Endometrial cancer: experimental models useful for studies on molecular aspects of endometrial cancer and carcinogenesis

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Abstract
There is definitely a need for the development of new drugs for the treatment and cure of endometrial cancer. In addition there are various new drugs or phyto-remedies under development which are intended for use in the treatment and prevention of breast cancer, for the treatment of menopausal symptoms and for hormone replacement therapy. The efficacy of novel drugs targeting steroid receptors in endometrial cancers has to be evaluated and the safety of other endocrine manipulations on endometrial cancers or on endometrial carcinogenesis has to be assessed. For these experimental purposes five main classes of experimental models are available: spontaneous endometrial tumorigenesis models in inbred animals (Donryu rats, DA/Han rats, BDII/Han rats), inoculation tumors from chunks of tumors (rat EnDA-tumor, human EnCa 101 tumor) or from inoculated tumor cell lines (rat RUCA-I cells, human Ishikawa and ECC-1 cells), developmental estrogenic exposure or chemical carcinogen exposure of CD-1 and ICR mice, transgenic approaches such as mice heterozygous regarding the tumor suppressor gene PTEN (pten<sup>+/−</sup>-mice) and endometrial tumor cell lines cultured under conditions promoting in vivo-like morphology and functions e.g. cell culture on reconstituted basement membrane. Although the number of models is comparatively small, most aspects related to functions of estrogenic or gestagenic substances are assessable, particularly if various experimental models are combined. Whereas models based on human endometrial adenocarcinoma cells are widely used, the properties and advantages of animal-derived models have mainly been ignored so far.

Introduction
Endometrial cancer is the most frequently diagnosed malignancy of the female genital tract. Incidence rates of endometrial cancer are described as 10 to 25 women per 100 000 with a clear geographic variation between e.g. European (Spain, UK, France) and North American (USA and Canada) countries, with a slightly higher incidence in North America (Parazzini et al. 1991). In 1998, an estimate of 36 000 new cases and 6300 deaths attributable to endometrial cancer were reported in the USA (Podratz et al. 1998) resulting in an overall frequency which is only exceeded by breast, lung and colon cancer (Li et al. 1999). However, the mortality rate of endometrial cancer if compared with other cancers is low and the prognosis is excellent if it is detected in the early stages (Creasman 1997). The present knowledge of the role of endocrine factors in the etiology of endometrial adenocarcinomas, their role in the regulation of tumor growth, invasion and metastasis of endometrial adenocarcinoma cells, as well as the potential value of established and novel endocrine manipulations for prevention and treatment of endometrial adenocarcinomas have been exhaustively reviewed in a recent review article (Emons et al. 2000).

In the concluding remarks of the same paper it was stated that neither progesterin treatment, the major hormonal therapy of endometrial carcinoma, nor cytotoxic chemotherapy showed substantial benefits in the adjuvant setting. Therefore, future research activities must evaluate new compounds and new treatment strategies.

The endometrium is a classical hormone-dependent tissue and most of the endometrial adenocarcinomas are hormone-dependent tumors. A high percentage of these tumors express the estrogen receptor(s) and/or progesterone receptors. Investigators have, therefore, aimed at targeting steroid hormone receptors by the development of novel sub-

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stances e.g. new antiestrogens, selective estrogen receptor modulators (SERMs) and aromatase inhibitors. For testing and characterization of these new substances appropriate models have to be available.

A further need for endometrial tumor models stems from safety considerations. Estrogens not only act as tumor promoters but also as carcinogens. This has consequences for the approval of endocrine active substances intended for use in tumor therapy e.g. breast cancer, tumor prevention or hormone replacement therapy. The concerns are that substances which are beneficial in one organ might harm another, e.g. tamoxifen, a common therapy in breast cancer, might increase the risk for endometrial cancer (Emons et al. 2000). Finally, plenty of phytoestrogens are recommended either as semipure substances or, as in the case of soy or red clover extracts, for use as supportive measures in hormone replacement therapy as well as for use in lifestyle medicine. None of these substances has been exhaustively investigated regarding its safety (Fugh-Berman & Kronenberg 2001, Balk et al. 2002, Burton & Wells 2002).

These examples clearly demonstrate the need for experimental endometrial tumor models. The aim of this paper is to review the knowledge of endometrial tumor models. Particular emphasis is given to their applicability in vivo and to their potential responsiveness to natural as well as synthetic estrogens and progestins. The latter feature is a prerequisite for the process of development and evaluation of endocrine active substances.

Spontaneous endometrial adenocarcinoma of rats

Longevity studies revealed that female animals of four rat strains die with an uncommonly high incidence rate for endometrial adenocarcinoma if kept to their natural life end. Due to their clinical, pathophysiological, histological and biochemical features they all represent excellent experimental models for endometrial carcinogenesis. Their potential as experimental tumor models has so far not been appreciated by the scientific community.

Han:Wistar rats

Already in 1981 Deerberg et al., published a report showing that virgin Han:Wistar rats die with an incidence rate of 39% from tumors of the uterus if kept to their natural life end. A total of 35% of these tumors were endometrial adenocarcinomas. This rat strain and the pathogenesis of its endometrial adenocarcinomas have not been investigated any further.

Donryu rats

Female Donryu rats have been presented as a hormone-dependent endometrial cancer model, an issue discussed in detail below. This model is not well characterized regarding histopathological properties and metastatic potential. Genetically, in spontaneous endometrial cancers point mutations of the K-ras locus have been found recurrently (Tanoguchi et al. 1999). The latter mutation also has a rather frequent occurrence in human endometrial adenocarcinoma (Boyd & Risinger 1991, Semczuk et al. 1998).

Female animals of the Donryu strain are characterized by a similar mortality rate for endometrial adenocarcinoma as Han:Wistar rats and exhibit an incidence rate of 35.1% for endometrial adenocarcinoma and a total of proliferative lesions of approximately 60% (Nagaoka et al. 1990). The pathogenesis of endometrial adenocarcinoma in this rat strain is characterized by some features which are discussed as risk factors contributing to endometrial carcinogenesis in humans. Donryu rats are characterized by an imbalanced (increased) estradiol/progesterone rate which at the age of 12 months is increased almost fivefold if compared, for example, with aged Fischer-344 rats (Nagaoka et al. 1990). In humans menstrual cycles reflecting the estrogen/progesterone imbalance are regarded as a typical risk factor for endometrial adenocarcinoma (Barakat et al. 1997).

It has been proposed that endometrial carcinogenesis in Donryu rats is hormone dependent (Nagaoka et al. 1994). However, this conclusion has been drawn from experimental observations which should not necessarily be interpreted as hormone dependency. The first piece of evidence derives from the above mentioned estrogen/progesterone imbalance in the serum of these animals. The other piece of evidence has been deduced from comparative studies with Fischer-344 rats which have a normal cyclicity and almost no advanced histological changes such as hyperplastic or neoplastic lesions (Nagaoka et al. 1994) or alterations in the proliferative capacity of the uterine epithelium (Ando-Lu et al. 1998). Further, reproductive experience reduced the incidence of endometrial and mammary carcinoma. This finding is analogous to the situation discussed for humans (Terry et al. 1999), although it only becomes apparent in Donryu rats after three periods of gestation. One- or twofold experiences were ineffective if compared with nulliparous animals (Nagaoka et al. 2000). However, the conclusive experiments proving hormone dependency, namely comparative studies in normal cyclic and ovariectomized animals are missing.

