Hormonal interactions in endometrial cancer

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Abstract
Endometrial cancer (EC) is the most frequent malignant tumor of the female genital tract. Increasing evidence suggests that at least two different types of EC exist. Type I is associated with an endocrine milieu of estrogen predominance. These tumors are of endometrioid histology and develop from endometrial hyperplasia. They have a good prognosis and are sensitive to endocrine manipulation. Type II EC is not associated with a history of unopposed estrogens and develops from the atrophic endometrium of elderly women. They are of serous histology, have a poor prognosis, and do not react to endocrine manipulation. Both types of EC probably differ markedly with regard to the molecular mechanisms of malignant transformation. This article reviews reproductive and lifestyle factors modifying the risk of developing type I EC, including the use of hormonal contraceptives, hormone replacement therapy and tamoxifen. The roles of established and novel therapies for precancerous lesions and for invasive EC in the adjuvant and palliative settings are discussed.

Introduction
Endometrial cancer (EC) is the most common malignancy of the female genital tract. In Western industrialized countries, annual incidence rates of between 10 per 100 000 women (UK, Spain, France) and 25 per 100 000 women (USA, Canada) are observed (Parazzini et al. 1991). In the USA, for 1998, an estimated 36 000 new cases and 6300 deaths will be attributed to endometrial cancer (Podratz et al. 1998). In Sweden, carcinoma of the endometrium is the third most frequent cause of cancer death in women, next after breast and colon cancer. In the USA, EC is ranked fourth after breast, lung and colon cancer (Li et al. 1999). The lifetime risk for a woman to develop EC is 1 in 38 in the USA. The incidence increases steadily with age and peaks between the ages of 70 and 74 years (112/100 000 women) before slightly falling (Ball & Elkadry 1998), making the disease a significant factor in women’s health in an aging population. EC is relatively uncommon in the younger age group, and women below the age of 45 represent only 2–14% of all cases (Vinker et al. 1999). About 20–25% of women with EC, however, are premenopausal (Creasman 1997). The prognosis for EC is excellent in the early stages, when the tumor is confined to the endometrium (Stage Ia: 5-year survival=91%) or to the inner 50% of the myometrium (Stage Ib: 5-year survival=88%) (Creasman et al. 1998). Five-year survival decreases to 81% when the tumor infiltrates the outer half of the myometrium (Stage Ic), to 77–67% when the cervix is engaged (Stage II) and to 60–52% when there are tumor manifestations outside the uterus (Stage III) (Creasman et al. 1998). In Stage IVb (distant metastases), only 5% of affected women survive 5 years (Creasman et al. 1998). Fortunately, most patients with EC present with early stage disease, because of typical early symptoms (bleeding disorders or vaginal discharge), resulting in an overall 5-year survival rate for EC of 73% (Creasman et al. 1998).

The mainstay of therapy for EC is the surgical removal of tumor manifestations. In the majority of cases this is achieved by total abdominal hysterectomy and bilateral salpingoophorectomy (Ball & Elkadry 1998). The role of diagnostic and therapeutic lymphadenectomy is currently the subject of debate (Ball & Elkadry 1998). The role of adjuvant postoperative radiation in the management of operable EC remains unresolved, though it is routinely performed in many centers (Cohen & Rahaman 1995, Ball & Elkadry 1998, Podratz et al. 1998). Patients who are not candidates for surgery, because of an advanced tumor stage or underlying medical problems, are often treated with radiotherapy alone (Ball & Elkadry 1998).

For patients with recurrent EC not eligible for surgery or radiotherapy or for those women primarily with advanced disease, a systemic treatment is the only remaining option. Chemotherapy for recurrent or advanced EC induces
response rates of up to 60% but is of limited benefit as progression-free survival is only in the range of some months (Moore et al. 1991, Burke & Gershenson 1996, Homesley 1996, Ball & Elkadry 1998, Quinn 1999). So far, there is also no evidence that adjuvant chemotherapy for the EC patient with a high risk of recurrence has any substantial benefit (Moore et al. 1991, Burke & Gershenson 1996, Homesley 1996, Quinn 1999).

Because EC develops from the endometrium, a classical hormone-dependent tissue, endocrine therapies have been the mainstay of systemic treatment for many decades. In addition, adjuvant endocrine therapies for high-risk disease have been commonly used. In this paper, we review the present knowledge on endocrine factors in the etiology of EC, their role in the regulation of proliferation and metastases formation, and the potential value of both established and novel endocrine manipulations for the prevention and treatment of EC.

Etiology

It has been acknowledged for more than 50 years that continuous exposure to estrogens in the absence of sufficient levels of progestogens promotes the development of EC. Typical risk factors are obesity, anovulatory states, early menarche and late menopause, nulliparity and unopposed exogenous estrogens (Table 1). EC in these cases develops via a characteristic sequence of hyperplastic changes of the endometrium with increasing premalignant potential (simple hyperplasia or complex hyperplasia, both types either with or without atypia) (Bokhman 1983, Deligdisch & Cohen 1985, Deligdisch & Holinka 1986, Nyholm et al. 1993a, Cohen & Rahaman 1995, Sivridis et al. 1998, Sherman 2000) (Table 1). Since the pioneer work of Bokhman (1983) and of Deligdisch and colleagues (Deligdisch & Cohen 1985, Deligdisch & Holinka 1986), a second type of EC has been identified which is not associated with hyperestrogenic states. It develops from the atrophic endometrium of older women, who do not have the classical risk factors for EC. These patients tend to be slim, are physically fit, and have never used estrogen-replacement therapy. These type II ECs are mostly of a high grade or have high-risk histology (serous-papillary or clear-cell carcinomas). On diagnosis, they are characterized by deep myometrial invasion and early lymph node or distant metastases. Type-II ECs rarely express functional estrogen and/or progestin receptors and their response rates to endocrine therapies tend to be low. Their prognosis is poor. Type-II ECs are estimated to represent 20% up to more than 50% of all endometrial cancers (Bokhman 1983, Deligdisch & Cohen 1985, Deligdisch & Holinka 1986, Nyholm et al. 1993a, Cohen & Rahaman 1995, Sivridis et al. 1998, Sherman 2000) (Table 1). Recently, a classification into three types of EC (I: endometrioid carcinomas associated

