The Status of Receptors in the Management of Endometrial Cancer

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Measurement of receptors in endometrial cancer would be clinically useful if it led to a decision about treatment that was different from the one that would have been made without the measurement or added information about prognosis that could not be obtained from other sources.

With respect to the first criterion, it is clear that a decision already has been made by consensus regarding the appropriate initial treatment of most cases of endometrial cancer. A recent survey of cases treated in the United States in 1981 determined that more than 90% of invasive cancers were confined to the uterus (81% International Federation of Gynecology and Obstetrics [FIGO] stage I and 11% stage II) and that almost half were histologically well-differentiated lesions. In almost 90% of all cases the treatment consisted of total hysterectomy and bilateral salpingo-oophorectomy, with sampling of lymph nodes or lymphadenectomy in 10%. Radiotherapy was administered in conjunction with surgery in half of the stage I cases and in a much higher percentage of those in stage II. The data of FIGO suggest that cure can be expected in nearly all of the patients with well-differentiated stage I disease and in many of the others as well. The survey confirms the impression that most cases are cured, quoting the American Cancer Society estimate of 39,000 new cases expected for 1984, with 2,900 expected deaths, or only 7% of the incidence. Current therapy, then, is appropriate and sufficient for most patients. By the same token, there is no need in most cases for an initial receptor assay in order to assign a patient to early hormone therapy. Indeed, it is not surprising to learn that current reviews of the literature find no convincing evidence for the value of adjuvant progestin therapy in endometrial carcinoma.2,3 The question of adjuvant hormonal therapy might be reopened, however, as applied to a controlled study of an accurately defined group of patients considered to be at high risk for early recurrence of their disease. Newer reports scrutinizing prognostic factors that have been neglected previously, such as the depth of myometrial invasion in relation to

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the risk of lymph node metastases,4 can be helpful in designing such studies. Meanwhile, the role of receptor assays in treatment decisions, if any, must be limited to cases of advanced or recurrent disease. Because most of the available data on receptors have been obtained from the early cases, however, we will discuss these observations and their relation to prognosis before returning to the problems posed by the advanced cases.

Data pooled from 258 cases in 10 reports in the literature showed that assays for cytosolic progesterone receptor with positive results were frequent in primary cancers of the endometrium (62%), the frequency being proportional to the degree of differentiation (undifferentiated—33%, moderately differentiated—56%, well-differentiated—81%).6 More recent studies have confirmed this. In one6 the frequency in more than 150 cases of progesterone receptor positivity in the three grades was 52%, 57%, and 87% (72% overall); that of estrogen receptor was 35%, 79%, and 96% (78% overall); and that of androgen receptor 32%, 70%, and 80% (62% overall). In a second recent study7 the frequencies of the presence of the progesterone receptor by grade were 64%, 98%, and 95%, while the estrogen receptor was present in 100% of cases. In a third study of 168 cases with clinical stage I and II disease,8 the frequencies for progesterone receptor positivity were 59%, 60%, and 82% (overall 70%) and for estrogen receptor 46%, 72%, and 82% (overall 73%). It is indeed surprising that the progesterone receptor is found so frequently in a patient population that has mostly postmenopausal women who are therefore not expected to have enough estrogen to maintain receptor levels.

The high frequency of receptor positivity and the uncertainty about the level that should be accepted as the threshold value in the assay has led to a quantitative analysis of receptor concentrations in cancer of different grades of differentiation, and the data have been compared with values obtained from normal endometrium. Higher receptor concentrations have been found in association with better-differentiated tumors as opposed to poorly differentiated ones, as might be expected,7,9,10 but there also seems to be a subgroup of poorly differentiated tumors whose levels are higher than the median for well-differentiated tumors. This was found to be true for estrogen receptor in 23% and for progesterone receptor in 4% of grade 3 carcinomas.10 In a comparison between endometrial adenocarcinomas and normal proliferative endometrium,11 the cytosolic receptor levels as measured by the dextran-coated charcoal (DCC) assay were lower in the cancers, but also more variable, while relatively more of the receptor was associated with the nucleus in the cancer cases.

In a few reports an effort has been made to correlate the presence of receptor concentrations with tumor type, with specific histologic features, and with evidence of invasiveness. In one study12 the concentration of progesterone receptor was found to be correlated positively with several characteristics of tumor differentiation: glandular as opposed to solid growth, absence of nuclear pleomorphism and of lymphocytic infiltration, and presence of cilia and of staining for glycoprotein.

