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Biomarkers in the Endometrium

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Abstract A number of oncogenes and tumor suppressor genes that may serve as surrogate biomarkers of transformation are altered during the process of endometrial carcinogenesis. Overexpression of HER-2/*neu* occurs in 10% of endometrial adenocarcinomas and correlates with intraperitoneal spread of disease and poor survival. The c-*myc* oncogene is amplified in 10% of cases. Point mutations in codon 12 of the K-*ras* oncogene have been reported to occur in 10–20% of endometrial cancers. K-*ras* mutations also have been noted in some endometrial hyperplasias, which may represent an early event in the development of some endometrial cancers. Mutation of the p53 tumor suppressor gene, with resultant overexpression of mutant p53 protein, occurs in 20% of endometrial adenocarcinomas. Overexpression of p53 is associated with advanced stage and poor survival. Because p53 mutations have not been observed in endometrial hyperplasias, this is thought to be a relatively late event in endometrial carcinogenesis. Microsatellite instability has also been noted in approximately 15% of sporadic endometrial cancers, but mutations in DNA repair genes have not yet been reported. Chemoprevention trials in endometrial cancer may be feasible due to the existence of a premalignant lesion and surrogate biomarkers.

Key words: Endometrial cancer, HER-2/*neu*, oncogenes, p53, *ras*, tumor suppressor genes

Although chronic stimulation of the endometrium by estrogens has been associated with the development of endometrial hyperplasia and adenocarcinoma, the underlying molecular mechanisms remain unclear. In addition, some endometrial cancers appear to arise in the absence of a premalignant hyperplastic phase. Recently, several lines of evidence have suggested that most human cancers arise from sequential damage to genes that encode proteins involved in regulation of cellular proliferation. Both oncogenes and tumor suppressor genes have been implicated in this process. Oncogenes encode proteins that participate in growth stimulatory pathways in normal cells. Conversely, tumor suppressor gene products normally inhibit unrestrained proliferation. A number of specific alterations in these genes may serve as surrogate biomarkers of malignant transformation in the endometrium.

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ONCOGENES

Cell membrane receptors that bind peptide growth factors are composed of an extracellular ligand binding domain, a membrane spanning region, and a cytoplasmic tyrosine kinase domain. Although numerous receptor tyrosine kinases have been identified, thus far most studies in endometrial cancer have focused on the epidermal growth factor (EGF) receptor, HER-2/*neu* and c-*fms*.

EGF and its receptor (EGFR) were among the first growth factor/receptor tyrosine kinases to be characterized at a molecular level. EGFR is present in glandular and stromal cells of the endometrium in both proliferative and secretory phases in cycling women [1]. Expression also is

maintained in atrophic endometrium after menopause. Although some squamous cancers overexpress EGFR due to amplification of the EGFR gene, amplification has not been noted in endometrial adenocarcinomas.

However, loss of EGFR may occur during endometrial carcinogenesis. Using a radioreceptor assay, Reynolds *et al.* [2] showed that grade 1–2 cancers had, on average, a 34% decrease, and grade 3 cancers a 90% decrease in EGFR expression relative to normal endometrium. In addition, immunohistochemical studies have demonstrated that EGFR is detectable in some, but not all, cases [1].

The HER-2/*neu* gene encodes a receptor tyrosine kinase similar in structure to the EGFR; a ligand that binds to HER-2/*neu* (heregulin) has been discovered. Amplification and overexpression of HER-2/*neu* has been noted in approximately 20–30% of breast and ovarian cancers [3]; in many studies, overexpresion has been associated with poor survival. In addition, several studies suggest that this oncogene product is overexpressed in 10–15% of endometrial cancers [4–6]. A study performed at the Mayo Clinic examined HER-2/*neu* expression in paraffin blocks from 247 endometrial cancers [5]. Expression was high in 15% of cases, mild in 58%, and absent in 27%; 5-year progression-free survival was 56%, 83% and 95% in these groups, respectively. Among Stage I cases, 26 (13%) had high expression; 5-year progression-free survival was 62% compared to 97% in cases with lesser expression. The incidence of overexpression was higher in advanced stage cases (11/44, 25%). In addition, multivariate analysis revealed that high expression was an independent variable associated with poor survival.