The overall yield of endometrial adenocarcinoma could be increased and the onset of endometrial adenocarcinoma could be considerably accelerated if animals were intraperitoneally treated four times with a combination of estradiol dipropionate and N-methyl-N-nitrosourea (Nagaoka et al. 1993) or if they received a single intra-uterine administration of N-ethyl-N’-nitro-N-nitosoguanidine via the vagina (Ando-Lu et al. 1994). High fat diets also led to an earlier onset of endometrial carcinogenesis which was paralleled by an earlier onset of imbalance in estrogen/progesterone levels (Nagaoka et al. 1995). Transplacentation administration
of diethylstilbestrol on days 17 and 19 at a dose of 0.1 mg/kg led to an increase in lesions of the female reproductive tract of Donryu female offspring, including an increase in endometrial adenocarcinoma (Kitamura et al. 1999). Although the overall process of carcinogenesis is accelerated by these chemical carcinogens or the tumor promoting estrogen, it is questionable whether results from studies applying this approach can be interpreted more accurately than studies using the spontaneous protocol. Treatment with chemical carcinogens induces mutations which unpredictably add to genetic alterations already present in Donryu rats, like the frequent mutation of the Ki-ras locus (Tanoguchi et al. 1999).

So far two therapeutic studies have been described using this model in the situation of spontaneous endometrial carcinogenesis. Treatment with dietary indole-3-carbinol reduced the incidence rate of endometrial carcinogenesis considerably. The study showed a clear correlation between the induction of estradiol 2-hydroxylation and a more normalized estrogen/progesterone level (Kojima et al. 1994). On a mechanistic level this means chemoprevention is due to induction of estradiol 2-hydroxylation. The other chemoprevention study was performed on N-ethyl-N’-nitro-N-nitrosoguanidine-treated Donryu rats. Tamoxifen treatment significantly reduced the number of proliferative lesions in the uterus which also was limited to a few hyperplastic lesions (Yoshida et al. 1998). The authors claimed that tamoxifen acted as an antiestrogen, because of decreased serum estradiol levels. However, in none of the papers were any comments on estrogen receptor involvement made. Receptor involvement is crucial for any antiestrogenic function. In addition, the finding mentioned above does not reflect the situation in humans. For the human situation it is well accepted that tamoxifen increases the relative risk for endometrial carcinogenesis (ACOG Committee Opinion 1996, Love et al. 1999), a calculation from epidemiological findings substantiated by the stimulation of proliferation in cultured endometrial adenocarcinoma cells by tamoxifen (Gusberg 1994, Creasman 1997).

DA/Han rats

DA/Han rats are characterized by an inbred background. Specific gene alterations are not yet documented. The overwhelming majority of tumors are moderately well differentiated which makes the tumor phenotypically similar to most of the human endometrial adenocarcinomas. This model exhibits a highly metastatic phenotype (Fig. 1), but has not so far been used for intervention studies, because the tumor can be transplanted into syngenetic animals. There it gives rise to metastasizing endometrial adenocarcinomas which by histopathological and biochemical means (expression of estrogen receptor α (ER-α)) are undistinguishable from the parental tumor (Horn et al. 1993, 1994).

Female DA/Han animals die from endometrial adenocarcinoma with an incidence rate of >60% if kept to their natural life end at 24–27 months of age. Ovariectomy prior to onset of cyclicity prevents endometrial carcinogenesis (Deerberg et al. 1985). This has to be interpreted as a clear indication of hormone dependency of the carcinogenic process. Sixty-three percent of all of the developing endometrial adenocarcinomas of the DA/Han rats metastasize into the lung, thereby using a lymphogenic pathway (Fig. 1; F Deerberg, unpublished observations). Lymphatic invasion is a characteristic of type II endometrial carcinoma (Emons et al. 2000). Distant metastases are rare in cases of human endometrial cancer (Cook et al. 1999); however, among those few cases the lung has been described as a frequent metastases site (Bouros et al. 1996).

So far, these spontaneously occurring tumors have not been used for experimental purposes. However, from these tumors a serially transplantable tumor called EnDA (Horn et al. 1993) and a cell line called RUCA-I (Schütze et al. 1992) which also gives rise to hormone-sensitive endometrial cancers if inoculated into syngenic rats or athymic nude mice (see below) have been established (Fig. 2).

BDII/Han rats

In terms of an endometrial cancer model the female rats of this strain are unique worldwide. Since being first described in 1987 (Deerberg & Kaspareit 1987) it took almost a decade before they were used for cancer treatment experiments, and an additional five more years before investigators started to unravel their genetic peculiarities which contribute to the high incidence of endometrial cancer. This tumor model is genetically well characterized (discussed in detail below), although some important genetic information is still missing e.g. mutation frequency of some tumor suppressor genes (e.g. APC, PTEN). Most histopathological properties known for human endometrial adenocarcinoma, e.g. tumor grading, are also found in this spontaneous animal model for endometrial adenocarcinoma (Deerberg & Kaspareit 1987). Furthermore, this model has been investigated for the occurrence of precancerous lesions and their immunohistochemical properties. In rat endometrial tissue the same precancerous lesions have been found which are also detectable in human specimens. In addition, as in hyperplastic and displastic human endometrium, tenascin-C, a stromal marker for proliferative epithelial disease, is strongly up-regulated in similar lesions of rat endometrial tissue (Vollmer et al. 1990, 1991, Sasano et al. 1993). These findings illustrate the large similarities in cell biological aspects in the pathogenesis of endometrial cancers in humans and rats. In experimental setups for the understanding of the molecular mechanisms of endometrial carcinogenesis this model is of high value and should be considered as one of the most important in vivo models. There are several experimental pieces of evidence that endometrial
carcinogenesis of female BDII/Han animals is hormone dependent and therefore represents an endometrial tumor model for spontaneous hormonal carcinogenesis.

If female animals of this strain are kept to their natural life end (around 27 months of age) they die from endometrial carcinoma or metastases with an incidence rate of >90%. The overwhelming majority (87%–97%) of all carcinomas in the various experimental groups were endometrial adenocarcinomas. The small remaining group consisted of anaplastic carcinomas, adenosquamous carcinomas and squamous cell carcinomas (Deerberg & Kaspareit 1987). This powerful study was set up with 50 animals per experimental group initially. In parallel experimental groups it was demonstrated that neither maintenance of animals in a germ-free environment nor feeding of a purified diet reduced the incidence rate of tumors. The group of retired breeders also exhibited an incidence rate of >90% but was characterized by a significantly longer life span. This finding is a clear indication of hormonal involvement in the carcinogenic process. Numbers of pregnancies are regarded as a life style factor inversely correlated to endometrial carcinogenesis in humans (Emons et al. 2000 and references therein). But even more importantly, if BDII/Han rats are ovariectomized prior to estrous cyclicity, they die from causes other than endometrial
Figure 2: The EnDA/RUCA model. This figure shows the experimental possibilities of the syngenic EnDA/RUCA model consisting of spontaneous endometrial carcinogenesis of DA/Han rats, of the EnDA inoculation tumor and of cultured and/or inoculated RUCA-I cells.
carcinoma, the incidence rate for this tumor decreasing to 0%. This is the strongest piece of evidence in favor of hormone-dependent endometrial carcinogenesis in BDII/Han rats (Deerberg & Kaspereit 1987). This latter finding was further substantiated in a more recent study in which life long treatment with the progestin, melengestrol acetate, in three different doses (0.1, 0.2 and 0.4 mg/kg/day) completely suppressed endometrial carcinogenesis (Deerberg et al. 1995). At the highest dose glucocorticoid-like side effects were observed.

