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Potential Criteria for Cohort Selection in Chemoprevention Trials of Uterine Adenocarcinoma

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Abstract Women at risk of uterine cancer include those with one or more of the following characteristics: obesity, nulliparity, late menopause, diabetes mellitus, prolonged unopposed estrogen use, and tamoxifen therapy. Risk is additionally increased by the presence of endometrial hyperplasia. The incorporation of biomarkers into the selection criteria of cohort groups at risk for developing endometrial cancer offers an innovative approach to the clinical design of chemoprevention trials of endometrial adenocarcinoma. Biomarkers that may be useful in cohort selection include nuclear morphometry, specific genetic abnormalities, and markers of proliferation and differentiation.

Key words: Biomarkers, chemoprevention, endometrial cancer, uterine cancer

The goal of a chemoprevention trial for endometrial adenocarcinoma is to reduce the likelihood of cancer in women at increased risk of this disease. Achievement of this goal in an affordable manner and in a reasonable period of time will depend on selecting cohort study groups that share three characteristics: (1) one or more epidemiological risk factors that place study subjects at increased risk of developing endometrial cancer; (2) a precursor lesion with a propensity to develop into cancer if untreated; and (3) biomarkers that parallel lesion severity in the spectrum of disease between normal and neoplastic endometrium.

RISK FACTORS

Epidemiological risk factors for endometrial adenocarcinoma include obesity, nulliparity, late menopause, and diabetes mellitus. Prolonged hormonal stimulation of the endometrium, whether from unopposed estrogen replacement therapy or tamoxifen, is also associated with increased risk. Risk is additionally increased by the presence of atypical adenomatous hyperplasia, a histologic precursor lesion for estrogen-dependent endometrial adenocarcinoma.

BIOMARKERS

Biomarkers that reflect the initiation and progression of pathologic lesions of the endometrium have been studied to a limited degree. Review of the literature suggests that alterations of general genomic markers, select genetic markers, and markers of proliferation and differentiation may parallel histopathologic diagnoses ranging from normal through hyperplastic to neoplastic endometrium.

Nuclear morphometry and ploidy are examples of general genomic markers. Nuclear morphometry includes parameters such as nuclear area, nuclear perimeter, the longest and shortest nuclear diameters, and the average feret diameter. Morphometric estimates are objective and reproducible measurements that reliably differen-

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tiate normal, hyperplastic, and neoplastic endometrium [1].

Aneuploidy is common in endometrial adenocarcinoma [2,3]. Abnormalities of ploidy are more common in moderately and poorly differentiated neoplasms. Aneuploidy has also been detected in endometrial hyperplasia but appears to be uncommon [4,5].

The human endometrium is a hormonally responsive tissue that undergoes cyclic proliferation, differentiation, and exfoliation in response to discrete changes in the expression of numerous genes [6]. Alterations of gene expression in abnormal endometrium, including preinvasive and invasive conditions, must be interpreted in the context of these changes.

The epidermal growth factor receptor (EGFR), encoded by the *erb*B protooncogene, is found in normal endometrium [7,8]. In response to estrogen, the levels of EGFR as well as EGF increase [9]. Expression of the EGFR is retained following neoplastic transformation, but the amount of receptor appears to decrease with increasing lesion severity [10]. The HER-2/*neu* oncogene, which is related to the gene that encodes EGFR, is also expressed by normal endometrium with no apparent variation during the menstrual cycle [11,12]. Sato [13] reported HER-2/*neu* over-expression in two of six atypical endometrial hyperplasia cases. HER-2/*neu* over-expression has been found in 10–15% of endometrial adenocarcinoma specimens [11,14].

The role of *ras* protein (p21) in normal endometrial function has not been established, although p21 is recognized as an important component of the intracellular signal transduction cascade. Although the total number of specimens analyzed to date is modest compared with the numbers of other solid neoplasms studied, it appears that *ras* mutations in endometrial adenocarcinoma occur in approximately 20% of cases and most commonly involve the Ki-*ras* gene. Mutations of the Ki-*ras* gene may be an "early" event [15–17]. Codon 12 Ki-*ras* mutations have been found in simple, complex, and atypical endometrial hyperplasia, as well as endometrial adenocarcinoma.

Alterations of c-*myc* gene structure and expression have not been extensively evaluated in endometrial hyperplasia or adenocarcinoma. Bai *et al.* [18] reported that the intensity of c-*myc* immunohistochemical staining occurred during the transition from normal to hyperplastic to carcinomatous endometrium.

Increased immunostaining of the p53 gene product, reflecting p53 point mutations, occurs in approximately one-third of endometrial adenocarcinomas. Kohler *et al.* [19] reported immunohistochemical detection of the p53 protein in 22 of 107 endometrial adenocarcinoma specimens. Bur *et al.* [20] reported a strong diffuse immunohistochemical staining pattern in 14 of 47 endometrial adenocarcinoma specimens [20]. Ambros *et al.* [21] found normal p53 expression in normal endometrium with increased expression in 2 of 13 biopsies of atypical adenomatous hyperplasia and in 23 of 59 adenocarcinoma specimens.

