very small vessels resulting from neovascularization explain why the patient experiences hemorrhaging. Neovascularization is an essential part of the definition of a poiyp. Polyps in themselves do not present a risk for the patient but they result in a decrease in compliance to treatment as a result of the hemorrhaging. Hyperplasia without atypia generally occurs in patients who do not comply with treatment or in patients who have not taken the treatment for a sufficient number of days. Hyperplasia without atypia is associated with vascular thrombosis and explains why the patient is hemorrhaging. Endometrial carcinoma is sometimes diagnosed at biopsy as an incident case but in most cases the carcinoma is probably present prior to treatment and has not been discovered before because the patient was asymptomatic and no baseline was performed. Alternatively, the biopsy may have been done blindly and it could therefore have missed a small carcinoma. In the case of carcinoma, it is usually the invasion of the stroma that causes the bleeding.

References

1. Grady 0, Gebretsadik T, Kerlikowake K at al. *Hormone replacement therapy and endometrial cancer risk: A meta.analysis.* Obstat Gynecol 1995; 85: 304-313.
2. Gushing KL, Weiss NS, Voigt LF at al. *Risk of endometrial cancer in relation to use of low-dose, unopposed estrogens.* Obstat Gynecol 1998; 91: 35-39.
3. Beresford SAA, Weiss NS, Voigt LF at al. *Risk of endometrial cancer in relation to use of estrogen combined with cyclic progestagen therapy in postmenopausal women.* Lancet 1997; 349: 458-461.
4. Gomarci JT, Fields A, Runowicz cD at al. *Continuous low-dose combined hormone replacement therapy and the dsk of endometrial cancer Gynecol* Oncol 1997; 64: 425-430.

Endometrial pathology of tamoxifen

F. Nogales

University of Granada, Spain.

Tamoxifen is a widely used long-term treatment for breast cancer. Although initially developed as a contraceptive, it was seen to have antiestrogenic actions beneficial to breast cancer sufferers, reducing recurrences or metastases by 50% and the appearance of tumors in the contralateral breast. Furthermore, a recent study has shown that the preventive administration of tamoxifen to women with a high risk of developing breast cancer reduces the incidence of malignant tumors by 45%.

The antiestrogenic effect of tamofixen seems to be target site specific because it behaves as a mildly estrogenic substance at bone level (preventing osteoporosis) and also, less advantageously, at endometrial level. Thus, rather than being a pure antiestrogen, it is better called a selective estrogen receptor modulator. The mechanism of its estrogenic action at endometrial level is unknown although several theories have been considered.

We have been able to demonstrate a substantially higher rate of proliferation markers in apparent atrophic endometria of control curettages in patients subjected to long-term tamoxifen treatment (LTTT). LTTT causes a marked decrease in estrogen receptors and a substantial increase in progestogen receptors, which explains the marked decidual change of endometria in LTTT patients when progestogen is administered simultaneously.

Clinically, LTTT uteri are large and a substantial increase in thickness is seen with ultrasonography. Diffuse cystic change is also frequently seen in as many as a third of all cases.

Benign changes

A quarter of all cases develop endometrial polyps that are different form the usual ones in that they have a wide base of implantation, are multicystic and exhibit a wide range of metaplasias in the glandular and stromal components. In these polyps we have also demonstrated an evident increase of proliferation markers.

Malignant changes

In the early 1980s it was noted that endometrial carcinoma was higher in LTTT patients. This is not surprising as higher doses (40 mg/day) were used in that era~. Experimentally, an enhancement of endometrial cancer was noted. The risk of developing an endometrial cancer was double after an accumulative treatment of 9-15 g, triple after 15 g and quadruple after concurrent irradiation. Latency period was approximately 2 years.

