despite the advances that have been made in the early detection and treatment of this disease, both the annual incidence and the death rate associated with endometrial cancer appear to be rising (Somoye *et al.* 2005, Sonoda & Barakat 2006).

The four basic types of treatment for women with endometrial cancer are surgery, radiation therapy, hormonal therapy, and chemotherapy. Surgery is the main treatment for most women with this type of cancer. Most patients present with localized disease that can be treated by surgery followed by radiotherapy. Fortunately, this is also one of the most curable cancers when detected in the early stages. For patients with advanced-stage or recurrent disease, who have already received tumor debulking and/or radiotherapy, hormonal treatment with progestins or combination chemotherapy is administered (Creasman *et al.* 2001). If discovered at an early stage, patients with endometrial adenocarcinoma have a good prognosis; nearly 90% of women survive at least 5 years. However, patients with recurrent endometrial adenocarcinoma (stage III–IV) face a dismal prognosis with an overall survival of only 7.7%. To date the results of treatment of advanced and/or metastatic endometrial cancer with either chemotherapy and/or hormone therapy have been quite disappointing in adjuvant setting (Markman 1992, Neijt 1994). Since the majority of relapses develop in distant sites (Gadducci *et al.* 2004, Kew *et al.* 2005), the pharmacological treatment plays a major role for the management of recurrent disease.

Chemotherapy and hormone therapy (treatment options for advanced and recurrent disease)

One of the major goals of cancer therapy is to trigger selective tumor cell death. Chemotherapy is rather a general term used to describe the treatment of tumors, as each chemotherapeutic agent acts via different pathways. The slow development of chemotherapy in endometrial cancer is probably due to both the frequent use of progestins in patients with advanced or recurrent disease and the suggestion that many patients with this malignancy are too old or too sick to tolerate chemotherapy (Dows & Boente 2004). It is now well documented that most cytotoxic anticancer agents cause tumor cell death by causing irreparable DNA damage that ultimately triggers apoptosis

(Park *et al.* 2002, Kaufmann & Vaux 2003, Wang & Lippard 2005, Cepeda *et al.* 2007). Cisplatin is the most effective cytotoxic agent. In combination chemotherapy for endometrial cancers, usually a platinum-based drug is coupled with either doxorubicin or paclitaxel. Indeed, a number of randomized studies were carried out on adjuvant chemotherapy in endometrial cancer. GOG-177 was the first phase III study on chemotherapy in advanced or recurrent endometrial cancer that showed a survival advantage. In this study, the Gynecologic Oncology Group showed that paclitaxel–doxorubicin–cisplatin was better than doxorubicin–cisplatin, but the toxicity of the three-drug regimen has precluded general acceptance (reviewed in Hogberg 2008). The combination of cisplatin with paclitaxel resulted in an impressive response rate and the longest median survival in a small group of heavily pre-treated patients (Gebbia *et al.* 2001).

These drugs have different mode of action. The action of cisplatin is thought to be associated with its ability to form inter- and intra-strand DNA cross-links causing G1 arrest (Kelland 2000, Un 2007). Recent reports suggest that apoptosis may be the cellular underpinning of cisplatin-induced cell death and that the DNA-damaging effects of cisplatin are also associated with expression of specific death genes and down-regulation of ‘survival’ counterparts (Li *et al.* 2000, 2001, Fraser *et al.* 2003a). As for doxorubicin, it interacts with DNA by intercalation, thereby inhibiting macromolecular biosynthesis and generating free radicals and hydrogenperoxide which activate mitochondria-induced apoptosis (Mizutani *et al.* 2005), whereas paclitaxel interferes with the normal function of microtubule growth (Schiff & Horwitz 1980). Finally, these phenomena ultimately lead to the activation of apoptotic cell death in tumor cells and consequently the efficacy of cancer treatments depends not only on the cellular damage they cause but also on the cell’s ability to respond to these damages by inducing the apoptotic machinery. However, the clinical impact of chemotherapy remains modest because of a high degree of inherited and/or acquired chemoresistance.

The endometrium is a hormone-responsive tissue that proliferates in response to estrogen exposure and the endometrial growth is arrested by PG during the menstrual cycle (Shafi *et al.* 2000). Therefore, hormone therapy is a systemic treatment that uses PG to balance the effects of estrogen and reduces tumor growth. The effectiveness of hormone therapy depends on the presence of proteins called hormone receptors e.g. progesterone receptors (PRs) in cancer cells.

Therefore, prior to treatment, a hormone receptor test may be performed to determine if the endometrial tissue contains these proteins. Receptor estimation is not yet accepted as the standard of care in endometrial cancer, and concerns remain that immunohistochemical analysis of PR alone may be insufficient for accurate prediction of hormone response (Richer *et al.* 2002). A wide range of hormonal agents has been assessed in endometrial cancer since the early studies by Kelley & Baker (1965) who were the first to use PG to treat patients with advanced endometrial cancer. The most commonly used therapeutic hormones are natural and synthetic PG based agents, but a variety of other hormonal interventions have been evaluated. Tamoxifen, an antiestrogen with clear beneficial effect on survival and recurrence in hormone-sensitive breast cancer, was identified as a therapeutic agent in endometrial cancer in the early 1980s. As a single agent, tamoxifen has more modest activity than progestogens (Moore *et al.* 1991), and several *in vitro* and *in vivo* studies provided the rationale for the clinical use of tamoxifen sequentially with progestogens for increased therapeutic benefit (Fiorica *et al.* 2004). A third generation of selective estrogen receptor modulators has now been developed, with one study showing the estrogen antagonist arzoxifene demonstrating significant activity in endometrial cancer (Burke & Walker 2003). In a recent study, we have also demonstrated that the antiestrogens like raloxifene also exerts partial agonistic/antagonistic activity on endometrial cancer cells (Leblanc *et al.* 2007).