The *fms* oncogene also has been shown to encode a receptor tyrosine kinase, which serves as a receptor for macrophage colony stimulating factor (M-CSF). Kacinski *et al.* [7] examined expression of *fms* in 21 endometrial cancers using *in situ* hybridization. Expression of *fms* complementary mRNA was found to correlate with advanced stage, poor grade, and deep myometrial invasion. The association of *fms* expression with adverse prognostic factors was confirmed by Leiserowitz *et al.* [8] at the Mayo Clinic. Subsequently, it was shown that *fms* and M-CSF usually were co-expressed in endometrial cancers and it was proposed that this receptor-ligand pair might mediate an autocrine growth stimulatory pathway [9]. In support of this hypothesis, M-CSF serum levels are increased in patients with endometrial cancer. In addition, M-CSF increases the invasiveness of cancer cell lines that express significant levels of *fms*, but has no effect on cell lines with low levels of the receptor [10]. It remains unclear, however, whether increased production of M-CSF or other peptide growth factors plays a role in eliciting malignant transformation or results from other unrelated alterations.

In many types of cancers, *ras* genes often undergo point mutations in codons 12, 13 or 61, causing a constituitively activated molecule. Boyd *et al.* [11,12] examined the *ras* genes in 11 immortalized endometrial cancer cell lines and 10 primary endometrial cancers. Mutations in codon 12 of K-*ras* were seen in four cell lines and one primary cancer. Subsequent studies of primary endometrial adenocarcinomas have confirmed that codon 12 of K-*ras* is the most frequent site of mutations. Enomoto *et al.* [13,14] reported from Japan that 15/52 (29%) endometrial cancers had mutations in codon 12 of K-*ras*. In two studies of American endometrial cancers, 3/30 (10%) and 7/60 (12%) cases had mutations in codon 12 [12,15].

This apparent difference in frequency of codon 12 K-*ras* mutations between Japanese and American endometrial cancers was confirmed by Sasaki *et al.* [16] in a study which examined cases from both countries. Mutations were seen in cancers from 5/36 (14%) White Americans, 0/5 Black Americans, and 10/43 (23%) Japanese. Striking differences exist in the incidence of endometrial cancer between America (approximately 20 cases per 100,000) and Japan (approximately 5 cases per 100,000). In contrast, mortality from the disease is roughly equivalent in the two countries. Epidemiologic studies have suggested that the higher mortality per case in Japan occurs because few Japanese women are obese and most develop estrogen-dependent, well-differentiated favorable lesions. In view of this, K-*ras* mutation may be associated with poorly differentiated lesions with unfavorable prognosis. In one Japanese study, survival was 93% in 43 cases with normal K-*ras* compared to 50% in 6 cases with mutations [17]. In contrast, Enomoto [14] did not find a correlation with histologic grade, and an American study [15] did not find a correlation

between K-*ras* mutation and survival. Furthermore, Sasaki *et al.* [16] found that none of the 22 patients in their study who died of recurrent disease had K-*ras* mutations. In summary, although it appears that the incidence of codon 12 mutations in endometrial cancers is significantly higher in Japan than in America, there is no obvious association of mutation with specific pathologic features or prognosis.

Finally, K-*ras* mutations have been identified in some endometrial hyperplasias [13,15,16]. The frequency of mutations in hyperplasias is similar to that seen in endometrial cancers, which suggests that K-*ras* mutation may be a relatively early event in the development of some endometrial cancers. Mutations are found more frequently as the severity of the hyperplasia increases, from 10% in simple to 14% in adenomatous to 22% in atypical adenomatous hyperplasias [16]. Since only a minority of endometrial hyperplasias contain K-*ras* mutations, other genes also must play a role in their development.

Among the nuclear transcription factors involved in stimulating proliferation, amplification of members of the *myc* family has most often been implicated in human cancer development. It has been shown that c-*myc* is expressed in normal endometrium [18], with higher expression in the proliferative phase relative to the secretory phase. Monk *et al.* [19] found that c-*myc* was amplified in 11% of 37 frozen endometrial cancers, and amplification correlated with poor grade.

TUMOR SUPPRESSOR GENES

Tumor suppressor genes encode proteins that normally inhibit proliferation. Because inactivation of both copies is required to eliminate the inhibitory effect of a tumor suppressor gene, these genes are referred to as recessive cancer-causing genes. Loss of tumor suppressor function may occur via several mechanisms, including complete deletion of a gene, mutations or partial deletions that cripple the gene product, lack of gene transcription, or inactivation of the corresponding protein.

