Histological Evaluation of in Vitro Responses of Endometrial Adenocarcinoma to Progestins and Their Relation to Progesterone Receptor Levels

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ABSTRACT

In vitro responsiveness of endometrial adenocarcinoma to progestins was evaluated histologically by incubation of tissue fragments in medium containing 10^{-6} M medroxyprogesterone acetate.

In a series of 19 experiments, formation of sub- and supranuclear vacuoles, which reflects accumulation of glycogen in response to medroxyprogesterone acetate added to the medium, was observed in well-preserved glandular epithelial cells only when the level of cytosolic progesterone receptor was above 300 fmol/mg protein. Previously, we have reported similar results obtained in in vivo experiments.

The present findings suggest that simple organ culture and histological procedures can be used to identify specimens of endometrial cancer that have functional progesterone receptors and are capable of responding to progestins. They also indicate that levels of progesterone receptor required to obtain responses to progestins are considerably higher than those necessary for analytical detection and that therefore the quantity and not merely the detectability of progesterone receptors must be taken into consideration for the prediction of responses to progestins.

In addition, in vitro responses to progestins may indicate the presence in endometrial cancer tissue of functional estrogen receptors and potential responsiveness to antiestrogens, since estrogen stimulation appears to be needed for the synthesis of progesterone receptors.

INTRODUCTION

Several reports have indicated that progestins can produce histological changes in endometrial adenocarcinoma which are characteristic of the transition from proliferative to secretory endometrium, namely, formation of subnuclear vacuoles, corresponding to glycogen, and a generally quiescent appearance of the epithelial cells, characterized by an absence of mitoses and a low nuclear-cytoplasmic ratio. These changes have been observed in vivo after administration of progestins to patients (1, 7, 10, 13) and in vitro during incubations of neoplastic tissue in medium containing progestins (9, 14). Since the hormonal effects are detected in only some of the specimens of endometrial adenocarcinoma, it is conceivable that a lack of responsiveness of the tumor could identify patients who would not benefit from progestin therapy, although such a correlation can only be established by comparing results from these tests with objectively evaluated responses to treatment.

Responsiveness to progestins is expected to depend on the presence of progesterone receptors in the tissue. In fact, previous reports by Young and Ehrlich (23) and other groups (3) have shown that the presence of receptors in recurrent endometrial cancer may be a useful predictor of responsiveness to treatment.

On the basis of these considerations, the present study aimed to correlate in vitro responses of primary endometrial cancer to progestins, evaluated by light microscopy, with levels of progesterone receptor in the tumor. Although comparisons of progesterone receptor levels in uterine and metastatic endometrial cancer have been reported in only a few cases, the available data indicate a rather good agreement between the receptor content of primary and recurrent tumors (20), as is the case in breast cancer (17).

The choice of a histological end point of progestin responsiveness offers several advantages over the quantitative determination of progesterone receptors. The test may be performed as a routine procedure at any laboratory equipped for cell or tissue culture work. It is less time consuming, technically simpler, less expensive in necessary equipment and supplies, requires fewer technical skills, and can be performed on amounts of tissue smaller than those needed for progesterone receptor analysis. Furthermore, the progesterone-stimulated glycogen accumulation indicates that the progesterone binder present in the tissue functions as a receptor capable of eliciting hormonal actions.

MATERIALS AND METHODS

Studies on responsiveness in vitro were carried out by incubating fragments of endometrial carcinoma curedt after hysterectomy. After separation from blood clots, mucus, and debris, the tissue was cut with ophthalmic scissors into small pieces (approximately 1 cu mm) under a laminar flow hood and divided into 3 portions, one to be used for progesterone receptor determinations, another for histological examination, and the rest for incubations. The tissue fragments used for incubations were placed on lens paper disks resting upon stainless steel grids distributed in polystyrene dishes, 35 mm in diameter (Falcon Plastics, Los Angeles, Calif.), which contained 2 ml of Ham’s F-10 medium with 10% fetal bovine serum, insulin (10 μg/ml), glucose (5 mg/ml), and 1% of an antibiotic-antimycoctic mixture (Grand Island Biological Co., Grand Island, N. Y.). Serum-free medium was used in Experiments 304 and 347. Tissues were only partially immersed to facilitate oxygenation. Some of the dishes contained 10^{-6} M MPA. This concentration was chosen because it had been found previously to result in maximal stimulation of 17β-HSD activity of proliferative endometrium in organ culture. 