Finally, all these drugs and agents ultimately lead to the activation of apoptotic cell death in tumor cells. Although, it is important to remember that inherent resistance at the molecular level is possibly the most serious barrier to successful chemo- and hormone therapy, other issues including tumor cell kinetics, drug metabolism, drug penetration within the tumor, and side effects on normal tissues are also critical factors in determining how an individual may respond to these therapies. However, it is important to remember that no biochemical pathway stands on its own. It is accepted that the process of drug-induced apoptosis is governed not only by the upregulation of pro-apoptotic factors or tumor suppressors, but also by modulation of cell survival factors. Therefore, a detailed understanding of the molecular mechanism by which these chemotherapeutic drugs and hormones induce cell death should provide a fundamental approach for increasing the sensitivity of endometrial cancer cells to these anticancer agents.

Molecular determinants of therapeutic resistance in endometrial cancer

Cell death (apoptotic) pathways

Apoptosis is a physiological mode of cell death that can be induced by adverse conditions, such as cellular stress, cross-linking of death receptor, and exposure to anticancer drugs (Reed 2000). Apoptosis occurs through two main pathways i.e. extrinsic or death receptor pathway and the intrinsic or mitochondrial pathway (as shown in Fig. 1). Both pathways converge to a final common pathway involving the activation of a cascade of proteases called caspases that cleave regulatory and structural molecules, culminating in the death of the cell.

The extrinsic death receptor pathway

The extrinsic pathway is activated by members of two protein families, the tumor necrosis factor (TNF) family and the receptors for these ligands (TNFR). For example, FasL binds to Fas; TNF binds to TNFR1; Apo3 ligand binds to DR3 and TRAIL binds to DR4 and DR5 (Locksley *et al.* 2001). The salient point of death receptor signaling is the formation of the death inducing signaling complex (DISC; Walczak & Sprick 2001). Upon activation of death receptors, such as Fas, the cytoplasmic adaptor molecule Fas-associated death domain (FADD) interacts with the intracellular regions of the receptor proteins. The apoptotic signal is then

transduced to pro-caspase-8 upon the interaction of FADD with pro-caspase-8 (Donepudi *et al.* 2003). The pro-domain of caspase-8 remains at the DISC, while active caspase-8 dissociates from the DISC, which is further released into the cytosol to propagate the apoptotic signal by initiating intracellular members of caspase family, mainly caspase-3. Several inhibitory proteins for this pathway have been characterized, like FADD-like interleukin-1 β converting enzyme/caspase-8 inhibitory protein (FLIP), Fas associated phosphatase-1 (FAP-1), and soluble Fas (sFas; Cheng *et al.* 1994, Sato *et al.* 1995, Irmeler *et al.* 1997). Overexpression of any of these molecules can lead to resistance to Fas mediated apoptosis in endometrial cancer (Lee *et al.* 1999, Abe *et al.* 2006, Llobet *et al.* 2008).

Fas-mediated apoptosis has also been shown to be important for endometrial remodeling/cycling and accumulating evidence suggest that dysregulation of the Fas/FasL interactions may have an important role in the development of endometrial cancer (Song *et al.* 2002, Atasoy *et al.* 2003, Abe *et al.* 2006). Fas gene mutations and single-nucleotide polymorphisms (SNP) in the promoter region have also been described for endometrial cancers (Ueda *et al.* 2006). However, the impact of these mutations on response to therapy and prognosis is not well defined and is yet to be understood in endometrial cancer. There are reports in other gynecological cancers that can lead to interesting avenue of research. In ovarian cancer cell

Figure 1 Apoptosis signaling and the PI3K/AKT survival pathway activation in response to chemotherapeutic drugs and hormones.

lines, the expression of Fas has been shown to be upregulated by chemotherapeutic agents like cisplatin (Uslu *et al.* 1996, Muller *et al.* 1997). Moreover, cisplatin was also shown to induce Fas redistribution in the membrane thus leading to increased cell apoptosis (Mansouri *et al.* 2003).

Recently, a number of studies have been designed with the aim of overcoming tumor resistance by combining conventional drugs to various biomolecules in search of new treatment modalities (Krosnick *et al.* 1989, Mizutani & Bonavida 1993, Uslu *et al.* 1996). For example, combining ligation of death receptors with exposure to drugs increased tumor cell death in both drug resistant cell lines and primary ovarian carcinoma cells, even though these cells were not sensitive to death receptor ligation alone. Sadarangani *et al.* (2007) have demonstrated that TRAIL at concentrations able to kill cancerous cells does not mediate apoptosis of normal cells. Similarly, Kato *et al.* (2007), reported that the estrogen metabolite 2-methoxyoestradiol alone or in combination with TNF-related apoptosis-inducing ligand mediates apoptosis in cancerous but not healthy cells of the human endometrium thus suggesting that TRAIL may be an effective treatment for endometrial cancer.