Only recently has the hormonal carcinogenesis of inbred BDII/Han rats been recognized as a model system to study genes involved in endometrial carcinogenesis. Using them as models for the understanding of genetic elements involved in the ontogeny of endometrial adenocarcinoma in general, genetic changes occurring in the course of endometrial carcinogenesis could be demonstrated in crossbreeding experiments applying comparative genomic hybridization (Helou et al. 2001). The most common aberration was amplification of the proximal region of rat chromosome 4. The genes Cdk6 (cyclin dependent kinase 6) and Met (hepatocyte growth factor receptor) were found to be located in the core of each amplified region and amplified most recurrently and at the highest level among the genes tested (Walentinsson et al. 2001). These data suggested that up-regulation of Cdk6 and/or Met contributes to the development of endometrial cancers in BDII/Han rats. The human homolog of Cdk6 has been postulated to be an important player in cell cycle control. Evidence exists that Cdk6 provides the link between growth factor stimulation and onset of cell cycle progression (Meyerson & Harlow 1994). However, the only documentation of Cdk6 overexpression in association with Cdk6 amplification comes from human gliomas (Costello et al. 1997). The Met protooncogene encodes a transmembrane growth factor which binds the cytokine hepatocyte growth factor/scatter factor (HGF/SF). The highest levels of this tyrosine kinase receptor are found in epithelial tissues. The ligand, in contrast, is predominantly expressed in mesenchymal tissues suggesting a paracrine mode of action (Gherardi & Stoker 1991). The major effect of HGF/SF function mediated by Met is thought to be epithelial cell proliferation and motility (Sugawara et al. 1997), including endometrial epithelium as well (Wagatsuma et al. 1998). Further, it has been shown that HGF/SF can stimulate invasion of endometrial adenocarcinoma cell lines if they express MET (Bae-Jump et al. 1999).

Extra copies of chromosomal regions were found for chromosome 6 and eight cancer-related genes were predicted to be located in this chromosomal region of BDII/Han rats. These genes comprised the N-myc protooncogene, apolipoprotein B, the DEAD box gene, ornithine decarboxylase, propiomelanocortin, ribonucleotide reductase, M2 polypeptide and syndecan. Amplification of N-myc was by far the highest suggesting that Mycn amplification and overexpression contributes to the development of this hormone-dependent tumor (Karlsson et al. 2001). In addition, three major chromosomal regions representing multiple susceptibility genes involved in the development of endometrial adenocarcinoma in rat could be identified (Roshani et al. 2001). Loss of heterozygosity analysis revealed three major losses of heterozygosity regions on chromosome 10 (Behboudi et al. 2001). By radiation hybrid mapping and single and dual color fluorescence in situ hybridization techniques a detailed chromosomal map of the proximal part of chromosome 10 could be established. With this approach the regional localization of 14 genes, most of them cancer related, and of 5 microsatellite markers could be determined (Behboudi et al. 2002).

Finally, in crossbreeding experiments of female BDII/Han rats with males of two strains with a low incidence of endometrial adenocarcinoma, three chromosomal regions, which give rise to susceptibility for endometrial adenocarcinoma, could be identified (Roshani et al. 2001). This is a clear indication that endometrial carcinogenesis of BDII/Han rats exhibits heritable features. Interestingly, the genes affecting susceptibility to endometrial adenocarcinoma were different in the two crosses, suggesting that genes behind the susceptibility in BDII/Han animals may interact with various genes in different genetic backgrounds. Data in the literature suggest that this feature of heritability of endometrial carcinogenesis in BDII/Han rats is shared by at least two forms of human endometrial cancer (Sandels et al. 1992, Gruber & Thompson 1996, Lynch et al. 1996). Whether or not this heritability of endometrial carcinogenesis resembles the heritable phenotype in human endometrial adenocarcinoma of patients with hereditary non-polyposis colorectal cancer (HNPPC) remains open. The latter phenotype would, at least, require microsatellite instability, mutation or alteration of promoter methylation of mismatch repair genes and mutations of the pten locus (Kuismanen et al. 2002, Whelan et al. 2002, Zhou et al. 2002a,b). A corresponding genetic analysis of endometrial adenocarcinoma of BDII/Han rats is still missing.

Mouse models

Mouse models historically have proved to be particularly useful in studying the effects of developmental exposure to hormones, particularly estrogens. This topic will be discussed in more detail below. In addition, the ICR mouse model which responds to chemical carcinogens by induction of endometrial cancer is described.

Transgenic mouse models are emerging tools for carcinogenesis in general. In the context of endometrial carcinogenesis pten+/− transgenic mice have to be discussed. These animals exhibit a phenotype strongly resembling human Cowden syndrome, a syndrome associated with high incidence of breast and endometrial neoplasia (Stambolic et al.
Developmental hormone (estrogen) exposure

In various mouse and rat strains estrogens increase the incidence of tumorigenesis in several organs (Liehr 2001 and references therein), including the uterus, particularly during developmental exposure. In humans, epidemiologists have recognized prolonged estrogen action or estrogen use unopposed by gestagens as a risk factor for endometrial carcinogenesis (Key & Pike 1988, Weiderpass et al. 1999). From all these data the question has been raised whether estrogens act as carcinogens (Boyd 1996). From mechanistic and molecular studies more and more pieces of evidence have accumulated to strengthen this hypothesis (reviewed by Liehr 2000, 2001).

The neonatal mouse has been proposed as a model for hormonal carcinogenesis of the endometrium (Newbold et al. 1990). If outbred mice are treated neonatally (days 1–5) they will develop endometrial adenocarcinoma in a dose-dependent manner and dependent on the strength of the estrogen. In the initial paper, Newbold et al. (1990) showed that diethylstilbestrol and derivatives thereof are more potent than estradiol. This model has been further characterized in terms of cellular differentiation and gene expression in the epithelium (Yoshida et al. 2000). In the meantime, this model has been successfully applied to test for the developmental toxicity of tamoxifen (Newbold et al. 1997), catecholestrogens (Newbold & Liehr 2000) and genistein (Newbold et al. 2001). All these substances induce endometrial adenocarcinoma if injected in neonatal mice during postnatal days 1–5. Whether this model can be replaced by transgenic mice overexpressing ER and which exhibit an accelerated onset of endometrial carcinogenesis (Couse et al. 1997) remains to be elucidated, because these tumors are characterized by a more aggressive phenotype and may therefore be less representative of the human situation than endometrial carcinogenesis in wild-type animals.

In summary, CD-1 mice appear to be an excellent model for testing developmental toxicity of estrogens, thereby evaluating the capacity of test substances to induce hormone-dependent endometrial cancers.

Chemical carcinogen-induced endometrial cancers in ICR mice

ICR mice respond to treatment with N-methyl-N-nitrosourea (NMU) or estradiol with the development of endometrial cancers within 30 weeks. This process is significantly accelerated if ICR mice are treated with both agents simultaneously (Niwa et al. 1991). The enhancing effects on endometrial carcinogenesis initiated by NMU are not only detectable for estradiol but also for estrone and estriol (Niwa et al. 1993).

In comparison to human endometrial carcinogenesis, histopathological examinations revealed that these tumors develop from various preneoplastic, hyperplastic lesions, resembling the human situation (Niwa et al. 1991). Interestingly, the carcinogen and estradiol induce different preneoplastic lesions. Estradiol induces glandular hyperplasia, while adenomatous hyperplasia is induced by NMU. For the development of atypical hyperplasia the cooperative action of estradiol and NMU is favorable (Niwa et al. 1996).