Loss of p53 tumor suppressor gene function due to mutation of this gene is the most frequent genetic event described thus far in human cancers. Because of their resistance to degradation, mutant p53 proteins overaccumulate in the nucleus and can be detected immunohistochemically. We found that mutant p53 protein was overexpressed in 20% of 107 frozen primary endometrial adenocarcinomas, including 9% of Stage I/II and 41% of Stage III/IV cancers [20]. Overexpression of p53 in endometrial adenocarcinomas was associated with several known prognostic factors, including poor grade and advanced stage [20]. In addition, survival rates of patients whose cancers overexpressed p53 were poorer than those of patients whose cancers did not overexpress p53. A correlation with poor survival also has been demonstrated in an immunohistochemical study of paraffin blocks from Japanese endometrial cancers [21]. Overall, 21% of 221 cases were found to overexpress p53. Among patients with Stage I/II disease, recurrence developed in 50% of 22 patients whose cancers overexpressed p53 compared to only 15% of 156 patients whose cancers did not overexpress p53.

Endometrial cancers that overexpress p53 protein have been shown to harbor point mutations in conserved regions of exons 5–8 of the gene, resulting in amino acid substitutions in the protein [20,22]. Only the mutant allele was transcribed in four of five endometrial cancers with mutations in our study, indicating that the wild-type allele was likely deleted. Other groups have also noted mutations in exons 5–8 of the p53 gene in endometrial adenocarcinomas [22]. It has been postulated that mutations in this region are particularly effective in negating the normal tumor suppressor function of the p53 gene product.

Using the technique of single-stranded conformation polymorphism analysis, we found no p53 mutations in any of 117 endometrial hyperplasias, including 41 atypical adenomatous cases [23]. Consistent with this finding, no p53 immunostaining was seen in any of 44 endometrial hyperplasias. In another study, Enomoto [14] found p53 mutations in only 1/13 atypical hyperplasias. The rarity of p53 mutations in endometrial hyperplasias and the correlation of p53 alterations with extrauterine disease in invasive endometrial cancers suggests that mutation of the p53 gene is a relatively late event in endometrial carcinogenesis. Alternatively, it is possible that acquisition of a p53 mutation leads to development of a virulent endometrial cancer that does not pass through a phase of hyperplasia and is associated with rapid spread of disease.

DNA REPAIR GENES

Our group and others [24,25] have noted that some endometrial cancer DNA samples contain microsatellite alleles that do not correspond to either allele from the matched normal DNA. Our study found that 17% of 36 sporadic endometrial cancers in which numerous markers were examined had widespread evidence of new microsatellite alleles throughout the genome [24]. Endometrial cancers that exhibited microsatellite instability were diploid and had a favorable prognosis. Similarly, Duggan *et al.* [25] found microsatellite instability in 9/45 (20%) cases. In their study, mutations in the K-*ras* oncogene were more common in cases with instability (56%) compared to cases in which instability was not seen (14%).

Microsatellite instability initially was noted in colon cancers of patients with hereditary nonpolyposis colon cancer (HNPCC), also known as Lynch Syndrome Type II. Endometrial cancer is the second most common malignancy observed in these families, but ovarian, breast, and gastrointestinal tract malignancies also occur. We found that 3/4 endometrial cancers from members of HNPCC families had microsatellite instability [24]. Subsequently, it was shown that 60% of affected individuals in HNPCC families were shown to have germline mutations in the MSH2 gene on chromosome 2p, which is involved in DNA mismatch repair. An additional 30% of these families carry mutations in another DNA repair gene on chromosome 3p (MLH1) [26]. In bacteria and yeast, mutations in these DNA repair enzymes also lead to microsatellite instability, confirming the cause and effect relationship between these events.

In addition to causing microsatellite instability, the inability to repair DNA may lead to an increased rate of genetic damage throughout the genome, which may increase the likelihood of tumorigenesis due to alterations in oncogenes and tumor suppressor genes. Thus, DNA repair genes represent a new family of cancer-causing genes, distinct from those previously described. Because microsatellite instability has been noted in some sporadic endometrial cancers, several groups are attempting to identify acquired mutations in DNA repair genes. Although these studies have been relatively unrewarding, it is believed that additional, though unidentified, DNA repair genes exist.

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