We have recently shown that TNF- α can efficiently induce apoptosis in endometrial carcinoma cells. Furthermore, we identified a key role for X-linked inhibitor of apoptosis (XIAP) in regulating cellular sensitivity to TNF- α -induced apoptosis, and highlighted the potent sensitizing effect of protein kinase C (PKC) inhibition. Therefore, local administration of recombinant TNF- α , in combination with PKC inhibitors or other agents targeting XIAP could represent an alternative therapeutic approach for the treatment of endometrial cancers (Van Themsche *et al.* 2008). However, the mechanisms responsible for reversal of drug resistance by combination treatments are not fully understood. Chen *et al.* (2005) suggested that c-FLIP might contribute to the carcinogenesis and aggressiveness of endometrial carcinoma and might be a useful prognostic factor. Modulation of FLIP expression has also been implicated in chemoresistance, and it has been shown that cisplatin significantly decreased FLIP protein level, induced cleavage of caspase-8 and caspase-3, and increased apoptosis in a concentration dependent manner in cisplatin-sensitive but not -resistant cells (Abedini *et al.* 2004). Since activation of the Fas/FasL system is an important mechanism of cisplatin-induced apoptosis, the possibility that FLIP is differentially regulated in chemoresistant endometrial cancer cells can not be excluded. This cell survival factor may be an important

determinant in chemoresistance in endometrial cancer and may serve as a molecular target for the development of novel therapies for chemoresistant endometrial cancer.

Expression of FAP-1, a negative regulator of Fas mediated apoptosis has also been investigated in endometrial cancer (Lee *et al.* 1999, Rey *et al.* 2000). However, the correlation between expression and response to therapy has yet to be investigated. In an *in vitro* study, transfection with FAP-1 antisense RNA was combined with carboplatin which resulted in more pronounced apoptosis as compared with 'carboplatin' treated SKOV3 cells, which implies that interference with FAP-1 expression may reverse drug resistance in ovarian cancer (Wang *et al.* 2004). Increased serum levels of soluble TNF- α (Gadducci *et al.* 1996, Chopra *et al.* 1997) and sFas (Konno *et al.* 2000) have been reported in endometrial cancer where they have been correlated with more invasive and advanced stage tumors. However, recently the role of sFas as a predictor of chemotherapy response have been investigated in ovarian cancer (Chaudhry *et al.* 2008), where the higher sFas levels significantly correlated with chemoresistance in the patients. Thus, we cannot rule out the possibility that sFas could also have a crucial role in determining the chemoresponsiveness of endometrial cancer cells. Moreover, most of the work regarding chemoresistance has been performed in ovarian cancer and given that the ovarian epithelium and endometrium share a common embryologic origin and similar reproductive and hormonal risk factors for malignancy, these therapeutic regimens have been proposed to have similar biological effects in the endometrium as those in the ovary. This however, needs to be confirmed and validated.

The intrinsic mitochondrial pathway

The intrinsic pathway is usually activated in response to intracellular stress signals (DNA damage and high levels of reactive oxygen species) and extracellular apoptotic stimuli (u.v.- and γ -radiation, chemotherapeutic drugs, the removal of cytokines, viral infection, and the detachment from extracellular matrix (Danial & Korsmeyer 2004). The intrinsic apoptotic pathway is dominated by the Bcl-2 family of proteins, which regulate the release of cytochrome *c* from the mitochondria (Puthalakath & Strasser 2002). Following a death signal, the pro-apoptotic Bax and Bak undergo a conformational change resulting in their integration into the mitochondrial outer membrane where it appears that they antagonize the function of pro-survival Bcl-2 proteins (Verrier *et al.* 2004).

Bax homodimers and heterodimers then interact with a voltage-dependent anion channel in the mitochondrial outer membrane to release cytochrome *c* by increasing mitochondrial membrane permeability via opening of the mitochondrial permeability transition pore (Acehan *et al.* 2002). The release of cytochrome *c* results in the formation of an apoptosome complex (~1.4 MDa), which consists of apoptosis activating factor-1 and procaspase-9 in the presence of dATP/ATP. Procaspase-9 promotes its self-activation (Salvesen & Duckett 2002). The activated initiator caspase i.e. caspase-9, leads to activation of one of the effector caspases, which can be caspase-3, -6 or -7. The active caspase further cleaves an inhibitor of caspase-activated DNase lamins, several cytoskeleton binding proteins and poly (ADP-ribose) polymerase (PARP). Cleavage of these proteins causes DNA fragmentation, inhibition of DNA synthesis and repair, nuclear membrane disruption, chromatin condensation, and cytoskeleton collapse.

Activation of caspases can be blocked by members of the inhibitor of apoptosis protein (IAP) family, which bind to and inhibit these death proteases. Eight human IAP family members have been identified so far: c-IAP1, c-IAP2, XIAP, NAIP, survivin, apollon, ML-IAP/livin, and ILP-2 (Verhagen *et al.* 2000). IAPs can inhibit activation of executioner caspases activated by extrinsic or intrinsic pathways. Thus, the release of cytochrome *c* does not necessarily result in apoptosis, and under certain circumstances cells survive. Important molecules able to inhibit IAP functions are Smac/Diablo and Omi/HtrA2 (Verhagen *et al.* 2000, Suzuki *et al.* 2001). When released from mitochondria, Smac/Diablo and Omi/HtrA2 trigger the sequestration and/or degradation of IAPs, therefore, ensuring full activation of apoptosome thus directly activating caspase-3 (Suzuki *et al.* 2001).