Alterations of cellular biology, including proliferation and function, reflect general genomic and specific genetic alterations in hyperplastic and neoplastic endometrial specimens. Nucleolar organizer regions (AgNORs), which reflect cellular and nuclear activity, have been studied to a limited extent in endometrial adenocarcinoma. Wilkinson *et al.* [22] reported no significant differences in the mean number of AgNORs per nucleus among normal proliferative and hyperplastic endometrium, and endometrial adenocarcinoma. However, Papadimitiou *et al.* [23] reported that malignant cells demonstrated higher AgNOR counts and a larger maximum nuclear diameter and mean area compared to normal and hyperplastic endometrium, suggesting that these parameters are useful criteria to differentiate these histopathologic diagnoses. However, other indices of cellular proliferation do not appear to consistently differentiate normal, hyperplastic, and neoplastic endometrium. Kysela *et al.* [5] reported a significantly lower S-phase fraction in normal endometrium than in adenocarcinoma specimens. Sizeable variation was noted in the S-phase fraction values of hyperplastic and adenocarcinoma specimens. Similar results have been reported by Thornton *et al.* [4]. Sato [13] found no difference in proliferative activities between atypical adenomatous hyperplasia and carcinoma based on PCNA and S-phase fractions. Ito *et al.* [24] reported no significant differences in PCNA labelling index between hyperplastic and carcinomatous specimens. Punnonen *et al.* [3] found the highest S-phase fractions in moderately and poorly differentiated carcinomas; the lowest values were found in atypical hyperplasia and well-differentiated carcinomas.

Pirog and Czerwinski [25] have studied the mitotic index in normal and abnormal endometrial specimens. Significant differences were not identified among normal endometrium, hyperplastic endometrium, and well-differentiated adenocarcinoma specimens (4.35 ± 3.4, 4.19 ± 6.0, and 4.01 ± 4.2, respectively).

Degradation of the basement membrane may be an early event in endometrial cancer development. Bulletti *et al.* [26] observed immunostaining for collagenase in adenocarcinoma specimens but not in normal and hyperplastic endometrium. A progressive increase in urokinase-type plasminogen activator, TGF-α, and EGFR were observed in the histopathologic transition from proliferative to hyperplastic to neoplastic endometrium.

Sato [13] reported that the intensity of immunohistochemical staining of cathepsin D, laminin, type IV collagen, tenascin, and CD44 differed between normal endometrium, atypical adenomatous hyperplasia and adenocarcinoma. Furness and Lam [27] also reported differences in laminin staining patterns between endometrial hyperplasia and endometrial adenocarcinoma.

Gold *et al.* [28] studied growth factor expression in normal and abnormal endometrial tissue. Glandular epithelium exhibited a statistically significant stepwise increase in the expression of TGF-β1,2,3 during the progression from normal to proliferative to simple hyperplasia to complex hyperplasia. No additional increase was observed in adenocarcinoma specimens. Immunoreactive basic fibroblast growth factor expression was negligible in normal and simple hyperplasia, but increased in complex hyperplasia with an additional increase noted in carcinoma specimens.

Tenascin is an extracellular matrix glycoprotein that appears to play a role in epithelial and stromal cell interactions during oncogenesis. Sasano *et al.* [29] reported that tenascin expression was absent in secretory endometrium and weakly present in only 50% of proliferative phase endometrial specimens. Approximately 60% of hyperplastic endometrial specimens exhibited weak, irregular periglandular tenascin expression that did not correlate with the degree of cellular atypia. Adenocarcinomas exhibited intense and diffuse staining around the neoplastic cells although the intensity of staining was not related to cellular differentiation.

Teni *et al.* [30] studied the immunohistochemical expression of inhibin in normal and abnormal endometrial tissue. Inhibin positivity was localized to the cytoplasm of epithelial cells in malignant and hyperplastic endometrium. There was no evidence of inhibin activity in early proliferative endometrium but it was present on the luminal border of the glandular epithelium in the mid and late proliferative phase. In comparison, secretory endometrium exhibited strong inhibin activity.

Charpin *et al.* [31] described immunostaining patterns observed using the monoclonal antibody 1BE12. This antibody binds to a poorly characterized antigen found in a variety of normal and abnormal tissues. In the endometrium, 1BE12 immunoreactivity correlated with increasing cell proliferation and malignancy. When frozen sections were studied, 1BE12 staining correlated with Ki-67, EGFR, HER-2/*neu*, and cathepsin immunostaining intensity.

The immunostaining patterns of CA19-9, CA-125, and CEA have also been studied [32]. Proliferative endometrium does not express CA19-9 and CA-125. CA19-9 is expressed in roughly 50% of atypical hyperplasias and in over 90% of carcinomas. Among adenocarcinomas, CA19-9 expression decreases as the grade of differentiation decreases. CA-125 is expressed in approximately 50% of atypical hyperplasias and carcinomas with no significant variation with cellular differentiation. CEA is infrequently expressed in normal and hyperplastic endometrium. In adenocarcinoma specimens, the expression of CEA increases with loss of neoplastic differentiation.

CONCLUSION

Cohort selection for a chemoprevention trial of endometrial adenocarcinoma should include epidemiological risk factors such as prolonged unopposed estrogen or tamoxifen therapy. The study group selection can be further focused by including women who have endometrial hyperplasia. There is no single genetic, morphologic, or biochemical alteration that reliably presages neoplastic endometrial transformation. To assess the efficacy of a chemopreventive agent, some combination of biomarkers that appear to parallel the severity of endometrial pathology should be monitored. Potential biomarkers of interest in endometrial adenocarcinoma include nuclear

morphometry, EGFR expression, Ki-*ras* mutations, and the expression of HER-2/*neu*, c-*myc*, fibroblast growth factor, tenascin, CA19-9, CA-125, and TGF-α and -β.

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