Whereas the histopathology of NMU-induced endometrial tumors in ICR mice resembles the situation described for endometrial carcinogenesis, the limited genetic analysis described for the mouse model is different to human endometrial carcinogenesis. In specimens of human endometrial cancer the mutation of various Ras loci (Boyd & Risinger 1991, Ignar-Trowbridge et al. 1992) and the p53 gene (Risinger et al. 1992) are found quite often. These kinds of mutations are very rare in NMU-initiated endometrial tumors in ICR mice (Murase et al. 1995).

With regard to steroid receptor expression and protein levels this model is poorly characterized. In a short-time protocol in ovariectomized ICR mice, animals respond to estrogen treatment by up-regulation of steady state mRNA levels of the proto-oncogenes fos and jun (Niwa et al. 1998).

The NMU-induced and estradiol-enhanced endometrial carcinogenesis proved to be particularly useful for treatment studies. Several hormonal and traditional treatment procedures were tested leading with one exception to inhibition or prevention of all tumors in the ICR model. Carcinogenesis induced by a combination of estradiol and NMU was inhibited by gestagen treatment (medroxyprogesterone acetate; Niwa et al. 1995) or by treatment with the antiestrogen, tamoxifen (Niwa et al. 2002). In contrast, treatment with the selective estrogen receptor modulator, tamoxifen, stimulated endometrial carcinogenesis in NMU-treated animals with or without the combined treatment with estradiol (Niwa et al. 1998).

Prevention of endometrial carcinogenesis in the above described mouse model including a reduction of hyperplastic foci was detected following treatment of animals with danazol (Niwa et al. 2000) or following treatment with traditional Japanese or Chinese remedies such as extracts from Glycyrrhiza radix (Niwa et al. 1999) and Juzen-taiho-to (Niwa et al. 2001). Shimotsu-to was identified as the active principle in Juzen-taiho-to (Lian et al. 2002). Finally, prevention studies with isoflavones revealed that both genistein and daidzein exhibited an inhibitory effect on endometrial carcinogenesis in the estradiol/NMU ICR mouse model, particularly on the indices of atypical endometrial hyperplasia (Lian et al. 2001).

In summary, NMU-induced endometrial adenocarcinomas in ICR mice are particularly sensitive to various hormonal treatment procedures. The limitation of this model is its poor characterization of hormone responsiveness at a
molecular level. Neither the levels nor the qualities of expressed steroid hormone receptors are known nor is there any information on hormonally triggered signal transduction cascades or responsive genes except for fos and jun.

**Heterozygous mouse models with pten<sup>−/+</sup>-mice as an example**

PTEN/MMAC1/TEP1 has been detected as one of the most commonly mutated tumor suppressor genes in human cancer (Li et al. 1997, Steck et al. 1997). A significant rate of PTEN mutations has also been reported for human endometrial adenocarcinoma (Risinger et al. 1997, 1998, Tashiro et al. 1997). This mutation has been found in up to 80% of cases of human endometrial adenocarcinoma, and is already detectable in 33–55% of the precancerous lesions (Latta & Chapman 2002, Konopka et al. 2002). In addition to a mutation promoter, hypermethylation is an alternative way to inactivate tumor suppressor genes. There is one report in the literature showing that the frequency of promoter hypermethylation of the PTEN tumor suppressor gene in endometrial adenocarcinoma is around 19% (Salvesen et al. 2001).

Although the importance of a functional PTEN protein has been known for several years, the association of PTEN expression with a prognosis is not yet clear. It appears as if loss of PTEN expression is associated with metastatic disease (Salvesen et al. 2002) and may serve as an independent prognostic marker for patients who undergo postoperative chemotherapy (Kanamori et al. 2002).

In order to understand the biological role of this dominant tumor suppressor gene, transgenic PTEN knock-out mice have been created. A PTEN<sup>−/−</sup> mutation is lethal presumably due to defective chorio-allantoic development (Suzuki et al. 1998).

Surprisingly, the mutation of one allele is sufficient to cause neoplasia in multiple organ systems in these pten<sup>−/+</sup>-mice (Podsypanina et al. 1999). A particularly high incidence has also been detected in breast as well as in endometrial neoplasia and preeclampsia, strongly resembling human Cowden syndrome (Stambolic et al. 2000). Indeed, in the human situation and in addition to the occurrence of PTEN mutations in sporadic tumor, germ-line mutations are believed to cause related autosomal dominant hamartoma syndromes, among them Cowden syndrome (Liaw et al. 1997, Eng 1998). Although the phenotype detectable in pten<sup>−/+</sup>-mice in terms of the development of Cowden syndrome and the association with the high incidence of development of precancerous and cancerous lesions of breast and endometrium is strikingly similar to the human situation, some differences do exist. Unlike the human situation, in pten<sup>−/+</sup>-mice neoplasia of the skin and brain were notably absent, whereas the observed changes in the endometrium were very consistent (Podsypanina et al. 1999).

Endometrial carcinoma, except colon carcinoma itself, is the most common malignancy in patients with hereditary non-polyposis colon cancer (HNPCC). This disease is characterized by germ-line mutations in mismatch repair genes and by microsatellite instability (Fishel et al. 1993, Leach et al. 1993, Peltomäki et al. 1993, Bronner et al. 1994, Nicolaides et al. 1994, Papadopoulos et al. 1994). Surprisingly, patterns of microsatellite instability differ between endometrial and colorectal tumors from patients with HNPCC (Kuismanen et al. 2002).

To learn more about the involvement of these genes in the pathogenesis of endometrial carcinogenesis, tumor specimens from patients with HNPCC and an established mutation record of either the MLH1 or MSH2 missmatch repair gene locus were subjected to analysis of the PTEN locus. Mutation of the mismatch repair loci and PTEN gene were very common in HNPCC, with PTEN mutation presumably preceding mutations of the mismatch repair loci (Zhou et al. 2002b). Interestingly, crosses of pten<sup>−/+</sup>- with mlh1<sup>−/−</sup>-mice were characterized by an accelerated endometrial tumorigenesis (Wang et al. 2002).

In summary, endometrial cancers from pten<sup>−/+</sup>-mice with or without mlh1<sup>−/−</sup> may serve as an excellent model for hereditary endometrial cancer, because of the strong resemblance with human Cowden disease and human HNPCC. In addition, Stambolic et al. (2000) proposed pten<sup>−/+</sup>-mice as a model for endometrial adenocarcinomas which develop in women with unopposed estrogen stimulation. These patients rather commonly suffer from loss of heterozygosity at the PTEN locus and/or mutations in all stages of endometrial hyperplasia (Risinger et al. 1997, 1998, Tashiro et al. 1997).

Whether pten<sup>−/+</sup>-mice can serve as a hormone-dependent cancer model or if they are suitable as models for hormonal treatment protocols remains open, because no data are available on these issues. However, despite the lack of the latter information it has to be stated that pten<sup>−/+</sup>-mice represent a promising new endometrial cancer model, because of the similarity to hereditary human endometrial cancer.

**Inoculation tumors**

These tumors are derived by inoculating chunks of tumors or defined numbers of cultured cells e.g. subcutaneously or into the fat pad of syngenic animals, athymic nude mice or rats. It is the scope of this paper to focus primarily on steroid hormone dependent or at least steroid hormone sensitive in vivo models. For this reason in the following sections evidence for hormone responsiveness from in vitro data is briefly summarized prior to the review of in vivo application data of either model.
The RUCA-I/EnDA model

From a spontaneous endometrial adenocarcinoma a transplantable tumor called EnDA-tumor (Horn et al. 1993) and a cell line called RUCA-I (Schütze et al. 1992) have been derived. These models have primarily been used to test novel antiestrogens, potential agonistic activities of phytoestrogens and o,p′-DDT as an example of an endocrine-disrupting chemical.