The role of the mitochondrial pathway, associated with Bcl-2 and Bax proteins, in the pathogenesis of endometrial cancers was well established in previous immunohistochemical studies. Specifically, Bcl-2 is down-regulated and Bax is up-regulated in the sequence of events from endometrial hyperplasia to endometrial carcinoma (Wehrli *et al.* 1998, Kokawa *et al.* 2001, Bozdogan *et al.* 2002). Some authors have suggested that a frameshift mutation of *Bax* may be involved in the genesis of endometrial carcinoma (Burks *et al.* 1994, Catusus *et al.* 1998, Wehrli *et al.* 1998, Kokawa *et al.* 2001). Overexpression of anti-apoptotic members of the Bcl-2 family such as Bcl-2 and Bcl-XL have been implicated in cancer chemoresistance, whereas high levels of pro-apoptotic proteins such as Bax promotes apoptosis and sensitize tumor

cells to various anticancer therapeutics (Burks *et al.* 1994, Reed 1997, Catusus *et al.* 1998). However, most of the work correlating chemoresistance and chemoresponsiveness has been performed in cancer derived cell lines. In a study using ovarian cancer cell lines, Yang *et al.* (2002) have reported that cisplatin resistance was associated with the overexpression of anti-apoptotic protein Bcl-2 and down regulation of caspase-3 activity, but not associated with the expression of Bax and Bcl-XL (Burks *et al.* 1994, Reed 1997, Catusus *et al.* 1998). Doxorubicin has been shown to upregulate the expression of Bax protein and downregulate the expression of antiapoptotic Bcl-2 proteins (Leung & Wang 1999). Similar results are also observed with paclitaxel which causes a decrease in Bcl-2 expression and increase in Bax expression (Tudor *et al.* 2000). As Bcl-2 down-regulation is regarded as a major factor of chemosensitivity, an antisense Bcl-2 (Genasense) is presently undergoing phase I/II clinical trials also in combination with taxanes (Chi *et al.* 2001, Tolcher *et al.* 2005).

As regards for the inhibitory molecules like IAPs, XIAP is the most studied. XIAP is shown to be overexpressed in endometrial cancer cells using immunohistochemistry (Wu *et al.* 2005, Van Themsche *et al.* 2008). Moreover, Wu *et al.* (2005) have also suggested that the degree of XIAP staining of tumor cells can help in identifying the most therapy-resistant cases (i.e. those with strong XIAP expression). In an *in vitro* study, we have observed that the three drugs (cisplatin, doxorubicin, and paclitaxel) reduced XIAP content and XIAP RNAi has also shown to increase the sensitivity to cisplatin and doxorubicin but not to paclitaxel in endometrial cancer cells (Gagnon *et al.* 2008). Another IAP called survivin that plays an important role in the development of ovarian cancer, could be a useful prognostic maker for patients with ovarian cancer and endometrial cancer (Ai *et al.* 2006, Kleinberg *et al.* 2007). Ai *et al.* (2006) have reported that inhibition of survivin by RNA interference reduced cell proliferation and induced apoptosis in endometrial Ishikawa cancer cells by activating caspase-3 and caspase-8 thus suggesting that survivin may be an attractive target for endometrial cancer treatment.

p53

The p53 network is involved in the cellular defense against various stress signals, such as DNA damage, oncogenes or aberrant growth factor signaling and stress caused by u.v. radiation, hypoxia or chemotherapeutic drugs (Vogelstein *et al.* 2000). Inactivation

of p53 was detected in 30 to 80% of ovarian cancer (Milner *et al.* 1993) and 10 to 20% in endometrial cancer (Saffari *et al.* 2005). SNP at codon 72 of p53 gene has also been correlated to lower overall survival and responsiveness to adjuvant chemotherapy in ovarian cancer (Saffari *et al.* 2005, Gadducci *et al.* 2006) and endometrial cancer (Saffari *et al.* 2005). Stimulation of disabled p53 pathways has been suggested as a potential mode of therapy for cancer. Since p53 is implicated in the control of cell cycle progression, p53 status is an important determinant of the sensitivity to chemotherapeutic drugs. However, the relationship between p53 status and tumor responsiveness to chemotherapy is still unclear since conflicting results have been reported. Cisplatin up-regulates p53 in a number of systems (Li *et al.* 2001, Qin & Ng 2002). In an *in vivo* study, p53 could predict response to chemotherapy in ovarian cancer (Lavarino *et al.* 1997). However, the precise mechanisms behind this phenomenon are not clear. It has been demonstrated that while cisplatin increases p53 content in chemosensitive p53 wild-type ovarian cancer cells, it fails to do so in a chemoresistant, p53 wild-type variant (Lavarino *et al.* 1997, Fraser *et al.* 2003b). Cisplatin, γ -irradiation, and other DNA damaging agents induce stabilization and nuclear translocation of p53 and thereby leading to induction of apoptosis (Kastan *et al.* 1991, Kuerbitz *et al.* 1992, Fritsche *et al.* 1993). It has also been postulated that p53 status might influence sensitivity to taxol resulting from increased susceptibility to apoptosis after persistent G2M arrest (Wahl *et al.* 1996). Since p53 inactivation is associated with upregulation of Bcl-2 and downregulation of Bax (Perego *et al.* 1997), it is possible that taxol treatment may modulate the relative levels of these apoptosis regulators (e.g. Bcl-2 phosphorylation). Yang *et al.* (2006) have shown that cisplatin induced mitochondrial p53 accumulation, the mitochondrial release of Smac, cytochrome *c*, and HTR/Omi, and apoptosis in chemosensitive but not in resistant ovarian cancer cells. However, it should be noted that the effects of p53 are complex and difficult to study. It is estimated that between 200 and 300 human genes are transcriptionally activated by p53 (Tokino *et al.* 1994). p53 can also act as a repressor of transcription (as for example, with Bcl-2) and there is presently no estimate of the number of additional genes that may be repressed by p53. The effects of many of these genes could potentially offset one another. Thus, additional investigations remain to be done in order to clarify the precise role of p53 as a determinant of chemosensitivity.

