Molecular and Pathologic Aspects of Endometrial Carcinogenesis

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ABSTRACT

Endometrial cancer is the most common gynecological malignancy, with 41,000 new cases projected in the United States for 2006. Two different clinicopathologic subtypes are recognized: the estrogen-related (type I, endometrioid) and the non–estrogen-related types (type II, nonendometrioid such as papillary serous and clear cell). The morphologic differences in these cancers are mirrored in their molecular genetic profile with type I showing defects in DNA-mismatch repair and mutations in PTEN, K-ras, and beta-catenin, and type II showing aneuploidy and p53 mutations. This article reviews the genetic aspects of endometrial carcinogenesis and progression. We will define the precursor lesion of type I endometrioid cancer and the role of genetics and estrogen in its progression.

INTRODUCTION

Current concepts of endometrial cancer successfully integrate traditional histopathology with pathogenetic mechanisms. Endometrial cancers have long been classified into two major divisions (types I and II) based on light microscopic appearance, clinical behavior, and epidemiology.1 Type I, those with endometrioid histology, comprise 70% to 80% of newly diagnosed cases of endometrial cancer in the United States.2 They are associated with unopposed estrogen exposure and are often preceded by premenopausal disease. In contrast, type II endometrial cancers have nonendometrioid histology (usually papillary serous or clear cell) with an aggressive clinical course. Hormonal risk factors have not been identified, and there is no readily observed premalignant phase. The morphologic and clinical differences are paralleled by genetic distinctions, in that type I and II cancers carry mutations of independent sets of genes (Table 13-8). Most were identified as candidate genes by analogy with other tumor systems, and were confirmed subsequently to be altered in endometrial cancer. Open-ended gene discovery by comparison of tumor subsets using genome-wide methods such as expression profiling has further broadened our understanding of relevant genetic basis for these differences.9,10 The result is an enhanced understanding of early genetic events, reinforcement of the clinicopathologic subgroups originally defined by histologic and clinical features, and development of biomarkers informative in identification of previously unknown or poorly described precursor lesions.

In the last decade, progress has been greatest in molecular and histologic resolution of precursors of type I cancer, resulting in a cohesive model of endometrial carcinogenesis encompassing both genetic and hormonal factors, revised precancer diagnostic criteria, and novel prevention strategies. We will primarily focus on these advances and will describe ongoing attempts at genetic substratification within the endometrioid group.

GENETICS OF TYPE I CANCERS

While most serous (type II) cancers contain mutations of p53,3 endometrioid (type I) adenocarcinomas demonstrate larger numbers of genetic changes in which the temporal sequence of mutation, and the final combination of defects differ substantially between individual examples. Common genetic changes in endometrioid endometrial cancers include, but are not limited to, microsatellite instability (MSI),11-15 or specific mutation of PTEN,16-21 K-ras,12,22-28 and β-catenin genes.13,29-31

MSI

Approximately 20% of sporadic endometrioid endometrial cancers of all grades demonstrate a molecular phenotype referred to as MSI.11,12,15,32 Microsatellites are short segments of repetitive DNA bases that are scattered throughout the genome; they are found predominantly in noncoding DNA. MSI is the propensity to develop changes in the number of repeat elements as compared with normal tissue due to DNA repair errors made during replication. MSI is rare (< 5%) in type II endometrial cancers,33,34 where the primary genetic defect is in the
p53 gene and genetic instability is manifest globally at the chromosomal rather than microsatellite level. Thus, type II tumors frequently demonstrate high-order aneuploidy while having an intact mismatch-repair mechanism.34,35