To test for antiestrogenic activities of antiestrogens chunks of EnDA-tumors or RUCA-I cells have been inoculated into syngenic DA/Han rats or athymic nude rats (Fig. 2; Horn et al. 1993, Vollner & Schneider 1996). Tumor growth from chunks of EnDA-tumors at the ectopic site as well as formation of lymphogenic and pulmonary metastasis are estrogen-sensitive processes, because the tumor growth rate at the ectopic site, as well as the weight of ipsilateral axillary lymph nodes and the number of lung metastases is reduced in ovariectomized animals if compared with intact animals. These effects most likely are mediated by the ERα, which unlike the progesterone receptor is expressed in these tumors. This experimental model has been successfully applied to study, for example, the antiestrogenic activity and function of ZK119010 in an endometrial-derived experimental tumor model (Horn et al. 1993, 1994). As indicated above an endometrial adenocarcinoma cell line, the RUCA-I cell line, has been established from this transplantable EnDA-tumor. This cell line is characterized by the expression of the ERα and by its estrogen responsiveness in vitro and in vivo (Fig. 2). If RUCA-I cells are cultured on reconstituted basement membrane (matrigel) they respond to treatment with estradiol by stimulation of proliferation and alteration of gene expression e.g. by the upregulation of complement C3 expression (Vollmer et al. 1995a) which is the major estrogen responsive gene in the rat uterus (Sundström et al. 1989), as well as by down-regulation of fibronectin expression (Vollmer et al. 1995b) and up-regulation of clusterin gene expression (Wünsche et al. 1998). In vivo, if inoculated subcutaneously into the hind limb of syngenic DA/Han rats RUCA-I cells form estrogen-sensitive metastasizing adenocarcinomas which by histological and biochemical means are indistinguishable from tumors arising from chunks of EnDA-tumors. Tumor growth and metastasis in both experimental setups are very fast. After approximately 30–35 days animals are clinically ill from the metastatic disease if 0.5–1 × 10⁶ cells are inoculated at the ectopic site (Horn et al. 1994, Vollmer & Schneider 1996).

In this transplantation approach RUCA-I cells at the inoculation site as well as metastases of these cells at lymphogenic sites and in the lung react very sensitively to antiestrogen treatment. The weight of the primary tumor at the ectopic site, the weight of the axillary ipsilateral lymph nodes as well as the number of lung metastases were found to be reduced compared with control animals in response to antiestrogen treatment of inoculated animals (Vollmer & Schneider 1996). In ectopically grown tumors, in lymph node metastases and in the normal uterus (the latter organ was analyzed for control purposes) expression of estrogen-dependent genes was downregulated significantly following treatment of animals with the pure antiestrogen ICI 182,780 (Wünsche et al. 1998), amongst these genes were matrix metalloproteinases (Tushaus L, Hopert AC & Vollmer G, unpublished observations).

Inoculation of RUCA-I cells into ovariectomized DA/Han rats for the purpose of screening for estrogenic properties of phytoestrogens and endocrine-disrupting chemicals in an endometrial-derived tumor model led to unexpected results. Although tumor weight increased following oral application of ethinyl estradiol in a 28-day treatment protocol, the weight of the tumor tissue remained almost unaffected following treatment of animals with a selected phytoestrogen, genistein (Diel et al. 2001) or a representative industrial chemical o,p′-DDT (Diel et al., in preparation).

Unlike ethinyl estradiol-treated animals, there was no significant alteration of gene expression detectable in tumor tissues of animals treated with genistein or o,p′-DDT. Control investigations in the normal rat uterus showed that this organ responded very sensitively to treatment with ethinyl estradiol, genistein and o,p′-DDT, both with an increase in tissue weight as well as up-regulation of estrogen-dependent gene expression (Diel et al. 2001).

In conclusion, RUCA-I cells if inoculated into syngenic DA/Han rats represent a very sensitive estrogen-responsive tumor model for the purpose of antiestrogen screening and testing in an endometrial-derived tumor model. The RUCA-I cell-derived model is of limited value for testing of agonistic estrogenic properties of e.g. suspected phytoestrogens or endocrine-disrupting chemicals. Experimentally, the EnDA/RUCA-I model shows a unique feature: it is possible to work in syngenic animals which means it is not necessary to use athymic nude mice or rats having an imperfect immune system which have to be kept in germ-free surroundings.

Ishikawa cells

Hormonal responsiveness of Ishikawa cells in vitro

Since their first description (Nishida et al. 1985) Ishikawa cells, a human endometrial adenocarcinoma cell line expressing the estrogen and the progesterone receptors, are the most widespread human endometrial-derived cell culture model. Later on, applying the limited dilution method eighteen different clones of Ishikawa cells were established (Nishida et al. 1996), fifteen of them being estrogen receptor positive. However, in terms of population doubling time, plating efficiency or saturation density there was no significant
difference among the clones (Nishida et al. 1996). In addition to the expression of functional estrogen receptors (Holinka et al. 1986a, b) and progesterone receptors (Lessey et al. 1996), Ishikawa cells express the androgen receptor (Lovely et al. 2000) and the receptor for aryl hydrocarbons (Wormke et al. 2000). There exists a large body of evidence for the hormonal responsiveness of Ishikawa cells in vitro. In summary, the evidence for hormonal responsiveness is based mainly on hormonal modification of proliferation, cellular functions or gene expression (for summary see Table 1 and references therein).

In vitro the cells are in use for the elucidation of molecular mechanisms of hormone action e.g. in drug development, in the drug discovery process, for testing of potential agonistic functions of antiestrogens or selective estrogen receptor modulators in an endometrial-derived model (Labrie et al. 2001), in studies of ligand independent activation of the estrogen receptor, in anchorage independent tumor growth (Holinka et al. 1989), in studies on factors controlling hormonal receptivity (Appa Rao et al. 2001), in environmental toxicology studies on the function of phytoestrogens (Markiewicz et al. 2001), in studies on factors controlling hormonal receptivity (reviewed in Holinka et al. 2000). These experimental findings provide evidence in favor of the assumption that high levels of ERα may be beneficial in the control of endometrial cancer due to its inhibitory effects on angiogenic pathways.

Finally, endometrial carcinoma tumorigenicity could be suppressed following introduction of chromosome 18 into Ishikawa cells (Yamada et al. 1995). This chromosome is known to harbor the presumptive tumor suppressor gene DCC and therefore experimental evidence is provided for the hypothesis of DCC being a candidate for an endometrial carcinoma tumor suppressor gene.

In summary, Ishikawa cells represent a combined in vitro/in vivo human endometrial tumor model which is particularly suitable for the study of hormonal growth control. In addition, it may be useful in characterizing organ specificity of natural and synthetic estrogens, like phytoestrogens or endocrine-disrupting chemicals.

Ishikawa cells as an in vivo tumor model

Because of their hormone responsiveness in vitro Ishikawa endometrial adenocarcinoma cells have also been developed as an estrogen sensitive in vivo tumor model (Nishida et al. 1986). As mentioned above, eighteen subclones were isolated, all of these subclones were found being transplantable into athymic nude mice (Nishida et al. 1996). This model has mainly been used for two purposes: (1) to elucidate general mechanisms in tumor biology, particularly towards the understanding of estrogen and progesterone function in endometrial cancer and (2) as an endometrial model for hormonal treatment of endometrial cancer.