The clinicopathological types of tumor developing in LTTT situations have given rise to much controversy. While population controlled studies showed that most tumors corresponded to well-differentiated endometrial cancers in low stages, other studies from referral centers showed a predominance of unusual, high-grade; aggressive tumors, such as papillary serous carcinoma. Our study comprising more than 175 cases of control biopsies of LTTT patients and 150 studies of material from symptomatic patients showed 35 cases of endometrial cancer with a predominance of low-grade endometrioid adenocarcinoma, opposed to four cases of papillary serous carcinoma, one clear cell carcinoma, five cardnosarcomas and four sarcomas. An interesting feature of the three cases of carcinosarcoma was a unusual botryoid pattern, reminiscent of the morphology of chorionic villi. Additionally, three cases of atypical polypoid adenomyoma associated with LTTT were found for the first time.

Due to the high incidence of endometrial polyps in LTTT, a special interest was taken in the malignant tumors developing in them. Papillary serous carcinomas associated with polyps were seen in only two cases, while in the three other cases, two endometriold carcinomas and one carcinosarcoma were found.

Despite the obvious possibility of developing an endometrial carcinoma with LTTT, the improvement in the survival and quality of life in breast cancer patients is clearly worth the risk. In numerical terms, 30 times more lives are saved by tamoxifen than are lost by its side effects. However, a LTTT patient needs a close follow-up with ultrasonography and biopsy if changes are detected. Alternative selective estrogen receptor modulators such as raloxifen may prove to be an answer to this problem in the near future.

References

1. craig Jordan u. *Designer estrogens.* Scientific Am 1998; 279: 36.
2. craig Jordan u. *Thmoxifen: The herald of a new era of preventive therapeutics.* JNGI 1997; 69: 747.
3. small SM. *The effects of tamoxifen on the uterus.* curr Opin Obstet Gynecol 1996; 8:27-31.
4. Magriples U, Naftolin F, Schwartz PE at al. *High grade carcinoma in tamoxifen-treated breast cancer patients.* J Glin Oncol 1993; 11: 485-490.
5. Murphy U. *Growth factors and steroid hormone action in endometrial cancer* J Steroid Biochem Molac Biol 1994; 48: 419-423.
6. Boss 0, Whitehead M. *Hormonal manipulation and gynsecological cancer: The tamoxifen dilemma.* Curr Opin Obstat Gynecol 1995; 7: 63-68.

7. Silva EG, Tornos CS, Follen-Mitchefl M. *Malignant neoplasrns ofthe uterine corpus in patients treated for breast carcinoma: The effects of tamoxifen.* mt J Gynecol Pathol 1994; 13: 248-258.
8. Van-Leeuwen FE, Benraadt J, Coebergh JW *et* al. *Risk of endometriat cancer after tamoxifen treatment of breast cancer* Lancet 1994; 343: 448-452.

Advances in molecular biology in the endometrium

T. L6ning, A.M. Bamberger and L. Riethdorf

Institute of Pathology Dept. of Gynecopathology, Eppendorf University Hospital, Hamburg, Germany.

The endometrium is the classical target tissue for ovarian steroids. Both epithelial and stromal cells contain estrogen receptors and progesterone receptors (1) and the ovarian steroids play an essential role in regulating the growth and differentiation of endometrial cells. This influence is partially preserved in endometrial tumors, especially in the early and well-differentiated stages, which are more frequently receptor-positive than the more advanced stages (2, 3). Endometrioid carcinoma is considered to develop in a stepwise fashion from endometrial hyperplasia, under the influence of unopposed estrogenic stimulation. Most endometrioid carcinomas are associated with endometrial hyperplasia and are estrogen and progesterone receptor positive, p53 negative and express low Ki67 (4). Several oncogenes have been suggested as playing a role in prototypic endometrial cancer (5, 6). Among these oncogenes are activated Ki-ras, erb-B2 (HER/neu) and the overexpression of the epidermal growth factor receptor, which correlate with the lack of progesterone receptor and with poor prognosis. Also, suppression of insulin-like growth factor-I binding protein and high expression levels of c-fins messenger RNA (mANA), the receptor for colony stimulating factor-i, have been described (5). Our own results regarding alterations of p53 in endometrial carcinoma, as well as expression of the cell cycle inhibitors p27, p1 6/MTS1 and retinoblastoma protein are summarized below. Finally, data on expression patterns of the adhesion molecule CD66a in the normal and neoplastic endometrium are included.