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Type 1</th>
<th>Type II</th>
</tr>
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<tbody>
<tr>
<td>Menstrual history</td>
<td>Anovulatory bleedings</td>
<td>No disorders</td>
</tr>
<tr>
<td>Fertility</td>
<td>Reduced; infertility</td>
<td>No disorders</td>
</tr>
<tr>
<td>Age at menopause</td>
<td>&gt;50 years</td>
<td>&lt;50 years</td>
</tr>
<tr>
<td>Vaginal cytology during postmenopause</td>
<td>Estrogen activity</td>
<td>Atrophic</td>
</tr>
<tr>
<td>Age at diagnosis</td>
<td>Perimenopausal</td>
<td>Late postmenopause/senium</td>
</tr>
<tr>
<td>Ovarian histology</td>
<td>Stromal hyperplasia, polycystic ovarian disease, estrogen-producing tumors</td>
<td>Fibrosis</td>
</tr>
<tr>
<td>Status of tumor-free endometrium</td>
<td>Hyperplasia</td>
<td>Atrophic</td>
</tr>
<tr>
<td>Myometrium</td>
<td>Leiomyositis, fibroids</td>
<td>No abnormality</td>
</tr>
<tr>
<td>Obesity</td>
<td>Often present</td>
<td>Absent in most cases</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>Often present</td>
<td>Absent in most cases</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>Mostly long</td>
<td>Mostly short</td>
</tr>
<tr>
<td>Duration of symptoms</td>
<td>&gt;80% G1, G2</td>
<td>&gt;60% G3 or ungraded</td>
</tr>
<tr>
<td>Grading of the tumor</td>
<td>Mostly endometrioid cancers</td>
<td>Often serous papillary, clear cell, squamous carcinomas</td>
</tr>
<tr>
<td>Histological type</td>
<td>Mostly superficial</td>
<td>Mostly deep</td>
</tr>
<tr>
<td>Myometrial invasion</td>
<td>Rare</td>
<td>Common</td>
</tr>
<tr>
<td>Lymphatic invasion</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Expression of progesterone receptor</td>
<td>Favorable</td>
<td>Poor</td>
</tr>
<tr>
<td>Prognosis</td>
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with hyperplastic endometrium; II: endometrioid carcinomas associated with atrophic endometrium; III: non-endometrioid carcinomas associated with atrophic endometrium) has been suggested (Sivridis et al. 1998). The authors, however, point out that this subdivision, though possibly important pathogenically, does not have any therapeutic implications. Non-endometrioid adenocarcinomas and deeply invasive or poorly differentiated endometrioid carcinomas require aggressive treatment, but well-differentiated endometrioid carcinomas arising from an atrophic background can be treated in the same manner as those of a similar grade associated with an endometrial hyperplasia (Sivridis et al. 1998).

In a very recent paper, Sherman (2000) also suggests the existence of two major types of EC: (1) the most common type, endometrioid adenocarcinoma, represents over 80% of ECs that develop from endometrial hyperplasia in the setting of excess estrogen exposure and pursues an indolent clinical course; and (2) a minority of ECs, best represented by serous carcinoma, do not seem to be related to estrogenic risk factors or elevated serum hormone levels. These type II tumors seem to develop from atrophic rather than hyperplastic epithelium (Sherman 2000). Apart from these two well-defined types of EC, Sherman also acknowledges a third group, where endometrioid carcinomas are associated with atrophic endometrium (29% of all endometrioid ECs). In addition, mixed endometrioid/serous carcinomas exist, which, in 46% of cases, are associated with endometrial hyperplasia (Carcangiu & Chambers 1992). Finally, 5–8% of pure serous ECs were found to be associated with endometrial hyperplasia (Carcangiu & Chambers 1992, Ambros et al. 1995). Although the definition of this third group of ECs is still unsatisfactory from an etiologic point of view, these cancers usually share the high-risk characteristics of non-endometrioid tumors and are to be treated accordingly (Sivridis et al. 1998, Sherman 2000). Because of the lack of data on this third group, the present review will focus on the much better-defined ‘estrogen-driven’ endometrioid ECs and the estrogen-unrelated non-endometrioid ECs, best represented by serous carcinoma.

Etiology of estrogen-associated endometrial cancer (type I)

The association between an endocrine milieu of estrogen predominance, resulting in hyperstimulation of the endometrium, and an increased incidence of EC was first formally reported in 1947 (Gusberg 1947). Later, pathologists defined the cytologic and architectural patterns of endometrial hyperplasia more precisely; this led to a better definition of the premalignant potential of ECs. In a series of 170 patients who received no therapeutic intervention other than diagnostic curettage, Kurman et al. (1985) found that 23% of patients with atypical endometrial hyperplasia developed carcinoma compared with only 2% of patients with other types of hyperplasia.

Hormone-replacement therapy

The association between an estrogen-dominant endocrine milieu and type I EC is most comprehensible for the example of hormone-replacement therapy in peri- and postmenopausal women. A meta-analysis of 30 evaluable studies defined a relative risk (RR) for EC of 2.3 (95% confidence interval (CI): 2.1–2.5) for women on exclusive estrogen-replacement therapy (ERT) compared with women receiving no hormone substitution (Grady et al. 1995). ERT given for 10 and more years led to an increase of RR for EC to 9.5. The increase in the RR for EC remains elevated even after discontinuation of ERT for 5 and more years (Grady et al. 1995).