The Ishikawa endometrial adenocarcinoma model proved to be useful for studies on the regulation of growth control by steroid hormones in endometrial adenocarcinoma in vivo (Gong et al. 1994). The most important result of this study was that 4-hydroxytamoxifen, like estradiol, stimulates endometrial tumor growth in vivo. This effect most likely coincides with alteration of levels of expression of transforming growth factor (TGF)-α and TGF-β. In a similar approach following inoculation of Ishikawa cells into the fat pad of nude mice it could be demonstrated that raloxif en exhibits a growth stimulatory potential in this assay (Barsalou et al. 2002).

Involvement of a switch in the angiogenic pathways during tumorigenesis has become a recent focus of interest. The participation of the ERα in this process was studied in Ishikawa cells following overexpression of the ERα protein. Overexpression of ERα not only significantly inhibited the growth of xenografted cells, but also down-regulated vascular endothelial growth factor expression in tumor xenografts, resulting in a decreased vascularization of the tumors and the inhibition of the angiogenic agent integrin αvβ3 (Ali et al. 2000). These experimental findings provide evidence in favor of the assumption that high levels of ERα may be beneficial in the control of endometrial cancer due to its inhibitory effects on angiogenic pathways.

Finally, endometrial carcinoma tumorigenicity could be suppressed following introduction of chromosome 18 into Ishikawa cells (Yamada et al. 1995). This chromosome is known to harbor the presumptive tumor suppressor gene DCC and therefore experimental evidence is provided for the hypothesis of DCC being a candidate for an endometrial carcinoma tumor suppressor gene.

In summary, Ishikawa cells represent a combined in vitro/in vivo human endometrial tumor model which is particularly suitable for the study of hormonal growth control. In addition, it may be useful in characterizing organ specificity of natural and synthetic estrogens, like phytoestrogens or endocrine-disrupting chemicals.

Enca101 tumor and ECC-1 cells

ECC-1 cells as an in vitro model

ECC-1 endometrial adenocarcinoma cells (Satyaswaroop & Tabibzadeh 1991) have been derived from a transplantable endometrial adenocarcinoma called EnCa101 which was established immediately after the technique of tumor growth in athymic nude mice became available (Satyaswaroop et al. 1981). Most importantly, ECC-1 cells form tumors with glandular structures if inoculated into athymic nude mice (Satyaswaroop & Tabibzadeh 1991).

The EnCA101 tumor and the ECC-1 cell line respond to estrogen treatment (Satyaswaroop et al. 1983) and are particularly rich in progesterone receptors (Clarke & Satyaswaroop 1985). In addition, tumors respond to tamoxifen treatment by stimulation of growth (Jordan et al. 1991, Tonetti et al. 1998), a feature which from epidemiological studies is expected to increase the risk for sporadic human endometrial cancer during breast cancer therapy with tamoxifen (Emons et al. 2000 and references therein). There are many pieces of experimental evidence proving the responsiveness of this model to treatment with sex steroid hormones (for summary see Table 2). For these reasons the EnCA101/ECC-1 tumor is the most widely used in vivo human endometrial adenocarcinoma model.
Table 1  Hormonal responsiveness of Ishikawa cells

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Ligand(s)</th>
<th>Functional parameter</th>
<th>Regulation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER</td>
<td>E2</td>
<td>Proliferation</td>
<td>Stimulation</td>
<td>Holinka et al. (1986a,b)</td>
</tr>
<tr>
<td>ER</td>
<td>E2</td>
<td>Proliferation, colony formation</td>
<td>Stimulation</td>
<td>Holinka et al. (1989)</td>
</tr>
<tr>
<td>ER</td>
<td>4-OHT</td>
<td>Proliferation</td>
<td>Stimulation</td>
<td>Anzai et al. (1989)</td>
</tr>
<tr>
<td>ER</td>
<td>4-OHT</td>
<td>Proliferation</td>
<td>Stimulation</td>
<td>Croxall et al. (1990)</td>
</tr>
<tr>
<td>ER</td>
<td>E2</td>
<td>Alkaline phosphatase</td>
<td>Stimulation</td>
<td>Holinka et al. (1986c)</td>
</tr>
<tr>
<td>ER</td>
<td>EM652, EM800</td>
<td>Alkaline phosphatase</td>
<td>No regulation</td>
<td>Labrie et al. (2001)</td>
</tr>
<tr>
<td>ER</td>
<td>E2</td>
<td>Migration through basement membrane</td>
<td>Stimulation</td>
<td>Fujimoto et al. (1996b)</td>
</tr>
<tr>
<td>ER</td>
<td>Resveratrol</td>
<td>E2 induced alkaline phosphatase</td>
<td>Down-regulation</td>
<td>Bhat &amp; Pezzuto (2001)</td>
</tr>
<tr>
<td>ER</td>
<td></td>
<td>ERα</td>
<td>Down-regulation</td>
<td></td>
</tr>
<tr>
<td>ER</td>
<td></td>
<td>ERβ</td>
<td>No regulation</td>
<td></td>
</tr>
<tr>
<td>ER</td>
<td>E2, Tam</td>
<td>Proliferation, plasminogen activator</td>
<td>Up-regulation</td>
<td>Hochner-Celnikier et al. (1997)</td>
</tr>
<tr>
<td>ER</td>
<td>E2</td>
<td>Glycogen metabolism, gelatinase</td>
<td>Up-regulation</td>
<td>Mizumoto et al. (2002)</td>
</tr>
<tr>
<td>ER</td>
<td>Tam</td>
<td>Glycogen metabolism, gelatinase</td>
<td>Down-regulation</td>
<td></td>
</tr>
<tr>
<td>ER</td>
<td>E2</td>
<td>Growth, cyclin D1, invasiveness, MMP-1, -7, -9</td>
<td>Up-regulation</td>
<td></td>
</tr>
<tr>
<td>ER</td>
<td>E2</td>
<td>AF2</td>
<td>Stimulation</td>
<td>Sakamoto et al. (2002)</td>
</tr>
<tr>
<td>AR</td>
<td>DHT, T</td>
<td>Alkaline phosphatase</td>
<td>Up-regulation</td>
<td>Markiewicz &amp; Gurpide (1997)</td>
</tr>
<tr>
<td>PR</td>
<td>P</td>
<td>Growth</td>
<td>Stimulation</td>
<td>Holinka &amp; Gurpide (1992)</td>
</tr>
<tr>
<td>PR</td>
<td>MPA</td>
<td>Growth</td>
<td>Suppression</td>
<td>Shiozawa et al. (2001)</td>
</tr>
<tr>
<td>PR</td>
<td>P; E2 + P</td>
<td>PAI-1</td>
<td>Up-regulation</td>
<td>Fujimoto et al. (1996a)</td>
</tr>
<tr>
<td>PR</td>
<td>P</td>
<td>Estrogen sulfotransferase</td>
<td>Up-regulation</td>
<td>Falty &amp; Falty (1996)</td>
</tr>
<tr>
<td>PR</td>
<td>P</td>
<td>α,β, integrin</td>
<td>Up-regulation</td>
<td>Lessey et al. (1996),</td>
</tr>
<tr>
<td>PR</td>
<td>MPA</td>
<td>Sex hormone binding globulin</td>
<td>Up-regulation</td>
<td>Castelbaum et al. (1997)</td>
</tr>
<tr>
<td>PR</td>
<td>MPA</td>
<td>8 products from differential display RT-PCR</td>
<td>Up-/down-regulation</td>
<td>Misao et al. (1998)</td>
</tr>
<tr>
<td>PR</td>
<td>P</td>
<td>VEGFs</td>
<td>Down-regulation of E2</td>
<td>Sakata et al. (1998)</td>
</tr>
<tr>
<td>PR</td>
<td>P</td>
<td>Osteopontin</td>
<td>Up-regulation</td>
<td>Appa Rao et al. (2001)</td>
</tr>
</tbody>
</table>

Abbreviations: ER, estrogen receptor; E2, estradiol; 4-OHT, 4-hydroxytamoxifen; Tam, tamoxifen; DHT, dihydrotestosterone; T, testosterone; P, progesterone; MPA, medroxyprogesterone acetate; MMP, matrix metalloproteinase; PAI, plasminogen activator inhibitor; VEGF, vascular endothelial growth factor.