p53

The distribution of p53 alterations has been analyzed by immunohistochemistry and temperature-gradient gel electrophoresis in endometriold carcinomas (n=120) of different grades and stages, as opposed to normal endometrium and various risk groups of hyperplasia (n=39). With increasing malignancy, the number of cases with p53 aberrations rose and ranged from 10-20% in hyperplasia, through 25% in low-risk carcinomas to 70% in high-risk carcinomas (7). Thus, the frequency of p53 aberrations in high-risk carcinomas is already close to the figure obtained in serous carcinomas and their precursors.

Cell cycle inhibitors

P27

Progression through the cell cycle and cellular proliferation is under the control of cyclins and cyclin-dependent kinase (CDK) complexes. In mammalian cells, the cyclin D-CDK4 and cyclin E-CDK2 complexes are active during late Gi phase and are implicated in GuS progression. p27 is a member of a group of proteins identified as CDK inhibitors, which cause Gi arrest when overexpressed in transfected cells.

Expression of p27 in endometrial carcinomas (n=41) was analyzed using immunohistochemistry with a specific antibody and was compared to expression in the normal endometrium throughout the cycle. Normal endometrial cells showed strong nuclear expression of p27. Expression was present throughout the cycle and was stronger during the secretory phase. Compared with the normal endometrium, we found strongly reduced or abolished expression of p27 in endometrial carcinoma (85% of the cases). Comparison of the p53 status showed that all tumors with strong p53 expression had low/negative p27 staining, while the tumors that were positive for p27 had negative/low p53 staining. Reduced or absent p27 levels were also observed by Western blot analysis both in tumor samples and in HEC-iB endometrial adenocarcinoma cells. We conclude that p27 expression is essential for the control of normal endometrial proliferation and that reduced or absent p27 expression could be an important step in endometrial carcinogenesis (8).

o 167MTS I and retinoblastoma protein

P16MTS1/CDKN1 and the retinoblastoma protein are both involved in negative regulation of G1/S progression in the mammalian cell cycle. Inactivation of one of these tumor suppressor genes is involved in many malignant tumors and in some studies, a negative correlation between p16 and retinoblastoma protein expression was found. In order to study the role of p16 and retinoblastoma protein inactivation in endometrial carcinogenesis, we investigated 36 endometrial carcinomas, 11 hyperplasias, 23 normal endometrial samples and two uterine carcinoma cell lines by immunohistochemistry or reverse transcription-polymerase chain reaction (RT-PCR). Retinoblastoma protein was expressed in normal endometrial epithelium, hyperplasias, cell lines and most carcinomas and negative immunostaining was only detected in one of 36 tumors. In contrast, p16 expression was weak in normal endometrium, increased in most hyperplasias but negative or minimally positive in 75% of the carcinomas and the Heci B adenocarcinoma cell line. A strikingly high p16 expression was found in foci of squamous metaplasia within hyperplastic or carcinomatous tissue. Deletion and mutation analysis of the p16 gene was performed in DNA from microdissected tumor samples and cell lines. No p16 deletion was found and mutations were detected in only one tumor sample and Skuti B uterine mixed mesodermal tumor cells. Our data indicate that in spite of the low or missing p16 expression, genetic alterations of the p16 and RB tumor suppressor genes are rare in endometrial carcinogenesis (9).

Expression of CD66a (BGR C-CAM

CD66a is an adhesion molecule of the carcinoembryonic antigen (CEA) family, which has been shown to be down-regulated in non-gynecological cancer. To determine its expression pattern in endometrial neoplasia, we performed immunohistochemistry using the 4D1/C2 monoclonal antibody on a series of 39 endometriold and eight serous-papillary carcinomas. Strong CD66a expression was observed in epithelial cells of the normal endometrium with a consistent localization at the apical poles of these cells. With increased malignancy grade, increased loss of expression was