At the ultrastructural level, tumors with high levels of progesterone receptor showed fewer characteristics of malignancy than those with low levels or absence of receptor: a similar distinction was not seen between estrogen receptor-positive and estrogen receptor-negative tumors.13 Adenosquamous and clear cell carcinomas did not differ from adenocarcinomas with respect to levels of estrogen and progesterone receptor in one study,6 while in another study clear cell and papillary tumors had distinctly low levels.8 The most
extensive correlation of pathologic changes with receptor values is that reported by Creasman et al., who, in their study of 160 patients with clinical stage I and II disease, found the presence of cytosolic estrogen receptor to be related inversely to histologic and nuclear atypicaity and the presence of proven extrauterine metastases. Simultaneous positivity or negativity of both cytosolic estrogen and progesterone receptors was associated significantly with all of these and with peritoneal cytology and histologic cell type as well. The stage of disease and the presence or extent of cervical or myometrial involvement were not related significantly to the presence or absence of receptor, but in other studies myometrial involvement was found to be related negatively to the concentration of cytosolic estrogen or progesterone receptor.

Do receptor measurements have a prognostic significance in the recurrence and survival of endometrial cancer that is independent of the many other known factors? Several studies indicate that they do. The detailed study of patients with stages I and II cancer reported by Creasman et al. included stepwise regression analysis of proportional hazards and showed cytosolic estrogen receptor, progesterone receptor, and the presence or absence of both together to be significant prognostic factors, replacing histologic assessment of glandular or nuclear differentiation. Other significant prognostic factors noted in their study were the age of the patient; the stage of disease; the presence of cervical involvement, myometrial invasion, or peritoneal malignant cytologic characteristics; and the histologic type, differentiation, or nuclear grade of the tumors. The survival advantage of receptor-positive lesions was said to be observable within all the other clinicopathologic categories.

In summarizing the receptor data with respect to prognosis, one must conclude that the patient that is most likely to need adjuvant therapy or treatment of recurrences is the one with the unfavorable case least likely to be receptor positive and, as a consequence, hormone sensitive. It would require a carefully designed trial to determine the cost-effectiveness of detecting patients who are the exceptions to this rule in order to treat them with hormones.

Returning to our first criterion of usefulness, how well do receptor assays predict a response to hormone therapy? The number of reports that have correlated response to progestin therapy with receptor status are few and the numbers are small (Table 1), as might be expected from the problems associated with obtaining adequate samples of tumor tissue from patients with advanced disease. The overall response rate of 34% shown in Table 1 is exactly the same as that derived from previous studies of progestin treatment in unselected cases. As in the case of breast receptor assays, a negative result in an assay for cytosolic progesterone receptor has a greater predictive value (92%) than a positive result (71%). It should be noted, however, that, in contrast to the primary cases discussed previously, only 41% of this group of advanced cancers had positive results when tested and that this number is close to the observed response rate of 34%.

<table>
<thead>
<tr>
<th>Author</th>
<th>Responders/PR Positive</th>
<th>Responders/PR Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Martin et al., 1979⑦</td>
<td>13/14</td>
<td>1/6</td>
</tr>
<tr>
<td>McCarty et al., 1979⑧</td>
<td>4/5</td>
<td>0/8</td>
</tr>
<tr>
<td>Benraad et al., 1980⑧</td>
<td>5/6</td>
<td>2/7</td>
</tr>
<tr>
<td>Creasman et al., 1980⑧</td>
<td>3/5</td>
<td>1/8</td>
</tr>
<tr>
<td>Ehrlich et al., 1981⑥</td>
<td>7/8</td>
<td>1/16</td>
</tr>
<tr>
<td>Kauppila et al., 1982⑥</td>
<td>2/4</td>
<td>1/17</td>
</tr>
<tr>
<td>Carlson et al., 1984⑥</td>
<td>2/3</td>
<td>0/3</td>
</tr>
<tr>
<td>Quinn et al., 1985⑥</td>
<td>3/10</td>
<td>0/13</td>
</tr>
<tr>
<td>Total</td>
<td>39/55 (71%)</td>
<td>6/78 (8%)</td>
</tr>
</tbody>
</table>