The combination of progestins with ERT markedly reduced the RR for EC to 0.8 (95% CI: 0.6–1.2) (Grady et al. 1995). When only cohort studies were analyzed, combined estrogen/progesterone replacement therapy even reduced the RR for EC to 0.4 (95% CI: 0.2–0.6), suggesting a protective effect compared with women receiving no hormone replacement (Grady et al. 1995). In a recent case-control study, Pike et al. (1997) found that the RR for EC was 2.17 (95% CI: 1.91–2.47) per 5 years of ERT use. For women who received sequential estrogen–progesterin replacement therapy, with the progestin being given for less than 10 days (effectively 7 days) per month, the RR was only slightly reduced, to 1.87 (95% CI: 1.32–2.65) per 5 years of use. However, when progestin was given for 10 or more days (effectively 10 days), there was essentially no increased risk (RR=1.07 per 5 years of use; 95% CI: 0.82–1.41). Continuous combined replacement therapy was also associated with essentially no increased risk (RR=1.07 per 5 years of use; 95% CI: 0.8–1.43). The authors concluded that the progestin in sequential estrogen–progesterin replacement therapy needs to be given for at least 10 days to block any increased risk of EC effectively. Continuous combined estrogen–progesterin therapy is similarly effective. Neither regimen reduced a woman’s underlying risk of EC (Pike et al. 1997).

Weiderpass et al. (1999a), when conducting a nationwide population-based, case-control study in Sweden found that 5 or more years of exclusive ERT was associated with a marked duration- and dose-dependent increase in the RR of EC. Five and more years of treatment had an RR of 6.2 for estradiol (95% CI: 3.1–12.6) and an RR of 6.6 for conjugated estrogens (95% CI: 3.6–12.0). Following combined estrogen–progestin use, the association was considerably weaker than that for estrogen alone; the RR was 1.6 (95% CI: 1.1–2.4) after 5 or more years of use. This increase in risk was confined to women with cyclic use of progestins, i.e. use for fewer than 16 days per cycle (most commonly 10 days per cycle): RR=2.9 (95% CI: 1.8–4.6 for
5 or more years of use). In contrast, continuous progestin use along with estrogens was associated with a reduced risk (RR=0.2; 95% CI: 0.1–0.8 for 5 or more years of use) (Weiderpass et al. 1999a).

Even low doses of unopposed estrogens or the oral use of a low-potency estrogen (estriol) increase the risk of EC. Cushing et al. (1998) found an RR of 5.4 (95% CI: 2.3–13) for EC in women who had taken 0.3 mg unopposed conjugated estrogens per day. The risk was particularly high in women whose use of this dose was both current and of more than 8 years’ duration (RR=9.2; 95% CI: 2.9–29) (Cushing et al. 1998). Weiderpass et al. (1999b) found that the oral use of estradiol (1–2 mg), which is assumed to have few, if any, adverse effects on the endometrium, increased the RR of EC and endometrial atypical hyperplasia. After at least 5 years of oral estradiol replacement, the RRs were 3.0 (95% CI: 2.0–4.0) for invasive EC and 8.3 (95% CI: 4.0–17.4) for atypical endometrial hyperplasia. Only weak associations were observed between vaginal application of estril formulations and the RR of endometrial hyperplasia (Weiderpass et al. 1999b). Women receiving ERT who develop endometrial cancer have traditionally been thought to develop good-prognosis tumors of low grade and early stage. However, a recent case-control study found that, women who took unopposed estrogens for 3 or more years had a fivefold increase in the risk of myometrial invasion and almost a threefold risk of high-grade tumors, risks which were negated by the use of cyclical or continuous progestins (Shapiro et al. 1998).

Although there are a few differences between the above observational studies, they are only marginal. These studies unequivocally find a significant time-dependent increase of the RR for EC induced by hormone-replacement therapy with unopposed estrogens during peri- and postmenopause. Combination with progestins significantly reduces this excess risk for EC caused by estrogen replacement. The protective effect of progestins seems to increase with their duration of use per month. The data suggest that progestins should be used for at least 10 days per replacement cycle, or even as a continuous combination with estrogens, to achieve a maximal reduction in the estrogen-induced excess risk for EC.

Disorders of the menstrual cycle

An endocrine milieu similar to that associated with unopposed ERT is found in women with anovulatory cycles or corpus luteum insufficiency (CLI), states which are characterized by a complete or relative deficiency of endogenous progesterone. CLI and anovulatory cycles occur not only in infertile women of reproductive age but are characteristic of the premenopausal period. Thus, a late menopause or a premenopause complicated by intensive bleeding disorders due to CLI or anovulatory cycles are typical risk factors for EC (Barakat et al. 1997).

Estrogen-producing tumors and polycystic ovary syndrome

Estrogen-producing tumors and polycystic ovary syndrome (PCOS) also induce an endocrine milieu of estrogen predominance. PCOS is characterized by the absence of ovulations and thus endogenous progesterone production and an increased secretion of ovarian androgens which are peripherally converted to estrogens (Barakat et al. 1997). Estrogen-producing tumors and PCOS carry a high risk for EC in young women (Cohen & Rahaman 1995, Barakat et al. 1997).

Obesity/metabolic syndrome

A comparable pathophysiologic mechanism explains the significant association between obesity and risk for EC. Adrenal androgens are converted to estrogens by aromatases in the adipose tissue (Gusberg 1994). Hyperinsulinemia, which is common in obese women, additionally stimulates androgen synthesis by postmenopausal ovaries; hypercorticism contributes to increased adrenal androgen production (Nagamani et al. 1988, 1992a, Goodman et al. 1997, Troisi et al. 1997). In addition, direct growth-potentiating activity of insulin in EC cells has been demonstrated (Nagamani & Stuart 1998).

Postmenopausal women with EC have significantly elevated levels of bioactive luteinizing hormone, a factor probably contributing to increased ovarian androgen production (Nagamani et al. 1992b). The aromatization of adrenal and ovarian androgens leads to significantly elevated plasma levels of estrone and estradiol (Nyholm et al. 1993b). As in breast cancer cells, aromatase activity in endometrial cancer cells or in the desmoplastic stroma surrounding the tumor cells might be of additional importance (Bulun et al. 1994, Watanabe et al. 1995).