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Culture (22). The dishes, each holding 10 to 15 mg of tissue, were placed in an incubator and kept for 2 days at 37°C in a humidified 5% CO₂-95% air atmosphere. At the end of the incubation period, tissue from individual dishes was used for histological examination.

Histological Examination. Tissue fixed in formalin was embedded in paraffin, sectioned, and stained with hematoxylin-eosin and, in some cases, with periodic acid-Schiff for glycogen. Accumulation of glycogen in glands was established by the appearance of subnuclear vacuoles. No glycogen vacuoles were observed in the control slides corresponding to fragments incubated in medium to which no progesterin was added. Sub- or supranuclear vacuoles were distinguishable from randomly located cytoplasmic vacuoles usually found in degenerating endometrial tumors and in poorly preserved tissue characterized by pyknotic or disrupted nuclei. Cytoplasmic periodic acid-Schiff-positive granules, sensitive to diastase treatment, were associated with well-preserved nuclei.

A response was considered to be positive when sub- or supranuclear vacuoles were observed in at least some of the glands in tissue incubated in the presence of MPA but were absent before incubation or in control dishes. The intensity of response was indicated by using the symbols + or ++. The histological response to MPA might be found in only some areas of the adenocarcinoma, while other areas were unaffected or showed evidence of degeneration. In most responsive cases, however, not only were subnuclear vacuoles present in almost all of the glands, but epithelial cells exhibited a quiescent appearance characterized by the absence of mitoses, a low nuclear-cytoplasmic ratio, a predominant arrangement in single rows, and a general resemblance of these cells to those of normal secretory glands. Nuclear pleomorphism, i.e., irregular shape, size, and staining of nuclei, was minimal in responsive glands.

Degree of differentiation of the adenocarcinoma was rated as described by DiSaia and Creasman (4).

In some cases, a papillary pattern was found to be associated with the glandular tumoral elements. It consisted of finger-like projections composed of a delicate fibrovascular stalk covered by epithelial cells (Fig. 1C). After incubation with MPA, all papillary structures failed to show subnuclear glycogen vacuoles, even in the cases in which the glands did show the progesterin-induced changes. The papillary structures displayed a stratified epithelium, devoid of polarity and glycogen vacuolization, even when adjacent to responsive glands (Fig. 1C).

Measurement of Progesterone Receptor Levels. Progesterone receptor levels were determined by incubating cytotox with 20 nM tritiated progesterone plus 2 μM cortisol in the presence or absence of 100-fold excess of unlabeled progesterone, followed by separation of unbound hormone with dextran-coated charcoal, according to the method of Bayard et al. (2). The tissue was assayed immediately after collection.

RESULTS

Table 1 shows the results obtained by incubation of 19 specimens of endometrial adenocarcinoma. Cytosol progesterone receptor levels ranged from nondetectable (<10 fmol/mg protein) to 2200 fmol/mg protein. No responses were observed in tissues with less than 300 fmol/mg protein, whereas all specimens with a receptor content above that level showed glycogen accumulation. The percentage of responsive specimens in the well, moderately, and poorly differentiated groups was 67% (n = 9), 29% (n = 7), and 0% (n = 3), respectively. This distribution is in agreement with the well-documented decline of progesterone receptor levels with loss of differentiation (16, 18). It should be noted, however, that these levels may be as low as 25 fmol/mg protein in a Grade 1 specimen and as high as 940 fmol/mg protein in a Grade 2 tissue. Therefore, degree of differentiation is not an adequate indicator of progesterone receptor levels or responsiveness of individual tumors to progestins.

Fig. 1, A to D, shows photomicrographs of a sample of moderately differentiated adenocarcinoma with papillary features before and after incubation in medium with or without MPA (10⁻⁶ M).