Cell survival pathways

The phosphatidylinositol 3-kinase/AKT pathway

The phosphatidylinositol 3-kinase (PI3K)/AKT represents one of the key pathways controlling survival, proliferation, and growth in cells (Fig. 1). This pathway can be activated by a diverse array of physiologic stimuli, including growth factor receptor signaling, several interleukins and stress, as well as by activated Ras proteins (Burgering & Coffey 1995).

PI3K belongs to a large family of PI3K-related kinases. PI3Ks are heterodimers with separate regulatory (p85) and catalytic (p110) subunits. A large numbers of the plasma membrane receptors, in particular those with tyrosine kinase (TK) activity, can activate class I PI3Ks and lead to receptor activation and autophosphorylation on tyrosine residues. This in turn leads, through an adaptor molecule to the recruitment of PI3K to the membrane. Once activated and localized to the membrane, PI3K phosphorylates phosphoinositol lipids on the D3 position of the inositol ring generating PtdIns-3-phosphates (PtdIns-3,4-P₂, and PtdIns-3,4,5-P₃; Fruman & Cantley 2002). The tumor suppressor PTEN antagonizes PI3K activity by converting the second messenger PIP₃ to its inactive state PIP₂. These specialized lipids serve to recruit pleckstrin homology (PH) domain-containing proteins such as the serine–threonine kinase AKT and phosphoinositide-dependent kinase 1 to the plasma membrane. Growth factor stimulation of PI3K activity leads to AKT activation. Conversely, PI3K inhibition (i.e. using chemical inhibitors such as wortmannin or LY294002) and PTEN mediated dephosphorylation of PtdIns-3,4,5-P₃, and PtdIns-3,4-P₂ results in inhibition of AKT. The serine–threonine protein kinase AKT (also known as protein kinase B, PKB) exists in three closely related isoforms, AKT1, AKT2, and AKT3. All of which share an N-terminal PH domain, a central kinase domain, and a serine/threonine-rich C-terminal region (Datta *et al.* 1999). After recruitment to the membrane, AKT is phosphorylated and consequently activated, by PDK at serine and threonine residues which in turn phosphorylates multiple downstream proteins. Proteins phosphorylated by activated AKT promote cell survival and control essential cellular processes, such as glucose metabolism and cell proliferation. Some proteins phosphorylated by AKT are Bad, caspase 9, Ikappa-B kinase, FKHR, MDM2, etc. Most importantly, AKT can exert effects on cell metabolism and growth through activation of the protein kinase ‘mammalian target of rapamycin’ (mTOR; Nave *et al.* 1999). Rapamycin is an mTOR inhibitor but due to poor water solubility and

stability in solution, it is not a good candidate for parenteral administration, and therefore some analogues of rapamycin, CCI-799 (tensirolimus), RAD-001 (everolimus), and AP-23573 (ARIAD), have been developed with improved pharmaceutical properties (Dutcher 2004). These agents are presently under evaluation in phases I–II trials in endometrial cancer. Additive effects of combined treatments of mTOR inhibitor RAD-001 and Tamoxifen on the growth and apoptosis of endometrial cancer cell lines has been reported (Treeck *et al.* 2006). The significance of this data in clinical situation has to be evaluated in further studies.

Recently, some of the genes contributing to the development of endometrial carcinoma have been elucidated, including PTEN which is the most frequently mutated gene ever reported in endometrial adenocarcinoma (Bilbao *et al.* 2006, Chow & Baker 2006). Genotyping studies indicate that ~50% of endometrial adenocarcinoma cells harbor a double allelic mutated PTEN gene (Hecht & Mutter 2006). PTEN is involved in the early phases of endometrial tumorigenesis, and it can be speculated that decreased PTEN expression with loss of differentiation in carcinoma can contribute to the emergence of tumors with a more aggressive phenotype (Kapucuoglu *et al.* 2007). Kanamori *et al.* (2001) showed that AKT was significantly phosphorylated in tumor tissue with a loss of PTEN expression, and that phospho-AKT (p-AKT) expression was negatively correlated with PTEN expression. This finding supports the basic evidence that AKT activation accompanied by PTEN inactivation is a key step in the development and/or progression of endometrial carcinomas. *PIK3CA* that encodes the catalytic subunit p110 of PI3K is also found to be frequently mutated in endometrial carcinoma and support the hypothesis that *PIK3CA* mutations may have an additive effect to PTEN monoallelic inactivation in endometrial carcinoma (Velasco *et al.* 2006, Ollikainen *et al.* 2007). Since, *PIK3CA* mutations were reported to be more common in tumors with *PTEN* mutations compared with those without *PTEN* mutations (Oda *et al.* 2005). Moreover, we know that loss of PTEN expression was significantly associated with positive p-AKT expression and this was also correlated to prognosis of endometrial cancer patients where it was observed that the patients with PTEN-positive and p-AKT negative expression clearly showed a higher survival rate (Uegaki *et al.* 2005). Endometrial cancer cells often have high levels of phosphorylated Akt seen in conjunction with a PTEN mutation or deletion. We have recently showed that the levels of PTEN in the endometrial cancer cells

are regulated by XIAP, which leads to the degradation of PTEN by ubiquitination thereby increasing AKT activity (submitted paper). Overexpression of AKT using a constitutively active AKT expression vector resulted in an up-regulation of cIAP-1 expression which suggested a pivotal role of AKT in the regulation of endometrial cancer cell survival through the up-regulation of a specific IAP (Gagnon *et al.* 2003). It has recently been demonstrated that PI3K/AKT pathway has a role to play in the resistance to anti-tumor agents. PTEN transfection significantly enhances doxorubicin chemosensitivity through effective induction of apoptosis by downregulation of the PI3K/AKT signaling pathway in Ishikawa cells (Wan *et al.* 2007). Furthermore, knock-down of PI3K p110- β by siRNA also induced decreased expression of cyclin E and Bcl-2, suggesting that PI3K p110- β stimulates tumor growth, at least in part by regulating cyclin E and Bcl-2. Thus, these results indicated that siRNA-mediated gene silencing of PI3K p110- β may be a useful therapeutic strategy for endometrial cancers with overexpression of PI3K p110- β (An *et al.* 2007).