MSI is due to inactivation of any of a number of intranuclear proteins that comprise the mismatch repair system, leading to accumulation of structural changes in coding and noncoding repetitive elements of many genes.36 MLH1 inactivation, a component of the mismatch repair system, is the most common mechanism in the endometrium and is accomplished by hypermethylation of CpG islands in the gene promoter, a process known as epigenetic silencing.5 This mechanism stands in contrast to colon cancer, where MSI is due to mutations in the MSH2, MLH1, and MSH6 genes.37 Inherited or somatically acquired mutations of MSH6, another mismatch repair element, are also common in patients with MSI endometrial cancers.74 A single nucleotide insertion (frameshift) mutation in MSH3 has been described less frequently in MSI endometrial cancers with MSI. This deletion occurs in a string of eight adenine residues (A₈). Because simple repeat sequences are unstable in cells with MSI, the observed mutation may be secondary to the MSI derived from defective MLH1 expression.38,39 Since MLH1 is important in repair of short segments (two to four bases), and MSH2:MSH3 serves to repair larger insertion-deletion mutations, the combined defect of the mismatch repair system results in inhibition of both small and large insertion-deletion mismatch repair.40

MSI, and abnormal methylation of MLH1, is a rare event in endometrial carcinogenesis that has been described in precancerous lesions.32,118,41 Once established within a tumor lineage, MSI leads to creation of genetic heterogeneity within carcinoma cells of a single tumor. MSI may specifically target for inactivation those genes which contain susceptible repeat elements, such as transforming growth factor β receptor type II, (TGF-βRII), BAX, insulin-like growth factor II receptor (IGFIR), and hMSH3, resulting in secondary tumor subclones with an altered capacity to invade and metastasize.42-46 Indeed, screening of MSI endometrial cancers has shown frameshift mutations in the coding region repeats of FAS, BAX, CASPS, and IGF-βRII genes that are infrequent in microsatellite stable cancers.47,48

PTEN

Inactivation of the PTEN tumor-suppressor gene (formerly known as MMAC1) is the most common genetic defect in endometrioid carcinoma and is seen in up to 83% of tumors that are preceded by a histologically discrete premalignant phase.16 PTEN is a tumor suppressor gene encoding a lipid phosphatase which acts to maintain G1 arrest and enable apoptosis through an AKT-dependent mechanism.49,50 The most commonly observed PTEN defect is inactivation of both alleles to generate a protein null, or complete loss of function, phenotype. There is some evidence that even a PTEN hemizygous inactivation leading to a protein deficient, rather than null state, may be functionally significant when combined with abnormalities of other genes which converge on its downstream pathway. PTEN acts in opposition to phosphatidylinositol-3-kinase (PIK3CA) to control levels of phosphorylated AKT. PIK3CA mutation, seen in 36% of endometrial carcinomas, is most frequent in those tumors which also bear PTEN mutation.51 This is consistent with a cooperative effect between these two elements in promoting neoplastic transformation.

PTEN inactivation is a functionally significant in carcinogenesis as is demonstrated by the high prevalence of endometrial cancer in PTEN knockout mice. Heterozygous PTEN inactivation produces an abnormal endometrial phenotype in mice, with 100% of mice developing hyperplastic lesions, and 20% of animals progressing to endometrial carcinoma.52 Humans with constitutive germline PTEN mutations may present with the heritable cancer syndrome of Cowden’s disease, which includes high rates of breast, thyroid, and other cancers in conjunction with hamartomas of multiple organs.6 There appears to be an increased risk of endometrial cancer in women with Cowden’s disease, but only small numbers of these patients have been available for study, and the magnitude of increased risk is modest.

PTEN inactivation may be caused by a variety of mechanisms. Mutation, or deletions resulting in loss of heterozygosity (LOH) at chromosome 10q23, are detected in 37% to 61% of cancers.6,53 The pattern of PTEN mutation is different in MSI and microsatellite stable cancers. MSI-positive tumors have a higher frequency of deletions involving ≥ three base pairs when compared with the MSI-negative group. In addition, the mutations observed in MSI tumors only rarely involve the polyadenine repeat of exon 8 that is the expected target (containing a microsatellite marker). The mechanism for these MSI related changes is unknown.40 Promoter methylation has been postulated an alternative transcriptional inactivating event, but has been difficult to prove experimentally because of technical limitations in quantification of methylation level and assay interference by a co-amplified pseudogene.