Overall: receptors positive in 55/133 or 41%, treatment responses in 45/133 or 34%, predictive value of a positive test is 39/55 or 71%, predictive value of a negative test is 72/78 or 92%.
Can the predictive value of the receptor assay for the response of advanced or recurrent disease to hormone therapy be improved? The answer to this question depends on two factors—improvements in the assay, on the one hand, and improvements in treatment, on the other. For the clinician the latter aspect is the most important. Is hormone therapy, in fact, still useful in treating advanced—recurrent endometrial cancer? A review of 17 studies also records a 34% response rate to progestins. There was no significant difference between the response rate to medroxyprogesterone acetate, megestrol, or hydroxyprogesterone, but there was evidence that better responses can be achieved with higher doses. Other hormonal agents appear to be effective even after treatment failure with progestins: in six studies of 38 patients, the estrogen-blocking agent tamoxifen induced responses in 85% of such patients, and in one study of 9 patients, aminoglutethimide, an agent that blocks steroid biosynthesis, induced responses in 22%. Danazol, a derivative of ethinyl testosterone that has a wide range of blocking activities on the reproductive system, induces progestational morphologic and biochemical changes in endometrial cancer that are similar to those induced by medroxyprogesterone acetate, which suggests that this agent might prove to be therapeutically effective. There is evidence that at least some endometrial cancers contain aromatase, an enzyme capable of synthesizing estrogen in situ from circulating androgens, so that compounds that block aromatase activity, such as testolactone, also might be useful. Finally, it is possible that in some cases progesterone receptor may be present, but in an insufficient amount for an optimal therapeutic response. One way to increase the concentration of this receptor is to administer estrogen. This measure has been tried and has proved effective but is obviously contraindicated because of the known mitogenic effect of estrogen. The antiestrogens, such as tamoxifen, on the other hand, can increase progesterone receptor levels without inducing growth responses. Thus far, however, treatment with the combination of tamoxifen and a progestin does not seem to be more effective than progestin alone, and in addition, the combination has been found to be associated with hepatotoxicity in some cases.

The alternative to hormonal therapy is cytotoxic chemotherapy, which can achieve a comparable response rate in unselected patients. Combination cytotoxic chemotherapy that includes progestin can achieve responses in poorly differentiated tumors that would not be expected to respond to the administration of progestin alone. Are such regimens also that best choice for patients with recurrences of more “favorable” lesions (better differentiated tumors and positive hormone receptor values)? Cohen et al. concluded, appropriately, that

with the technology for receptor studies now widely available it would seem reasonable to use progestational agents only in those patients in whom a favorable outcome can be predicted, and to apply cytotoxic chemotherapy to those patients with less favorable prognosis. Multidrug regimens should be reserved for those with poor differentiation, absent progesterone receptors, and reduced performance status.

Are receptor assays as reported in the literature adequate, or can the test itself be significantly improved? Some of the basic problems associated with receptor assays are outlined in Table 2. Some are obvious and do not merit further discussion, while some are questionable. The basic problem is the question whether the binding activity commonly observed is properly referred to by the name of receptor. These cellular binders do, for the most part, fulfill some
TABLE 2. Problems with Receptor Assays in Endometrial Cancer

<table>
<thead>
<tr>
<th>Problems</th>
<th>Remedies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Too many false positives:</td>
<td>a) Histologic check on sample</td>
</tr>
<tr>
<td>normal endometrium, stroma, endometrial hyperplasia in sample</td>
<td>b) Test recurrences, metastases only?</td>
</tr>
<tr>
<td></td>
<td>c) Use immunohistochemical assay?</td>
</tr>
<tr>
<td>Plasma binders (CBG, SHBG) included in measurement</td>
<td>Choose ligand or competitor that binds only to receptor</td>
</tr>
<tr>
<td>Low-affinity (Type II) binders included</td>
<td>Multiple-point DCC assay with Scatchard plot</td>
</tr>
<tr>
<td>Receptors may be nonfunctional</td>
<td>a) Accept only if both estrogen and progesterone receptors are present?</td>
</tr>
<tr>
<td></td>
<td>b) Raise threshold level above which sample is called positive?</td>
</tr>
<tr>
<td></td>
<td>c) Compare with immunohistochemical findings</td>
</tr>
<tr>
<td></td>
<td>d) Accept only if tamoxifen treatment results in a higher progesterone receptor level in a second sample?</td>
</tr>
<tr>
<td></td>
<td>e) Try another test for hormone sensitivity?</td>
</tr>
<tr>
<td>Some false negatives:</td>
<td></td>
</tr>
<tr>
<td>Mishandled tissue</td>
<td></td>
</tr>
<tr>
<td>Tumor necrosis or no tumor in sample</td>
<td>Fresh tissue, prompt storage at $-70^\circ C$14</td>
</tr>
<tr>
<td>Prior radiotherapy</td>
<td>Histologic check on assay sample</td>
</tr>
<tr>
<td>Masking of binding sites by circulating hormone</td>
<td>Samples must be obtained before treatment</td>
</tr>
<tr>
<td></td>
<td>a) In premenopausal patients, assay serum for estradiol, progesterone</td>
</tr>
<tr>
<td></td>
<td>b) Check history of hormone treatment</td>
</tr>
<tr>
<td></td>
<td>c) Preincubate samples with charcoal?</td>
</tr>
<tr>
<td></td>
<td>d) Use a nuclear exchange assay?</td>
</tr>
</tbody>
</table>