Although XIAP and the PI3K/AKT pathway are important cell survival factors, if and how they interact in the induction and maintenance of chemoresistance is not known. But potential interactions between XIAP and the PI3K/AKT pathway do exist. It has been demonstrated that treatment of chemosensitive ovarian cancer cells with cisplatin decreases XIAP content, activates caspase-3 and -9, and induces AKT cleavage and apoptosis. However, these phenomena are not observed in the chemoresistant counterpart (Asselin *et al.* 2001, Shaw *et al.* 2008). Recently, we have shown that XIAP overexpression also increases the expression of p-AKT (Gagnon *et al.* 2008). Thus, it appears that there is an intricate, coordinated regulatory system at play between XIAP and the PI3K/AKT pathway.

Taken together, these data indicate that the PI3K/AKT pathway is a critical target for cancer intervention and that activation of this pathway is associated with chemoresistance in human endometrial carcinoma and over-expression of PTEN may represent a novel therapeutic approach for chemoresistant phenotype. Overall, these findings render the combined use of PI3K inhibitors with conventional drugs, a promising new strategy for cancer chemotherapy that should improve the response rate in resistant tumors which affect a large percentage of cancer patients.

The EGFR pathway

The epidermal growth factor receptor (EGFR) family embodies four homologous receptors: the

EGFR/HER1/ErbB1, HER2 (ErbB2), HER3 (ErbB3), and HER4 (ErbB4). The receptors consist of three main domains: a ligand-binding extracellular domain, a transmembrane segment, and an intracellular TK domain (Klapper *et al.* 2000). Activation of a monomeric receptor occurs by dimerization between two identical receptors (homodimerization) or between different receptors of the EGFR family (heterodimerization; Klein *et al.* 2004). This results in activation of the TK domain with subsequent activation of interrelated intracellular signaling pathways such as the PI3K/AKT pathway as discussed previously. Out of all the growth factor receptors, human EGFR (HER)2/neu is the most studied in endometrial cancer. HER2 is a transmembrane receptor, which is encoded by the *c-erbB2* gene located on chromosome 17. The important intracellular signaling pathways activated by HER2 include PIP3 and the AKT pathway (Ou *et al.* 2008). EGFR and HER2 gene polymorphisms in endometrial cancer has also been reported (Kitao *et al.* 2007) but the clinical significance of these polymorphisms has yet to be identified. Overexpression of HER2 gene is associated with high grade and high stage of endometrial cancers (Villella *et al.* 2006). HER2 status of an endometrial tumor has been shown to be an independent prognostic indicator as HER2 overexpression was associated with a poor overall survival and a very low relapse-free survival time. Therefore, HER2 has been considered a candidate marker of worse overall prognosis in endometrial cancer (Santin *et al.* 2005, Benevolo *et al.* 2007). Although, overexpression of HER2 helps to predict both prognosis and chemoresistance in spite of that the use of Herceptin (trastuzumab), a monoclonal antibody directed against HER2, for the therapy of patients with HER2 positive endometrial tumors should be further investigated in clinical trials (Slomovitz *et al.* 2004a). Transfection of HER2 antisense RNA suppressed the mRNA and protein expression in HEC-1-A cells, caused cellular apoptosis and inhibited cell growth, suggesting that it may be a more useful gene therapy for endometrial cancer (Zhao *et al.* 2007). Moreover, other selective inhibitors targeting the intracellular TK domains (TK inhibitors) have been explored for their therapeutic potential in the treatment of endometrial cancer (Slomovitz *et al.* 2004b, Menczer *et al.* 2005, Konecny *et al.* 2008).

Cyclooxygenases and prostaglandins

Cyclooxygenases (Cox) are the rate-limiting enzymes in prostanoid synthesis, which convert arachidonic acid into prostaglandin H₂, a substrate for specific

prostaglandin synthases (Herschman 1996). Presently, two Cox isoenzymes are known i.e. Cox-1 and Cox-2. Prostaglandin E₂ (PGE₂) is a Cox-1 and Cox-2 metabolite and is an important mediator of immunoregulation and epithelial survival. PGE₂ exerts its multiple effects through four G protein-coupled receptors (GPCR). It has been shown previously that GPCRs are able to transactivate the EGFR pathway leading to the promotion of cancer cell growth and motility (Pai *et al.* 2002, Buchanan *et al.* 2003). The main Cox inhibitors are the non-steroidal anti-inflammatory drugs (NSAIDs) which promote cancer cell apoptosis and are therefore being evaluated for cancer chemoprevention and therapy (Dannenberg *et al.* 2001). Some other Cox-2 selective NSAIDs, such as celecoxib and etoricoxib are still on the market.