Sherman et al. (1997), when performing a case-control study of 328 patients with endometrioid EC and 26 with serous EC, found that both the estrogen and the androgen serum levels were elevated in women with endometrioid EC, compared with controls.

Diet and lifestyle

Women with diets high in energy and fat and low in complex carbohydrates including vegetables, fruits, cereals and grains were at increased risk of EC, a phenomenon which was more pronounced in obese women (Goodman et al. 1997). The association with fruit and vegetable intake is considered to be less clear by other authors, who found physical activity, number of pregnancies, and weight in young and middle age...
to be the most important lifestyle factors modifying EC risk (Terry et al. 1999).

Cigarette-smoking has been found to reduce the risk of EC significantly, according to a number of observational studies (reviewed by Parazzini et al. 1995). This phenomenon is explained by the induction of earlier menopause and by the induction of liver enzymes inactivating circulating estrogens. Although this ‘protective’ effect of cigarette-smoking is more than counteracted by the other carcinogenic effects of this habit, it supports the concept of the impact of estrogen dominance in the pathogenesis of EC (Parazzini et al. 1995).

**Hormonal contraception**

The use of oral contraceptives (OCs) significantly reduces the risk of EC. This protective effect increases with duration of use, is most prominent with continuously combined preparations, and persists for many years after discontinuation of OC use (La Vecchia et al. 1996, Brinton & Hoover 1997). A comparable protective effect has been attributed to the contraceptive use of depot medroxyprogesterone acetate (Lumbiganon 1994).

**Unopposed estrogens: mitogens or mutagens?**

The common denominator for the above phenomena is that a shift in the estrogen–progestin balance towards a predominance of estrogens favors the development of EC, whereas a relative or absolute increase in progestin exposure has a protective effect. (Fig. 1). Morphologically, an estrogen predominance induces the typical sequence of endometrial hyperplasia, finally resulting in the development of EC (Gusberg 1994). Estrogen stimulation of the endometrium increases mitogenic activity, whereas this activity is reduced by progestins (via different anti-estrogenic mechanisms, including a reduction in estrogen receptors and an increase in metabolic inactivation of estrogens). In addition, the mitotic activity of the endometrium is reduced by progestins through the induction of cellular differentiation, thus counteracting the development of hyperplasia (Lupulescu 1993, Gusberg 1994, Berchuk & Boyd 1995, Cohen & Rahaman 1995, Kumar et al. 1998). There is no evidence that physiological levels of estrogens or concentrations achieved with their therapeutic use are directly carcinogenic for human cells (Lupulescu 1993, Berchuk & Boyd 1995). Rather, it is assumed that the increased mitotic activity of the endometrium caused by estrogen predominance increases the probability of the accumulation of random mutations (activation of oncogenes, inactivation of tumor-suppressor genes), finally leading to malignant transformation. Thus, estrogens probably act as tumor promoters but not as carcinogens in EC. Their action is believed to be mitogenic but not mutagenic (Lupulescu 1993, Gusberg 1994, Berchuk & Boyd 1995).

Recently, it has been suggested that estradiol is additionally a weak carcinogen and mutagen capable of inducing genetic lesions with low frequency. Tumors may be initiated by the metabolic conversion of estradiol to 4-hydroxyestradiol and by further activation of this catechol to reactive semiquinone/quinone intermediates that cause the DNA damage. Tumors may develop by means of further hormone-receptor-mediated proliferation of such damaged cells (Liehr 2000). Liehr, however, stresses the point that the mutagenic activity of estradiol is weak, as high mutagenic and carcinogenic activity of estradiol would not have permitted the existence of many higher life-forms, including humans (Liehr 2000).

**Etiology of non-estrogen-associated endometrial cancer (type II)**

Histopathologic studies suggest that the majority of serous carcinomas develop from a distinctive lesion termed endometrial intraepithelial carcinoma, which appears to represent malignant transformation of atrophic surface endometrium (Ambros et al. 1995, Sherman 2000). In uteri containing serous carcinoma, the uninvolved endometrium is usually atrophic. It has been shown that when endometrial hyperplasia is identified in a uterus containing a carcinoma that is partly or exclusively serous, the hyperplasia and the carcinoma are usually topographically unrelated and appear distinct (Sherman 2000). In the case-control study by Sherman et al. (1997), obesity and exogenous hormone use were not related to risk for serous carcinoma. Serum estrogen and androgen levels for patients with serous carcinoma were similar to those in the controls. In contrast, levels of sex-hormone binding globulin, a circulating protein that reduces bioavailable estrogen, were higher among serous carcinoma patients than in controls or endometrioid carcinoma patients (Sherman et al. 1997). The only risk factors for non-estrogen-related EC that can be assumed today are age (Deligdisch & Cohen 1985, Cohen & Rahaman 1995, Sivridis et al. 1998, Sherman 2000) and pelvic irradiation (Sivridis et al. 1998). With advancing age, the probability of the accumulation of mutations leading to malignant transformation increases (Berchuk & Boyd 1995). Pelvic irradiation might also add to the accumulation of mutations. The declining competence of the immune system with advancing age has been suggested as a further possible reason (Deligdisch & Cohen 1985, Cohen & Rahaman 1995, Deligdisch & Holinka 1986) (see Fig. 2).

**Molecular findings regarding the etiology of endometrial cancer**

Most molecular studies of EC have consisted of small series reported from single institutions (reviewed by Berchuk &
Figure 1 Model of carcinogenesis of endometrial cancer type I (estrogen-related).
Endometrioid EC (type I)

In most cases, these cancers evolve from atypical endometrial hyperplasia as a clonal lesion (Mutter et al. 1995, 1996, Sherman 2000).