of the criteria for receptors (high binding affinity, specificity of binding limited to biologically active compounds, localization in target tissues, correspondence between the concentration of steroid that binds and the concentration that evokes a biologic response), but it has not been established that the observed binding is a true measure of the specific receptor protein that attaches steroid hormones to the genome in a manner that alters nuclear transcription. (It should be noted that one of the founders of the field, Elwood Jensen, consistently has avoided the term “estrogen receptor” in favor of his own coinage, “estrophilin”). The low-affinity type II binders may or may not be importantly associated with the mitotic effects of hormones—if so, they are clearly important to the cancer problem.23 Most assays are carried out by the DCC or sucrose density gradient (SDG) methods and are designed to measure type I binders exclusively. Single-point assays measure the sum of types I and II binders and run the risk of including binding that is nonspecific. Therefore, they may significantly overestimate the quantity of biologically significant binding activity.

Perhaps it is not enough to look at just the binders for estrogen and progesterone. Androgen binders, alluded to earlier, tend to be present more or less proportionally and may convey some information that we do not know how to use. Surprisingly, estrogen target tissues also contain separate and distinct binders for antiestrogens,24,25 perhaps we should be looking at these.

Table 2 alludes to immunohistochemical assays. At the moment, the only possibly reliable assays are those that are performed with the use of the monoclonal antibody against the estrogen receptor developed by G. L. Green in Jensen’s laboratory. This antibody detects receptor only in the nucleus. The generally accepted explanation for this is that the cytosolic re-
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cceptor hitherto reported is an artifact caused by leaching of receptor out of the nucleus during preparation, but it is also possible that the antibody is insensitive to receptor not bound to steroid. Immunohistologic localization of receptor has two main advantages: 1) only tiny samples of tissue are required, as opposed to the 200 mg or more needed for other assays; 2) binding to cancer cells can be distinguished from binding to noncancerous elements. Those who favor this method argue that biochemical assays are expensive and difficult. Immunohistochemical analysis, however, can be applied only to a few square millimeters of a tumor that may be very heterogeneous\textsuperscript{26,27} and requires a morphologic analysis involving arbitrary decisions about the counting of cells and the grading of staining intensity. Nevertheless, as applied to breast cancer it can carry a positive predictive value that is superior to that of the DCC assay when used by expert hands.\textsuperscript{28} The method has been applied to the study of normal endometrium\textsuperscript{29} and results of its use in endometrial cancer should soon be forthcoming. It is to be hoped that useful antibodies to other receptors, especially the progesterone receptor, will appear before long.

Perhaps the most important point made in Table 2 is the suggestion that receptor assays be restricted to samples of recurrent or metastatic endometrial carcinoma, because, after all, these are the appropriate targets of hormone therapy. The suggestion involves a great, hitherto unsolved, practical problem—the difficulty of obtaining adequate samples of such tissues, preferably from nonirradiated areas, safely and with a minimum of discomfort. If only needle biopsy specimens can be obtained, then clearly immunohistochemical analysis is the best method for their evaluation.

Finally, standardization of laboratory methods should be mentioned. Laboratories performing receptor assays now employ slightly different methods and different criteria for a test with positive results. A properly designed study would require a consensus about optimum methods, a standardization of methods, and the institution of quality controls.