Cox-2 is known to be associated with tumorigenesis in many cancers including endometrial cancer (Fowler *et al.* 2005), while there is substantial evidence for the tumorigenicity of Cox-1. We have reported that downregulation of Cox-2 with specific inhibition by NS-398 increased apoptosis in endometrial cancer cells (St Germain *et al.* 2004a). Since, AKT promotes gene expression of several pro-survival genes via nuclear factor kappa B (NF- κ B). Phosphorylation of I κ B kinase by AKT leads to activation of the transcription factor NF- κ B to oppose apoptosis. Activated IKKs phosphorylate I κ B thus targeting it for degradation by the proteasome. This allows NF- κ B to translocate to the nucleus and activate transcription of a variety of substrates including anti-apoptotic genes such as the IAPs c-IAP1, c-IAP2, and XIAP (Kane *et al.* 1999, Romashkova & Makarov 1999). We have demonstrated that regulation of Cox-2 by Akt is also mediated via NF- κ B in endometrial cancer cells (St Germain *et al.* 2004b). Knockdown of Cox-2 expression by shRNA or by using Cox-2 inhibitors (celecoxib) was also tested and showed potent antitumor activity and chemosensitization activity to taxanes in laryngeal cancer (Wang *et al.* 2008) and to doxorubicin in breast cancer (van Wijngaarden *et al.* 2007, Singh *et al.* 2008). Similar relationship between Cox-2 expression and chemosensitivity to cisplatin has also been investigated in colon cancer cell lines using selective Cox-2 inhibitor JTE-522 (Saikawa *et al.* 2004) and this hypothesis can also be tested in endometrial cancer, since Cox-2 has been shown to be involved in the development and progression of endometrial cancer (Ohno *et al.* 2005). Therefore, several biological agents like Cox-2 inhibitors or their synthetic derivatives show promise as apoptosis modulators, having the potential to place neoplastic cells into a more susceptible state for cell suicide. Knowledge about

the mechanisms by which these agents influence apoptosis pathways may suggest strategies for more effective and less toxic therapies.

Progesterone and progesterone receptors

In addition to chemotherapy, hormone therapy using steroid hormones or hormone antagonists is the other treatment option for recurrent endometrial cancer. These steroid hormones like estrogen and PG, act by diffusing through the plasma membrane and mediate their cellular actions through their specific receptors such as ER and PR which are members of the nuclear receptor superfamily, and following ligand binding are recruited to specific hormone response elements on the DNA. From these sites, they can stimulate or inhibit the transcription of a wide range of genes (Osborne & Schiff 2005). PG acts in reverse to the tumorigenic effect of estrogens and its effects are dependent on PR. The PR has two isoforms, A and B, which are expressed from a single gene (Feil *et al.* 1988). The relative expression of PR-A to PR-B has been shown to vary in the human endometrium during the menstrual cycle (Mangal *et al.* 1997). Many studies have been focused on finding the precise role of these two isoforms in endometrial carcinogenesis; however, it is clear that the relative expression of the two isoforms can represent an important prognostic factor in endometrial cancer (Saito *et al.* 2004). Arnett-Mansfield *et al.* (2001) reported a reduced PR expression level in tumors compared with normal glands in endometrial tissue. Promoter region polymorphism in PR have been associated with a risk of endometrial cancer and recurrence (De Vivo *et al.* 2002, Pijnenborg *et al.* 2005) and the use of DNA methyltransferase (*DNMT*) inhibitors (5-aza-20-deoxycytidine) and histone deacetylase (HDAC) inhibitors (Trichostatin A) leads to the restoration of PR gene expression in endometrial cancer (Ren *et al.* 2007). PR-B functions as a transcriptional activator in most cells, whereas PR-A is transcriptionally inactive and functions as a strong ligand-dependent transdominant repressor of steroid hormone receptor transcriptional activity. PG binding to the PR results in the association of the PG-PR dimer complex with specific coactivators and transcription factors. This activated complex binds to progesterone response elements in the promoters of target genes, resulting in modulation of transcription of those genes (Osborne & Schiff 2005) as shown in Fig. 2.

The association of hormone response and PRs has been known for many years (Kauppila 1984), but few studies in endometrial cancer have addressed the

Figure 2 Progesterone activation of progesterone receptor. Binding of progesterone to the inactive receptor complex induces a conformation change, which leads to hsp (heat shock protein) dissociation, receptor dimerization, DNA binding, and recruitment of coactivators to facilitate communication with the basal transcription apparatus. P4, progesterone; PR, progesterone receptor; PRE, progesterone response element (Adapted from Leonhardt & Edwards (2002)).

question of selection of cases based on the expression of ER and PR, which has been more extensively described in breast cancer (Carcangiu *et al.* 1990, Dowsett *et al.* 2005), where ER and PR are now routinely measured at pathological assessment of the primary tumor biopsy. PG therapy may act through the PR or indirectly through the ER by acting as an antagonist, and this may account for the contradictory observations about response to hormonal interventions in endometrial cancers. Epidermal growth factor (EGF) and vascular endothelial growth factor receptor levels are both associated with survival in endometrial cancer (Fournier *et al.* 2001, Miyamoto *et al.* 2004), and agents targeting both these pathways are under development.

Mifepristone (RU486) was the first PG antagonist developed that exhibited antiprogesterone activity in humans. Since the first clinical trial in 1982, RU486 has been used in many clinical studies in the gynecologic fields including endometrial cancer (Schneider *et al.* 1998). Navo *et al.* (2008) have tested RU486 in Hec-1-A and Ishikawa cell lines and upon treatment with RU486, PR expression increased in Hec-1-A cells with no change in ER α / β levels. Moreover, the expression of p53 and Cox-2 increased and Bcl-2 decreased in this set of experiments. However, in a single *in vivo* study, expression of steroid hormone receptors, TNF- α , P-glycoprotein and overexpression of c-erb-B-2 or EGFR were not associated with chemoresistance (Goff *et al.* 1998) but the expression of Bcl-2 protein correlated to response to endocrine therapy (Charpin *et al.* 1997).