Mismatch repair defects

The microsatellite instability or replication error repair (RER) phenotype has been reported in 9–45% of sporadic endometrioid carcinomas (Mutter et al. 1996, Gurin et al. 1999, MacDonald et al. 2000). Microsatellite instability was detected in atypical hyperplasia associated with carcinoma but not in atypical hyperplasia without associated carcinoma (Mutter et al. 1996), suggesting that mismatch repair defects may occur in the transition between the two lesions (Sherman 2000). Somatic mutational inactivation of known mismatch repair genes, however, does not account for the great majority of sporadic ECs with microsatellite instability. A significant fraction of these cases may be causally associated with hypermethylation of the MLH1 promoter, resulting in a loss of repair function without mutation (epigenetic effect) (Gurin et al. 1999).

ras mutations

ras mutations have been reported in 10–46% of endometrioid ECs (Berchuk & Boyd 1995, Jeyarajah et al. 1996a, Terakawa et al. 1996, Niederacher et al. 1999). ras mutations have been detected in 10% of simple, 14% of complex, and

<table>
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<tr>
<th>Event</th>
<th>Description</th>
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<tr>
<td>Overexpression/amplification of growth factors and/or their receptors</td>
<td>(EGF, IGF-I, IGF-II, TGF-α, PDGF, M-CSF, C Fms, HER-2/neu-protein)</td>
</tr>
<tr>
<td>Mutation of K-ras (proto-oncogene, signal transduction)</td>
<td></td>
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<tr>
<td>Amplification of other oncogenes (c-myc, int-2)</td>
<td></td>
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<tr>
<td>Mutations/loss of heterozygosity of tumor suppressor genes (TP 53, BRCA1, TCRD, DCC, PTEN)</td>
<td></td>
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<tr>
<td>Mutation of DNA-repair genes (MSH-2, MLH-1)</td>
<td></td>
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<tr>
<td>Loss of expression of estrogen/progesterone receptors,</td>
<td>mutations in these receptors, changes in the relationships of isoforms of estrogen and/or progesterone receptors</td>
</tr>
<tr>
<td>Overexpression of genes relevant to metastasis formation (cathepsin D, urokinase-type-plasminogen activator, CD 44 and others)</td>
<td></td>
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p53 mutations
p53 mutations are rare in endometrioid EC. Most endometrioid carcinomas that harbor p53 mutations are large high-grade tumors, which suggests that p53 mutation in endometrioid carcinoma is more closely related to dedifferentiation, as in the case of other tumor systems (Sherman 2000).

Hormone receptors
Estrogen and progesterone receptors are usually found in high concentrations in endometrial hyperplasia and endometrioid carcinomas of low grade and stage. Steroid receptor expression diminishes with increases in stage and grade (Kleine et al. 1990, Nyholm et al. 1992, Creasman 1997) (Fig. 1).

Non-endometrioid ECs (type II)
These cancers rapidly develop from endometrial intraepithelial carcinoma, which appears to represent malignant transformation of atrophic surface epithelium (Sherman 2000). Microsatellite instability and mutations in ras and PTEN are not found in serous carcinoma (Sherman 2000). The typical molecular features of this type of EC are mutations in the p53 tumor suppressor gene and accumulation of p53 protein, which are found in approximately 90% of both serous ECs and their precursor lesions, endometrial intraepithelial carcinomas (Sherman et al. 1995, Moll et al. 1996, Tashiro et al. 1997, Sherman 2000). Steroid receptors are not expressed in serous carcinoma (Moll et al. 1996, Sherman 2000) and endometrial intraepithelial carcinoma, although the atrophic endometrium in which these tumors arise is receptor-positive (Sherman 2000) (Fig. 2).

New findings on hormone receptors
Estrogen and progesterone receptors
At the end of 1995, a second ER (ERβ) with different regulatory functions was cloned. Estradiol may activate transactivation through the classic estrogen receptor (ERα) but inhibit transcription through ERβ (Enmark & Gustafsson 1996).
1998). First reports are now appearing on the expression of ER isoforms in gynecological tumors (Chu et al. 2000) including ECs (Saegusa & Okayasu 2000). The latter paper reports that relative amounts of ERα at both mRNA and protein level were significantly greater than those for ERβ in normal and malignant endometrial lesions. ERα mRNA showed a stepwise decrease from normal endometrium or grade (G) 1 to G3 tumor lesions, in line with changes at the protein level. In contrast, ERβ mRNA or protein expression did not alter, suggesting a shift in the ratio of the two ER subtypes during endometrial tumorigenesis. Progesterone receptor (PR) mRNA was significantly correlated with ERα, but not ERβ mRNA status (Saegusa & Okayasu 2000). Similarly, two distinct forms of PR receptors (PR-A and PR-B) exist in the female genital tract and might be differentially regulated in EC. Downregulation of PR-B in poorly differentiated human ECs might be involved in the resistance of these cancers to progestin therapy (Kumar et al. 1998).

Exon deletions and variants of human ER mRNA in endometrial hyperplasia and adenocarcinoma have been described recently, suggesting that differing ER proteins are present in EC and may influence estradiol signaling pathways (Horvath et al. 2000).

Receptors for gonadotrophin releasing hormone

Recently, a series of papers from different laboratories has demonstrated the expression of gonadotropin-releasing hormone (GnRH) and its mRNA in almost 100% of ECs and the expression of the GnRH receptor and its mRNA in about 80% of ECs (for a review see Emons et al. 1997). GnRH-agonists and -antagonists dose-dependently inhibit the proliferation of human EC cell lines that express GnRH receptors, probably through inhibition of mitogenic signal transduction (Emons et al. 1997, Gründker & Emons 1999, Gründker et al. 2000). In addition, spontaneous and growth-factor-stimulated proliferation of human EC cell lines can be inhibited by agonistic analogs of somatostatin through specific somatostatin receptors (Emons et al. 1996).