DISCUSSION

The levels of cytosolic progesterone receptor necessary to obtain in vitro glycogen accumulation under the influence of MPA appear to be about 300 fmol/mg protein. The receptor levels we find necessary to obtain responses to progestins during in vitro tests are similar to those that we found during in vivo studies in which MPA was administered to postmenopausal patients with endometrial adenocarcinoma after diagnostic curettage and before hysterectomy (9). They were higher, however, than the 50 fmol/mg protein (5) or the 10 fmol/mg protein (3, 15) levels used by other workers to discriminate between adequate and insufficient receptor concentrations. The distinction between presence or absence of progesterone receptors, as reported by other authors, is based on either the limit of sensitivity of the analytical method used, e.g., density gradient or dextran-coated charcoal analysis (16), or specific binding levels found in nontarget tissues (5).

One specimen of well-differentiated endometrial adenocarcinoma with papillary features obtained from a 70-year-old patient showed a level of progesterone receptor of 190 fmol/mg protein and glycogen accumulation (+) in response to MPA. This specimen, however, presented before incubation histological features characteristic of necrosis not seen in any of the other cases. Since dead cells and infiltrating leukocytes may contribute proteins but no progesterone-specific binding to the assay, their presence can be expected to result in an underestimate of receptor levels. For this reason, this specimen was not included in Table 1.

Histological testing of a specimen of endometrial adenocarcinoma incubated with progestins appears to provide evidence for responsiveness to progestins and information about levels of progesterone receptor in the tissue. The simplicity of the test, which involves techniques used routinely in pathology laboratories, justifies further evaluation of its potential.
The lack of histological response of the papillary structures to progestins is probably an expression of the dedifferentiation of this type of tumoral tissue. Adenocarcinoma, especially the well-differentiated type, is a tumor relatively similar to the tissue of its origin, i.e., the endometrial glands. The papillary structures represent an anaplastic phenomenon in which the tumor is composed of a more primitive Mullerian type of tissue, reminiscent of other papillary tumors of Mullerian-related origin, such as ovarian, endocervical, or Fallopian tube primary tumors. A recent report (8) emphasized that the virulence of these tumors is higher than that of the endometrial adenocarcinoma and closer to that of other papillary tumors of Mullerian-related origin, such as carcinosarcoma. The papillary structures to respond to progestin stimulation, even in the close spatial proximity with responding glands, is a feature related to the anaplasia and loss of functional orientation or polarity. Predominantly papillary endometrial carcinomas are unlikely to respond to progestin therapy. This histological feature, along with the grade of differentiation and invasiveness of the myometrium, may represent a discriminating criterion in the choice of therapeutic agents.

One of the effects of in vivo administration of progestins observed in some patients with endometrial adenocarcinoma is the stimulation of 17β-HSD activity in the tumor (7). However, in contrast to the consistent in vitro responses obtained in normal proliferative endometrium (21), we have not been able to detect significant increases in 17β-HSD in tumors incubated with MPA (9). The reasons underlying the lack of responsiveness of 17β-HSD in tumors containing high levels of progesterone receptors and accumulating glycogen in vitro under the influence of progestins (e.g., Cases 237 and 211 in Table 1) are not clear. It is likely, however, that partial necrosis during incubation lowers the estimated activity of the enzyme when expressed per mg of protein, whereas the histological evaluation of glycogen accumulation in well-preserved glands is not affected by this methodological problem.

In conclusion, the clear association of levels of progesterone receptors to histological responses in specimens of endometrial adenocarcinoma suggests that in vitro enhancement of glycogen accumulation can be considered a practical test, at least in well- and moderately differentiated tumors, for the presence of adequate amounts of progesterone receptors and therefore of functional estrogen receptors (11, 16). This histological test may be of potential usefulness in the prediction of responsiveness of endometrial adenocarcinoma to treatment with progestins (12) or antiestrogens (19).

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REFERENCES

Fig. 1. Histological evaluation of in vitro responsiveness to MPA of a specimen of moderately differentiated endometrial adenocarcinoma (Patient 250 in Table 1). Specimen before incubation (A) and after incubation for 2 days in the absence (B) and presence (C) of $10^{-6}$ M MPA. C illustrates responsiveness to MPA in the glandular part and its absence in the papillary component of the tissue. H & E, $\times$ 400.
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