EnCa101 and ECC-1 cells-derived tumors

In terms of hormonal regulation of tumor growth, inoculation of EnCa101 tumors or ECC-1 endometrial adenocarcinoma cells into nude mice appears to be the most complete model. It is responsive to estrogens and progestins and it grows following tamoxifen stimulation. In addition, the EnCA101 tumors exhibit another feature which is highly advantageous in experimental cancer research: it can be used as a multi-site transplantation model thereby saving animals (Heitjan et al. 2002). In these tumors the most interesting features of hormonal influences on endometrial tumor growth can be studied, e.g. testing of novel estrogen receptor antagonists, treatment of tamoxifen-stimulated tumor growth — a major concern as indicated above (ACOG Committee Opinion 1996, Love et al. 1999) — and characterizing the effects of progestins, these being the adjuvant therapy for endometrial carcinoma for decades (Emons et al. 2000 and references therein).

Once the estrogen dependency of tumor growth of EnCa101 tumors (Satyaswaroop et al. 1983, Jordan et al. 1991) and their estrogen-responsiveness as measured by gene expression, particularly of progesterone receptor (Clarke et al. 1987) and of c-fos, (Sakakibara et al. 1992) had been established, in a first series of larger experiments the effects of antiestrogens and progestins on the growth of these tumors were investigated. In fact, for progestins schedules for progestin administration were designed in this model (Mortel et al. 1990).

For antiestrogens it could be shown that almost all anti-estrogens tested stimulated tumor growth although to a lesser degree than tamoxifen (Gottardis et al. 1990) with the exception of the pure antagonist ICI 164,384 which was not only void of tumor growth stimulatory activity but also blocked tamoxifen-induced tumor growth.

During combination treatments with tamoxifen and progestins, resistance of EnCa101 tumors to treatment became apparent. This resistance was attributed to a desensitization
following down-regulation of the progesterone receptor (Satyaswaroop et al. 1992). Based on the results of another study in which progesterone levels, progesterone receptor levels and rates of tumor growth were assessed, it was predicted that intermittent progestin administration may result in better control of endometrial cancer growth in the nude mouse model (Mortel et al. 1990).

The EnCa101 tumor model is characterized by another feature – the estrogen-inducible expression of aromatase. Aromatase expression has been reported to be up-regulated in endometrial adenocarcinoma, particularly in the stromal tissue compartment (Watanabe et al. 1995, Sasano et al. 1996). Analyses of aromatase (Cyp19) gene polymorphism revealed that these polymorphisms might represent one genetic risk factor for endometrial cancer development (Berstein et al. 2001). Despite the clear up-regulation of aromatase activity in endometrial cancer, the use of aromatase inhibitors in a clinical setting has not been exhaustively investigated and the clinical outcome is questionable (Rose et al. 2000, Berstein et al. 2002).

Estrogen-inducible aromatase activity, however, is interesting for general tumor biology because the role of androgens in the growth of endometrial carcinoma is poorly understood. The question of androgen sensitive endometrial tumor growth was addressed in this particular tumor model. There was little detectable growth promoting activity of androgens which was lower by far than that of estrogens. Surprisingly, there was no difference in the effects of androgens on the growth of EnCa101 tumors if the effects of aromatizable and non-aromatizable androgens were compared (Legro et al. 2001).

In summary, the EnCa101/ECC-1 tumor model is the most complete endometrial tumor model because the effects of estrogens, progesterins and androgens can be assessed due to the stable and, in part, estrogen inducible expression of the estrogen receptor, the progesterone receptor and aromatase. These concluding remarks have recently been substantiated by studies on the characterization of some novel antiestrogens: the tamoxifen analog GW5638 and the piperidin derivative ERA-923. These substances, like tamoxifen, block growth of breast cancer cells and, in addition, more effectively block EnCa101 endometrial tumor growth (Greenberger et al. 2001, Dardes et al. 2002a).

### Table 2: Hormonal responsiveness of EnCa101 tumors and ECC-1 cells

<table>
<thead>
<tr>
<th>Cell line/tumor</th>
<th>Receptor</th>
<th>Ligand</th>
<th>Functional parameter</th>
<th>Regulation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>EnCa101</td>
<td>ER</td>
<td>E2</td>
<td>Growth in vivo</td>
<td>Increase</td>
<td>Satyaswaroop et al. (1993)</td>
</tr>
<tr>
<td>EnCa101</td>
<td>ER</td>
<td>Tam</td>
<td>Growth in vivo</td>
<td>Increase</td>
<td>Jordan et al. (1991)</td>
</tr>
<tr>
<td>EnCa101</td>
<td>ER</td>
<td>E2/Tam</td>
<td>Growth in vivo</td>
<td>Increase</td>
<td>Jordan et al. (1989)</td>
</tr>
<tr>
<td>ECC-1</td>
<td>ER</td>
<td>E2</td>
<td>Growth in vivo</td>
<td>Increase</td>
<td>Dardes et al. (2002a)</td>
</tr>
<tr>
<td>ECC-1</td>
<td>ER</td>
<td>GW5638</td>
<td>Growth in vivo</td>
<td>No effect</td>
<td>Dardes et al. (2002b)</td>
</tr>
<tr>
<td>ECC-1</td>
<td>ER</td>
<td>E2</td>
<td>Growth in vivo</td>
<td>Stimulation</td>
<td>Dardes et al. (2002b)</td>
</tr>
<tr>
<td>ECC-1</td>
<td>ER</td>
<td>Tam, Ral</td>
<td>Growth in vivo</td>
<td>No effect</td>
<td>Bergeron et al. (1999), Castro-Rivera et al. (1999), Dardes et al. (2002b)</td>
</tr>
<tr>
<td>EnCa101</td>
<td>ER</td>
<td>E2</td>
<td>Progesterone receptor</td>
<td>Stimulation</td>
<td>Clarke et al. (1987)</td>
</tr>
<tr>
<td>ECC-1</td>
<td>ER</td>
<td>E2, BPA</td>
<td>Progesterone receptor</td>
<td>Stimulation</td>
<td>Bergeron et al. (1999)</td>
</tr>
<tr>
<td>EnCa101</td>
<td>ER</td>
<td>E2, Tam</td>
<td>c-fos</td>
<td>Induction</td>
<td>Sakakibara et al. (1992)</td>
</tr>
<tr>
<td>ECC-1</td>
<td>ER</td>
<td>E2</td>
<td>Cathepsin D</td>
<td>Induction</td>
<td>Castro-Rivera et al. 1999</td>
</tr>
<tr>
<td>ECC-1</td>
<td>ER</td>
<td>E2</td>
<td>GREB1</td>
<td>Induction</td>
<td>Ghosh et al. (2000)</td>
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<td>ECC-1</td>
<td>ER</td>
<td>E2, Ral</td>
<td>ER-α</td>
<td>Down-regulation</td>
<td>Dardes et al. (2002b)</td>
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<td>ECC-1</td>
<td>ER</td>
<td>ICI182,780</td>
<td>ER-α</td>
<td>Degradation</td>
<td>Dardes et al. (2002b)</td>
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<td>E2</td>
<td>pS2, VEGF</td>
<td>Induction</td>
<td>Dardes et al. (2002b)</td>
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<tr>
<td>ECC-1</td>
<td>ER</td>
<td>E2</td>
<td>HER2/neu</td>
<td>Down-regulation</td>
<td>Dardes et al. (2002b)</td>
</tr>
</tbody>
</table>

Abbreviations: ER, estrogen receptor; E2, estradiol; Ral, raloxifen; Tam, tamoxifen; BPA, bisphenol A; VEGF, vascular endothelial growth factor; pS2, presenelin-2.