Hereditary forms of EC

The best-documented form of familial EC occurs as part of the Lynch II syndrome, which describes a subgroup of the hereditary non-polyposis colorectal cancer (HNPCC) syndrome in which colorectal, endometrial, breast and ovarian tumors are inherited in an autosomal dominant fashion. The molecular base for this syndrome are germ-line mutations of DNA-repair genes (MSH2 or MLH1) (Vasen et al. 1994, Jeyarajah et al. 1996a). Women from these families have a lifetime risk of developing EC of 20–30%. Typically, these ECs are diagnosed approximately 15 years earlier than those in the general population (Vasen et al. 1994). The Lynch II syndrome accounts for only a small proportion of ECs. It was shown, however, that first-grade female relatives of EC patients have an increased risk for EC (Lynch et al. 1994). The relative risk of EC as well as that for other cancers is significantly elevated in family members of breast cancer patients (Assikis & Jordan 1996, Jeyarajah et al. 1996a).

Tamoxifen and EC

For about 15 years, it has been known that the anti-estrogen tamoxifen (TAM) is able to stimulate the proliferation of certain EC cell lines (Gusberg 1994, Cohen & Ramahan 1995, Assikis & Jordan 1996, Homesley 1996, Creasman 1997). These findings attracted public concern when data from several large trials on TAM use in adjuvant therapy for early breast cancer suggested an excess risk of EC for women taking TAM as compared with controls (Assikis & Jordan 1996, Creasman 1997). It has also been proposed that TAM-associated ECs tend to be more often of the high-risk type compared with those in the general population (Assikis & Jordan 1996). After years of discussions, in the public and the scientific communities, which were stimulated by contradictory results of clinical trials (Creasman 1997), the Committee on Gynecologic Practice of the American College of Obstetricians and Gynecologists stated in 1996 that TAM time- and dose-dependently increases the relative risk for EC. Overall, there seems to be a two- to threefold increase in the incidence of EC in women treated with adjuvant TAM (ACOG Committee opinion 1996, Love et al. 1999), leading to an absolute annual risk of approximately 2 per 1000 patients (Barakat 1999). It is now generally accepted that stage, grade, histological type and other prognostic factors of TAM (20 mg/day)-associated ECs are not different from those of ECs found in the normal population (ACOG Committee opinion 1996, Homesley 1996, Love et al. 1999). It is generally accepted that TAM causes endometrial changes (benign polyps and hyperplasia in up to 40% of cases) (ACOG Committee opinion 1996). However, these hyperplasias are often located in the stroma and not in the endometrium (ACOG Committee opinion 1996, Assikis & Jordan 1996, Love et al. 1999). Transvaginal ultrasound detected a high incidence (41%) of apparent endometrial thickening in women treated with TAM, although 46% had atrophic endometrium on further assessment, and none of the remaining asymptomatic women had significant lesions (Love et al. 1999). Debate, however, continues about the value of screening for EC and of histological evaluation of endometrial abnormalities in women on adjuvant TAM (Barakat 1999, Ramondetta et al. 1999).

No increase in EC risk has been observed in adjuvant TAM trials from the UK (Baum 1998) and other countries...
(Creasman 1997). It remains unclear as to whether TAM, because of its estrogen agonistic properties, induces the formation of EC or whether it accelerates the growth of pre-existing EC. A detection bias must also be taken into account: gynecological symptoms induced by TAM (vaginal discharge, abnormal bleeding) and/or TAM-related ultrasound phenomena (‘endometrial thickening’) often lead to hysterectomy, endometrial sampling and/or dilation and curettage, which might detect latent EC that would not have been found if the woman had not shown these TAM-induced gynecological symptoms (Cohen & Ramahan 1995, Assikis & Jordan 1996, Creasman 1997, Baum 1998).

It has been suggested that TAM might be genotoxic in human endometrium (Hemminki et al. 1996), a hypothesis that was not corroborated by findings from analysis of endometrium from patients on TAM in another study (Carmichael et al. 1996). The uterine stimulatory effects of a new non-steroidal anti-estrogen, toremifene, seem not to be dissimilar to those of TAM (Tomas et al. 1995, Buzdar & Hortobagyi 1998, O’Reagan et al. 1998), while the steroidal pure anti-estrogen ICI 182, 780 inhibited the growth of TAM-stimulated human EC in nude mice, in both the presence and the absence of estrogen (O’Reagan et al. 1998). The authors concluded that ICI 182, 780 may be safe even if it is used following TAM, and that it may not result in an increased incidence of EC. They considered it possible that ICI 182, 780 may prove useful as an adjuvant agent in early-stage EC (O’Reagan et al. 1998). These views, however, have been contested (De Gregorio 1999, Mäenpää et al. 1999, Williams 1999).

Raloxifene, a non-steroidal anti-estrogen, which, being a benzothiophene, is chemically distinct from TAM and estradiol, is considered to bind to estrogen receptors and competitively block estrogen-induced DNA transcription in the breast and endometrium (Delmas et al. 1997, Cummings et al. 1999). So far, in women with osteoporosis using raloxifene (median follow-up, 40 months) neither effects on endometrial thickness nor an increased risk of EC has been observed (Delmas et al. 1997, Cummings et al. 1999).

Palliative hormonal treatment of invasive EC

Progestogens

For several decades, progestogens have been successfully used in the palliative treatment of advanced or relapsed EC. In early reports, objective response rates of 30–50% have been described, while more recent studies, using strict criteria, found objective response rates of 10–25% (Moore et al. 1991, Cohen & Rahaman 1995, Quinn 1999). On the basis of encouraging response rates in breast cancer with high-dose megestrol acetate (MA), the Gynecologic Oncology Group recently performed a phase II trial with 800 mg MA/day in patients with recurrent or advanced ECs. Of 63 patients entered into the trial, 54 were assessible for responses. Of 13 responders (24%), six had a complete response and seven had a partial response. Four of the responses lasted greater than 18 months. Twelve patients (22%) had stable disease. The response rate of patients with grade 1 or 2 lesions (11 of 30, 37%) was significantly higher than that of patients with more poorly differentiated tumors (2 of 24, 8%). Three deaths secondary to cardiovascular events were possibly related to therapy; diabetes was also a contributing factor in all three cases. The authors concluded that high-dose MA is active in endometrial carcinoma but appears to have no advantage over lower-dose progestins (Lentz et al. 1996).

