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Advances in molecular biology in the endometrium

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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

observed. The apical expression pattern of the normal epithelium was changed to an all-around" pattern. Northern blot analysis also showed a loss of mRNA expression in tumor samples and HEC-1 B endometrial adenocarcinoma cells. Loss of protein expression in the tumor samples was further observed by Western blot. In conclusion, CD66a protein expression is deregulated in endometrial carcinomas, showing reduction or loss of expression with increased malignancy grade and a change from the apical to a pencellular, membranous localization (10).

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Aggressive forms of endometrial carcinoma

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Clear cell carcinoma

Freouencv

Clear cell carcinoma accounts for 1-5% of all endometrial carcinomas.

Histovathology

Clear cell carcinoma consists of cells with clear, vacuolated cytoplasm due to the presence of glycogen, hobnail cells or flattened or cuboidal cells. In the latter cell types, the clear cytoplasm is inconspicuous and in the hobnail cells the nuclei protrude to the surface of the cells. The nuclei generally are pleomorphic with marked atypia. This tumor exhibits a variety of growth patterns, including papillary, solid, tubular and cystic. Usually, there is a mixture of the different growth patterns. About 15% of the cases are mixed with other subtypes of endometrial carcinoma, including serous papillary carcinoma.

Differential diagnosis

Differential diagnosis is with serous papillary carcinoma and secretory carcinoma.

Prognosis

Patients with clear cell carcinoma of the endometrium have an overall death rate significantly higher than that of patients with ordinary carcinoma and clear cell carcinoma tends to occur in older patients (mean age 66 years). In stage I disease, survival is related to the extent of myometrial infiltration, age and nonsolid type. Two-thirds of the patients relapse outside the pelvis (upper abdomen, lungs and liver).

Serous panillary carcinoma

Freouencv

The frequency of serous papillary carcinoma is 1-10% 01 all endometrial carcinomas.

Histovathology

The tumor grows in complex papillary fronds with central fibrovascular connective tissue cores identical to those seen in ovarian serous carcinoma or is composed of glands. The lumens have an irregular gaping appearance. Necrosis is common and psammoma bodies are present in about one-third of the cases. The tumor cells are smaller than the endometrioid cells, cuboidal or hobnail shaped with a greater nuclear/cytoplasmic ratio, marked nuclear pleomorphism and eosinophilic cytoplasm. Invasive foci of serous carcinoma often show small glands and cysts with intraluminal papillary projections. These tumors often invade the myometrium deeply and also invade the vessels. Endometrial serous intraepithelial carcinoma is seen in a number of cases and is the only lesion from which serous carcinoma is thought to arise. The surrounding nonneoplastic endometrium is usually atrophic and the transition between atrophic and neoplastic endometrium is abrupt.

Since these tumors are almost always composed of high-grade nuclei, nuclear grading is not necessary. The diagnosis of serous papillary carcinoma itself establishes the presence of a highly malignant endometrial carcinoma.

Differential diagnosis

A differential diagnosis may have to be made with villoglandular! papillary endometriold adenocarcinoma, clear cell carcinoma of the papillary type, spread from ovarian serous papillary carcinoma.

Prognosis

Serous carcinoma is a highly malignant tumor, even when superficial or confined to an endometrial polyp or in the early stages. It occurs in older women, with a mean age of about 70 years. The metastatic pattern is similar to that of its ovarian counterpart.

Small cell carcinoma

Frequency

The frequency of small cell carcinoma is <1%.