The PTEN locus on chromosome 10 may be flanked by other tumor suppressor genes that are codelleted with PTEN itself.54,55 The homeodomain containing gene EMX2 is a strong candidate for just such a gene.56 Increased native EMX2 expression is associated with diminished endometrial proliferative activity, a pattern that might be predicted for a tumor suppressor. Deletion of EMX2,
with commensurate decline in expression, is seen in some endometrial adenocarcinomas.

**K-ras**

*K-ras* mutations have been identified in 10% to 30% of type I endometrial cancers.26,27,57 There is a higher frequency of *K-ras* mutations in MSI cancers, and many of these are characterized as methylation related GC → AT transitions.38

**β-catenin (CTNNB1)**

Gain of function mutations in exon 3 of CTNNB1 gene at 3p21 are seen in 25% to 38% of type I cancers.29–31 These mutations in exon 3 result in stabilization of the protein, cytoplasmic and nuclear accumulation, and participation in signal transduction and transcriptional activation through the formation of complexes with DNA-binding proteins.58 β-catenin is a component of the E-cadherin-catenin unit essential for cell differentiation and maintenance of normal tissue architecture, and plays an important role in signal transduction. Increased nuclear levels of β-catenin produce transcriptional activation through the LEF/Tcf pathway.59 The APC protein downregulates β-catenin levels by cooperating with glycogen synthase kinase 3β (GSK-3β), inducing phosphorylation of serine-threonine residues coded in exon 3 of the β-catenin gene and its degradation through the ubiquitin-proteasome pathway.60,61

β-catenin mutation may represent a pathway to endometrial carcinogenesis characterized by squamous differentiation and independent of PTEN.59,61 Although MSI, PTEN, and K-ras mutations frequently coexist with each other, these molecular abnormalities are not usually seen in tumors with β-catenin alterations.58 When abnormal, β-catenin expression changes are usually seen throughout all tumor cells, and β-catenin changes are present in some premalignant lesions. This suggests that β-catenin mutation is an early step of endometrial tumorigenesis that is clonally represented in all tumor cells.62,3 However, changes in β-catenin activity may contribute to later tumor progression as well.63,64 Based on analogy with colon cancer, several genes may be potential targets for a dysregulated WNT/β-catenin pathway. For example, in colonic adenocarcinoma, elevated β-catenin levels caused by mutations in CTNNB1 or APC result triggers cyclin-D1 gene expression, and subsequently, uncontrolled progression of tumor cells into the cell cycle.67 β-catenin might regulate the expression of the matrix metalloprotease-7, which would have a role in the establishment of the microenvironment necessary for the initiation and maintenance of growth of the primary tumors and their metastasis.68

Nuclear localization (activation) of β-catenin is also induced by the WNT signaling pathway, which is abnormal in some MSI tumors. Gene expression profiling of cancers with and without MSI shows two members of the secreted frizzled related protein family (SFRP1 and SFRP4) as more often downgraded in MSI cancers.69 These proteins are negative regulators of the WNT signaling pathway. Fibroblast growth factor 18 (FGF18), a direct target of the WNT pathway, was upregulated in MSI cancers.

p53 is a nuclear phosphoprotein that induces proliferative arrest or apoptosis through induction of P21Waf1/Cip1 and hMdm2 in response to cellular stress. Although more common in serous and clear-cell type carcinoma, aberrant accumulation of inactivated p53 protein is seen in approximately 5% of endometrioid cancers.27 High protein levels correlate with high grade and stage, but are also an independent prognostic factor.70 Unlike serous cancers, p53 truncation mutations are rare in endometrioid tumors.71 In p53 “positive” endometrioid endometrial tumors, p53 protein accumulation may be secondary to changes in its upstream regulatory proteins rather than the p53 gene itself.72 Several genes, including MDM2 and p14 ARF, that regulate p53 levels have been shown to cause detectable levels of p53 in the absence of p53 mutation, and their dysregulation may be associated with adverse clinical outcomes.71–73 Alternatively, nonspecific DNA damage such as that induced by irradiation are also known to induce accumulation of wild-type p53.74