These conclusions have been confirmed by another trial, carried out by the Gynecologic Oncology Group, which was published recently (Thigpen et al. 1999). Two hundred and ninety-nine eligible women with advanced or recurrent EC were randomized to receive oral medroxyprogesterone acetate (MPA; either 200 mg/day or 1000 mg/day). Of 145 patients receiving the low-dose regimen, there were 25 (17%) complete responses and 11 (8%) partial responses, giving an overall response rate of 25%. The 154 patients receiving the high-dose regimen experienced 14 (9%) complete responses and 10 (6%) partial responses, giving an overall response rate of 15%. The median durations of progression-free survival were 3.2 months and 2.5 months for the low-dose and high-dose regimens respectively. The median survival durations were 11.1 months and 7.0 months respectively. Responses to progestin therapy were more frequent among patients with a well-differentiated histology and a positive PR status (>50 fmol/mg cytosol protein; response rate 37%). Patients with poorly differentiated tumors and/or with PR levels less than 50 fmol/mg cytosol protein had response rates of only 8–9%. Regardless of dose, oral MPA therapy was well tolerated, with thrombophlebitis, the most frequent adverse effect, occurring in only 5% of patients (Thigpen et al. 1999). Comparable trials, by the Gynecologic Oncology Group, using cytotoxic drugs resulted in response rates of 22% (doxorubicin) or 30% (doxorubicin plus cyclophosphamide). The median durations of progression-free survival were 3.2 or 3.9 months, and the median survival was 6.9 and 7.3 months respectively (Thigpen et al. 1994). In another protocol, the Gynecologic Oncology Group compared doxorubicin with a combination of doxorubicin plus cisplatin, which is considered to be the current cytotoxic regimen of choice (Quinn 1999). In terms of response, the combination yielded a statistically significant superior rate of 44% (which compares with 28% for the single agent). Progression-free survival was also superior for the combination regimen, but overall survival was not significantly different (Thigpen et al. 1993, Homesley 1996, Selman et al. 1998).

Summarizing the results of their trial on oral MPA, Thigpen and coworkers concluded that this study provides...
no evidence to support the use of 1000 mg MPA per day (orally) instead of 200 mg MPA per day (orally) (Thigpen et al. 1999). The use of 200 mg MPA per day (orally) is a reasonable initial approach to the treatment of advanced or recurrent endometrial carcinoma, particularly for those lesions that are well differentiated and/or PR-positive (>50 fmol/mg cytosol protein) (Thigpen et al. 1999).

**Tamoxifen (TAM)**

TAM has been used for the treatment of recurrent or advanced EC at doses of 10–40 mg/day. Response rates varying between 0 and 53% have been described. Of 257 patients treated in several small phase II trials, 56 (22%) had an objective response (Moore et al. 1991). TAM has been suggested as an alternative endocrine treatment for EC patients with obesity, hypertension, diabetes, and increased risk for thrombosis, for whom progestin treatment might be harmful. Taking into account the possible potential of TAM to induce EC (see above), this strategy has lost some of its attractiveness.

Both preclinical and clinical data suggest that TAM, when bound to the cytoplasmic ER, can stimulate the production of PRs and therefore, in theory, potentiate the activity of progestin therapy. No significant improvements of the efficacy of progestogen therapy, however, have been achieved by this combination with TAM (Moore et al. 1991).

**GnRH analogs**

Gallagher et al. (1991) treated 17 women with recurrent or advanced ECs which were refractory to progestin treatment with the GnRH analogs leuprorelin acetate (7.5 mg/month) or goserelin (3.6 mg/month). Six of these patients (35%) experienced an objective remission which continued for a median of 20 months with no adverse effects (Gallagher et al. 1991). In a later update of their series, now comprising 32 patients with recurrent ECs that had progressed through conventional treatment, an objective response was seen in nine patients (28%) with a median duration of 17 months (Jeyarajah et al. 1996b).

De Vriese and Bonte (1993) treated seven women suffering from advanced ECs with goserelin and achieved two objective remissions. Covens et al. (1997) treated 25 patients with recurrent or advanced ECs with leuprorelin acetate (7.5 mg/month) and achieved no objective remission. Markman et al. (1997), when treating 9 patients with leuprorelin, also observed no objective response. Given the experimental data on successful growth inhibition of ECs in vitro by GnRH analogs (Emons et al. 1996, 1997) and the low toxicity of these compounds, it seems appropriate that further clinical trials be undertaken.

**Other endocrine treatments of EC**

With the use of the aromatase inhibitor aminoglutethimide in 18 women with advanced EC, four objective responses were obtained (Murray & Pitt 1984). One case of successful treatment of a refractory small cell carcinoma of the endometrium with the somatostatin agonist octreotide has been reported (Verschraegen et al. 1999).

**Primary treatment of early EC with progestins**

Some small series have been reported in which grade 1, stage 1 ECs have been successfully treated with progestins alone (for reviews see Kim et al. 1997, Randall & Kurman 1997, Quinn 1999). A response rate of 66% has been calculated and 11 pregnancies (two after in vitro fertilization) have been reported (Paulson et al. 1990, Kim et al. 1997, Randall & Kurman 1997, Zuckerman et al. 1998). Vinker et al. (1999) reported the case of a young nulliparous woman with a well-differentiated adenocarcinoma of the endometrium that did not respond. Upon definite operation (including hysterectomy), a stage II EC was found. These authors point out that the differential diagnosis between a typical hyperplasia and well-differentiated adenocarcinoma is difficult and that a consultation with an endometrial pathologist is needed before a conservative treatment is discussed. In addition, these authors suggest that there might be a publication bias, as only those cases that had favourable outcomes have been reported (Vinker et al. 1999). However, older women with well-differentiated ECs and who are unsuitable for surgery or radiotherapy might be good candidates for primary progestin treatment.