Wang *et al.* (2003) have reported the upregulation of Fas/FasL expression as a part of the molecular mechanisms of progestin therapy for endometrial hyperplasia and deregulation of Fas/FasL expression

is suggested as one of the molecular mechanisms for resistance to progestin treatment. In a separate study, Song *et al.* (2002) showed that exposure to estrogen or PG decreased the expression of FasL with no change in Fas in endometrial cancer cells. On the other hand, upregulation of FasL was reported after Tamoxifen exposure (Nagarkatti & Davis 2003). However, these observations are not consistent for TNF- α . Moreover, the expression of TNF- α was not altered by estrogen, PG or both, in the endometrial epithelial cancer cells (Tabibzadeh *et al.* 1999). Overall, there is a paucity of studies on the relationship of the expression of the ER, PR, apoptosis regulators, and response to hormone therapy which thus prompts a need to explore this phenomenon in endometrial cancers.

Conclusion and future directions

Chemotherapy and progestin therapy represent the most commonly used pharmacological therapies for patients with advanced or recurrent endometrial cancer. Despite numerous attempts made to improve the therapeutic outcome for endometrial cancer in the past decades, chemoresistance remains a challenge for successful management of this malignancy. However, as yet there is no reliable means of predicting chemotherapy responsiveness. It has been established that a successful therapy for cancer requires readjusting the quantitative cell kinetic relationship so that cell death exceeds cancer cell proliferation. Thus, importance of cell death and cell survival is not restricted to tumor development but has relevance for cancer therapy and to understand the mechanism of resistance. Moreover, it has become clear that endometrial cancer survival rates will not improve by treating all patients uniformly according to standard guidelines. Even in those patients who respond, complete pathological response is rare due to the presence of a sub-population of chemoresistant cells in the heterogeneous environment of tumors. Therefore, the molecular characterization of the sub-population of *de novo* resistant cells in the tumors may provide an improved understanding of the mechanisms of chemoresistance and could lead to new, targeted treatment strategies for endometrial cancer.

The past few years have seen an enormous growth in our understanding of the mechanisms that regulate drug induced apoptosis, and thus influence chemosensitivity. The data in this review clearly showed that chemoresistance is a consequence of multiple factors, including, inhibition of apoptosis (Fas/FasL and Bcl-2 family proteins), stabilization of IAPs, constitutive activation of PI3K/AKT signaling and dysfunction of

the tumor suppressor gene p53. Comparisons of other components of the apoptotic and proliferative pathways in responding and non-responding cells may provide further mechanistic information and form the basis for more rational treatments and better drug development. Since the endometrium is a hormone dependent tissue, hormone therapy also has a crucial role to play. Recent studies also suggested that deregulation in the steroid hormone receptor levels can also lead to treatment failure.

Biomarkers are promising tools for the early detection and disease monitoring of endometrial cancer. Selective manipulation of these signal transduction pathways in combination with present chemotherapeutic drugs may lead to an increased potency in the clinic and efficacy of these agents. In addition, the development of advanced molecular techniques like genomic hybridization, real-time PCR, expression microarray, and proteomics will be crucial for the success of this endeavor. Much of this research regarding discovery of biomarkers and potential mechanisms of drug resistance has been done using cell culture models and far fewer data are available on the relevance of these studies to clinical samples. Efforts to standardize new techniques and set up large tumor banks will hopefully support patient tailored therapy in endometrial cancer since many studies were affected by small sample size. Besides, profiling the individual tumor to predict drug responsiveness, the inter-individual variability in drug response should also be taken into account. Furthermore, unraveling the molecular pathways of importance in the different histotypes may allow the development of more molecularly targeted therapies where specific tumors will be treated with specific drugs. One of the major drawbacks with the conventional chemotherapeutic drugs is the side effect because these drugs cannot selectively kill the tumor cells. The aim of drug targeting is to deliver drugs only to those sites needing treatment. When this objective is met, not only will the efficacy of the treatment be improved, but also toxic side effects will also be greatly minimized. Therefore, selective molecular receptors can be exploited to target these drugs. The estrogen receptor is a biological target for endometrial cancer that has attracted considerable attention over the years. The biological affinity between 17 β -estradiol and its cognate receptor can theoretically be used to direct a cytotoxic agent to the target cells. For instance, we have shown in a series of studies that 17 β -estradiol platinum(II) complex are highly toxic against ER⁺ breast and ovarian cancer cells and can kill platinum resistant endometrial and ovarian cancer cell lines (Gagnon *et al.* 2004).

The present era of molecular targeting has generated excitement in the field of anticancer drug development. Although, a large number of molecular targeted agents are presently in various stages of development and several are already being applied in anticancer therapy. However, no molecular targeted agents presently have a clinically proven role. In particular, mTOR inhibitors could represent a promising therapeutic option for endometrioid-type carcinomas that are often characterized by loss of PTEN function, whereas the anti-HER2 mAb trastuzumab (Herceptin) could have a role in the management of HER-2/neu overexpressing uterine papillary serous carcinomas. Future developments are likely to exploit specific molecular characteristics of endometrial cancers, including their hormone dependence, growth factor target overexpression, and PTEN loss. The next few years will likely be critical in determining the precise mechanisms controlling the onset and maintenance of therapeutic resistance, and will likely suggest several new methods for overcoming this enormous clinical problem.

Declaration of interest

We declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

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