### Differentiation of endometrial adenocarcinoma cells in vitro

Endometrial adenocarcinomas differ in their ability to form glandular structures; however most of the sporadic occurring endometrial adenocarcinomas are well to moderately well differentiated. In conventional cell culture on plastic in the presence of charcoal stripped fetal calf serum or in the presence of a serum-free defined medium, endometrial adenocarcinoma cells acquire a polygonal cell shape with a low degree of differentiation, and in a more narrow view cannot be regarded as correlated to the relatively high differentiated status of endometrial adenocarcinoma cells in vivo.

For cells derived from a variety of organs, particularly for normal and malignant mammary cells, a huge body of evidence has accumulated (for review see Hansen & Bissell 2000) that culturing of glandular cells on reconstituted...
Figure 3 Responses of endometrial adenocarcinoma cells to contact with reconstituted basement membrane. The upper part is a schematic drawing of the situation of an endometrial adenocarcinoma cell grown in conventional cell culture on plastic (dedifferentiated) and on reconstituted basement membrane (differentiated). This figure also indicates the presence of the stromal matrix underneath the basement membrane, which in cases of endometrial adenocarcinoma is particularly rich in tenascin-C (TN-C). The lower part shows the processes regulated by the basement membrane. icb-1, induced by contact to basement membrane; L3-PSP, L3-phosphoserine phosphatase; MMP, matrix metalloproteinase.

<table>
<thead>
<tr>
<th>Processes regulated by basement membrane</th>
<th>Reference</th>
</tr>
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<tr>
<td>Cellular morphology and ultrastructure</td>
<td>Satyaswaroop &amp; Tabibzadeh. (1991), Hopfer et al. (1996), Behrens et al. (1996), Pinelli et al. (1998)</td>
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<tr>
<td>Proliferation</td>
<td>Tan et al. (1999)</td>
</tr>
<tr>
<td>Hormone responsiveness</td>
<td>Vollmer et al. (1995a,b), Wünsche et al. (1998)</td>
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<tr>
<td>Gene expression in general</td>
<td>Strunck et al. (1996)</td>
</tr>
<tr>
<td>Expression of integrins</td>
<td>Strunck and Vollmer (1996)</td>
</tr>
<tr>
<td>Expression of cytoheratins</td>
<td>Pastor U, Strunck E &amp; Vollmer G, unpublished observations</td>
</tr>
<tr>
<td>Mitochondrial gene expression</td>
<td>Strunck E &amp; Vollmer G, unpublished observations</td>
</tr>
<tr>
<td>icb-1 expression (differentiation marker)</td>
<td>Treeck et al. (1998)</td>
</tr>
<tr>
<td>Expression of phosphatases (L3-PSP)</td>
<td>Strunck et al. (2001)</td>
</tr>
</tbody>
</table>

basement membrane induces differentiation processes, thereby increasing physiological responsiveness considerably if compared with cells kept under conventional cell culture conditions. The evidence for morphological and functional differentiation of human endometrial adenocarcinoma cells of Ishikawa (Pinelli et al. 1998), of the ECC-1 cell line (Satyaswaroop et al. 1991) and of the RUCA-I rat endometrial adenocarcinoma cells (Vollmer et al. 1995a) is summarized in Fig. 3. The most important observation is that the morphological differentiation strongly correlates with functional differentiation, the latter being most importantly evidenced by the induction of hormone responsiveness.

With the culturing of endometrial adenocarcinoma cells on reconstituted basement membrane a more pronounced physiological phenotype of these cells is achieved, allowing the assessment of physiological in vivo functions in an in vitro model. This assay can be modified into an in vitro invasion assay for the assessment of hormonal influences on tumor cell invasion through basement membranes (Fujimoto et al. 1996b). Finally, a modification of this assay has been described by reconstituting the natural situation even further and adding conditioned medium of endometrial stromal cells or cultured endometrial stromal cells to the system. In this way two important parameters in endometrial tumor growth can be assessed: the contribution of paracrine cellular interactions to tumor growth and the hormonal regulation of cells of the two tissue compartments in a single cell culture dish (Arnold et al. 2002).
Vollmer: Endometrial cancer

Conclusions

There is definitely a need for the development of substances for the treatment and cure of endometrial cancer. In addition, there are various new drugs or phyto-remedies under development which are intended to be used in the treatment and prevention of breast cancer, for the treatment of menopausal symptoms and for hormone replacement therapy. The efficacy of novel drugs targeting steroid receptors in endometrial cancers has to be evaluated and the safety of other endocrine measures on endometrial cancers or on endometrial carcinogenesis has to be assessed. For these purposes a relatively small number of animal models or combined in vivo/in vitro tumor models, based on human endometrial adenocarcinoma cells, are available. However, these models alone or in combination cover all needs for drug development or testing of drug safety in relation to endometrial cancer. While models consisting of human endometrial adenocarcinoma cells transplanted to athymic nude mice are commonly used, the potential of animal models consisting of spontaneous endometrial carcinogenesis or transplantation of rat endometrial adenocarcinoma cells to syngenic animals is largely ignored. First of all, with these models treatment protocols for chronic treatment of both primary tumors and metastases can be established. And even if the inoculation approach is used there is still a considerable advantage because syngenic animals can be used. Thus the use of the cost intensive athymic nude animal, a more artificial model which has to be kept in germ-free surroundings because of its imperfect immune system, can be avoided.

In the future, the number of studies using transgenic animals and therapy approaches in these animals will definitely increase. The advantage of these tools is that the genetic modification leading to the carcinogenic process is more precisely defined. In this way single genes or corresponding signal transduction pathways can be targeted in quite a selective way.

Acknowledgements

The author is grateful to Dr Deerberg for giving authorization for the use of unpublished data on lung metastasis of endometrial adenocarcinoma in DA/Han rats. The author wishes to thank Drs Susanne Starcke, Jannette Weber and Oliver Zierau for critical reading of the manuscript and for their helpful suggestions. In addition, special thanks are due to Dr Oliver Zierau for his assistance with the art work.

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Dedication

This paper is dedicated to honour the work of the recently deceased Dr Friedrich Deerberg, formerly of the German Institute for Laboratory Animals. Dr Friedrich Deerberg systematically investigated spontaneous carcinogenesis in inbred laboratory animals and developed experimental animal models useful for studying cancer treatment and prevention. Amongst these models are two spontaneous hormone-dependent endometrial cancer models which are exhaustively discussed in this paper. Personally, the author is indebted to Dr Deerberg for supplying him with the DA/Han and BD/Han rat endometrial cancer models and the cell lines developed thereof.

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Vollmer: Endometrial cancer


Vollmer: Endometrial cancer