**Primary endocrine treatment of endometrial hyperplasias**

The presence of complex hyperplasia with atypia in the premenopausal woman desirous of fertility poses an enormous problem. Up to 20% of hysterectomy specimens show an invasive carcinoma, one-quarter to one-third of these being associated with myometrial invasion (Quinn 1999). Also, for simple hyperplasia with atypias, a high frequency of malignant transformation must be expected (Kurman et al. 1985). A number of reports have attested to the efficacy of progestin treatment (Randall & Kurman 1997, Quinn 1999). They cover, however, only small series and there might be a publication bias. We consider extensive counseling about the risk of the presence of, or the progression to, invasive EC, as well as monitoring of the treatment effect by hysterectomy and formal dilatation and curettage, as prerequisites of conservative treatment of atypical hyperplasia. Recently, Agorastos et al. (1997) have shown that GnRH analogs, which suppress endogenous estrogen, also might be of use in the treatment of endometrial hyperplasias in women who wish to preserve their fertility. These authors also highlight the necessity of endometrial monitoring by formal curettings when complex or atypical
Adjuvant endocrine therapy of EC

Progestins have been used for two decades as adjuvant therapy for ECs that have been treated with a curative intention using surgery and (if indicated) additional radiotherapy (Martin-Hirsch et al. 1996). So far, seven randomized trials have been performed on this subject, altogether involving more than 4500 women. Six of these trials (Lewis et al. 1974, Malkasian & Decker 1978, MacDonald et al. 1988, Vergote et al. 1989, De Palo et al. 1993, Urbanski et al. 1993) have been subjected to a meta-analysis (Martin-Hirsch et al. 1996). Although one of the trials had shown a benefit of adjuvant progestin treatment (Urbanski et al. 1993), the meta-analysis demonstrated that overall survival was not improved by adjuvant progestogen therapy and even that it might have been adversely affected. Furthermore, this treatment was not associated with any reduction in relapse or death from EC (Martin-Hirsch et al. 1996). Most of the EC patients enrolled into these six trials had a low risk of recurrence, so any potential benefits of adjuvant progestin therapy in more severe cases might have been ‘diluted’ (Martin-Hirsch et al. 1996).

In 1998, the COSA-NZ-UK Endometrial Cancer Study Groups published their randomized trial on adjuvant MPA treatment (400 mg/day for 3 years) in 1012 patients with high-risk EC (grade 3 endometrioid, adenosquamous, clear cell or papillary serous cancer, any tumor >1/3 invasive or involving cervix or adnexa). When all patients were analyzed, there were significantly more relapses in patients who did not receive MPA ($P<0.05$), but there were no differences in survival. A secondary analysis, excluding those 112 patients considered ineligible following a central pathology review, was undertaken. Patients who received MPA had a significantly longer disease-free interval ($P=0.03$) and survival ($P=0.03$) than those who did not. Fifty-nine of the 96 women in the control group were given MPA upon relapse. The median survival in this group was 10 months, which compares with 4 months in those patients not given hormonal therapy, a factor that might have reduced the survival benefit of the group treated with MPA from the beginning of the trial. Also of interest was the failure of steroid-receptor status to predict survival advantage in the treatment group. These authors concluded that it seems apparent that the use of adjuvant MPA at this dose and for this length of time has little place in the management of patients with high-risk EC (COSA-NZ-UK Endometrial Cancer Study Groups 1998).

Conclusions

Because of the need for treatment for patients who are often elderly and medically unfit, and the present lack of agents that produce long-lasting responses in EC, future research must evaluate new compounds and approaches.

These treatments ought to include pure anti-estrogens (e.g. ICI 182, 780) and selective ER modulators (e.g. raloxifene and others), to assess whether these compounds are superior to TAM. With the availability of modern, specific and potent aromatase inhibitors (Dowsett 1999), their use in the treatment of EC should be evaluated. The anti-progestin RU 486 has been shown to inhibit EC cell lines in vitro at clinically achievable doses (Schneider et al. 1998). Intensive preclinical and clinical work is being performed by a number of groups on the application of GnRH-agonists and -antagonists for the treatment of EC (for reviews see Grundler & Emons 1999, Westphalen & Emons 1999). GnRH receptors expressed by more than 80% of ECs might be used for the targeting of cytotoxic drugs (Schally & Nagy 1999). The increasing knowledge on the regulation of EC via peptide hormones, growth factors, steroids and the respective agonistic and antagonistic analogs might open up new therapeutic approaches. In particular, new insights into the molecular mechanisms of steroid hormone action (Enmark & Gustafsson 1998, White & Parker 1998, Jordan & Morrow 1999), together with the development of tissue-specific modulators, might help to overcome the hormone resistance of ECs.

It is to be hoped that ongoing and future research will unravel the molecular mechanisms leading to the formation of virulent type II EC and will thus facilitate the development of efficacious and non-toxic systemic treatment modalities for this disease.

For the time being, progestins are the therapy of choice for patients with atypical endometrial hyperplasia and who wish to preserve their fertility, provided that careful follow-up is guaranteed. For patients with advanced or recurrent EC of a low grade and having positive PRs, progestins are still a reasonable initial approach. So far, neither progestins nor cytotoxic chemotherapy has been shown to have substantial benefits in the adjuvant setting. If the novel endocrine manipulations outlined above (e.g. pure anti-estrogens, selective estrogen receptor modulators, aromatase inhibitors) were shown to be efficacious in the treatment of advanced EC, their usefulness in the adjuvant situation ought to be evaluated.

References


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