Table 2 alludes to the possibility of testing the responsiveness of the tumor to hormones in terms of the administration of tamoxifen followed by assay of a second sample of cancer tissue to see whether it has responded to the stimulus. While this actually has been done in patients with early cancers and in nude mice harboring transplanted human cancer,\textsuperscript{30} it is not often likely to be a practical test for the patient with recurrent disease. Might there not be an assayable substance that could mark the response of endometrial cancer tissue to hormonal administration, preferably in vitro? A possible test substance for determining hormone responsiveness is 24K protein,\textsuperscript{31,32} originally identified in the human breast cancer cell line MCF-7 as a clearly hormone-dependent product. This protein has been purified and its mRNA identified and measured. It has been detected more recently by immunohistochemical means in normal human endometrial epithelium and in endometrial cancers. The amount of the protein in normal endometrium peaks with the cyclic estrogen peak in glandular epithelium and with the progesterone peak in surface epithelium. The histochemical studies show the protein first in the base of the epithelial cell and finally at the free surface, but it is not considered to be a secretion product. Its presence in endometrial cancer correlates with the degree of differentiation of the tumor. Detection in serum has not been reported.

The progestagen-associated endometrial protein (PEP) of Joshi\textsuperscript{33} is an antigen found in the epithelium of decidualized endometrium and in serum of the late luteal phase, where it increases in amount after serum progesterone has declined from its midluteal peak. It is found at still higher
levels in amniotic fluid and in pregnancy serum where its time course parallels that of human chorionic gonadotropin. It cannot be detected in the myometrium, cervix, fallopian tubes, or ovaries. Measurement of this protein in endometrial cancer tissue or in the serum of patients with endometrial cancer has not been reported.

Several intracellular proteins of the endometrium that are regulated by steroid hormones have been identified, notably the enzyme creatine kinase BB, which is sensitive to estrogen, and estradiol dehydrogenase (17-hydroxysteroid dehydrogenase, steroid 17 oxido-reductase), which is sensitive to progesterone. Attempts to prepare an in vitro assay using the latter enzyme have been unsuccessful. The addition of estradiol and progesterone to endometrial tissue in vitro has been shown to alter the production of several intracellular proteins formed from labeled amino acids.

The studies of Hirsch et al. and others have provided evidence that human uterine fluid is more than a transudate of serum. The presence of secretory component in uterine fluid and its increase during the secretory phase of the cycle conclusively demonstrates that the human endometrium is an organ of secretion. The only fully identified secretory product of human endometrium is prolactin, the in vitro production of which by decidual cells of the stroma is increased by the addition of progesterone and decreased by estradiol. Secreted proteins, however, also have been reported by Strinden and Shapiro, who, in experiments with human endometrium in organ culture, described five proteins that appeared to be progesterone regulated (some being increased, others decreased), and by Umapathy et al., who have described decidual cell protein(s).

As promising as all this research activity is, there remains a need for the identification of endometrium-specific, epithelium-specific, hormone-sensitive, and serum-detectable proteins. Our laboratory has been devoted for a number of years to a search for such proteins, studying aspirates of human uterine fluid and the media in which isolated human endometrial glands have been incubated in the presence and absence of hormones. By combining two-dimensional gel electrophoresis, a sensitive protein stain, radioautography, and a sensitive computer-assisted gel scanning system, we have described more than 20 proteins that are shed or secreted by normal endometrial glands and are not found in serum. These proteins provide a standard against which to compare proteins found to be associated with endometrial cancer.

We can conclude from all of this, then, that receptor assays obtained under appropriate conditions are useful for predicting responsiveness to hormone therapy. The assays should be standardized. They also can be improved, or eventually replaced, by other tests that reflect biologic responsiveness more accurately. Performing assays on primary endometrial cancers is not cost-effective, because most tumors can be expected to test positively and to do well when standard therapy is used. Tissue from primary cases could be stored routinely at \(-70^\circ\)C, however, until other prognostic factors such as grade of tumor and extent of disease have been assessed. Tissue from patients determined to be at high risk then could be assayed for receptors, and the patients could be offered a clinical trial that would test the usefulness of hormones as adjuvants and the predictiveness of receptor values. The patient with advanced or recurrent cancer should have a receptor assay performed on her tumor. This may be possible in most instances only by means of needle biopsy and immunohistochemical analysis of tissue sections. New hormone therapies, in addition to or replacing progestin administration, should be tested as adjuvants and for the treat-
ment of advanced cases. Finally, basic research needs to be done to unlock the secret of the hormonal response. From this will come both specific marker substances that will determine the ability of a tumor to respond to a hormone and the specific hormone therapies that will work best.

References


41. Strinden ST, Shapiro SS. Progesterone-altered secretory proteins from cultured