Other candidate prognostic markers investigated in endometrial adenocarcinoma have differing degrees of evidence supporting clinical relevance. Many have only been described in vitro models, and others fail to achieve a level of clinical outcome prediction independent of existing pathologic tumor classification. Expression of HOXB13, a member of the homeobox (HOX) gene family, is induced by estrogens in endometrial cancer cell lines and confers invasive potential.75 High rates of inactivation by promoter methylation of another homeobox gene HOXA11 correlates with tumor recurrence in stage I to II cancers.76 E-cadherin functions to maintain cell-to-cell adhesion and its loss is has been associated with invasive capacity in breast, esophagus and ovarian cancers.77 Inactivation of the E-cadherin gene by methylation is present in a subset of cancers and with increasing frequency at higher stage and grade. In addition to MSI, inactivation of MLH1 may create a selective growth advantage under conditions of oxidative stress or cross-linking agents via the deregulation of apoptosis.78 Comparison of expression profiling of endometrial cancer cell lines before and after treatment with a demethylation agent have revealed tumor suppressor genes that undergo epigenetic silencing through promoter methylation.79 Reported genes include Tazarotene-induced gene-1 (TIG1) and CCAAT/enhancer binding protein-alpha (C/EBPalpha).

**ROLE OF SEX HORMONES**

Estrogens and progestins act reciprocally on the hormonally responsive endometrial tissue field to modify endometrial cancer risk. Progestins have the ability to abrogate, or “oppose” the biologic effects of coexisting estrogens through down-regulation of the estrogen receptor itself. For this reason the biologic effects of admixtures of circulating progestins and estrogens are dominated by the gestational component. Women exposed to estrogens without opposing effects of progestins show a dose and duration dependent 2- to 10-fold increased cancer risk.80–83 The protective effects of progestins are evident in women using combined oral contraceptives, who have a 0.5 to 0.7 endometrial cancer risk relative to controls.84,85 There are several postulated mechanisms by which sex hormones affect endometrial cancer risk, and it is likely that all are relevant to varying degrees.

Large-scale expression profiling of endometrial tissue RNAs has enabled comparison of hormonally induced transcriptional changes in benign endometrium to those seen in carcinoma.86 The profile of
type I endometrioid carcinoma resembles the expression profile seen in estrogen-driven proliferative endometrium and lacks expression of those genes induced by progestins. Overall, cancers are characterized by a loss of gene relative to benign endometrium, accounting for eight of 10 discriminating changes in RNA abundance. Among the genes with reduced or lost expression in carcinoma are a small number of tumor-suppressor genes affected by primary mutation or deletion and a larger number of downstream target genes affected secondarily.

Estrogen promotes cell proliferation and inhibits apoptosis through a complex downstream cascade of transcriptional changes that may include modulation of tumor suppressor function. An example is PTEN expression in normal endometrial glands, which is greatly elevated by estrogens and reduced by progestins during hormonal fluctuations of the normal menstrual cycle. This expression pattern reflects the role of PTEN as regulator of mitosis and enabler of apoptosis in the estrogen stimulated proliferative phase. Unopposed estrogens thus act as positive selection factor for PTEN mutant cells, by unmasking the inability of mutant compared with wild-type cells to check the proliferative process. In the presence of circulating progestins, the proliferative advantage of PTEN mutant cells is lost, and as a result, PTEN mutant clones have a tendency to involute. The cancer protective effects of progestins are further mediated by induction of apoptosis through the increased expression of Bcl-2 and BAX. The ability of progestins to induce endometrial epithelial apoptotic cell death extinguishes after just a few days, but increases dramatically on withdrawal of progestins. The net effect cannot be simply viewed as simple opposition of estrogen and progesterone on a static gland population, but must also incorporate a dynamic element of changing tissue responsiveness over time. Estrogens may also increase the rate of mutagenesis through free radical formation, but the magnitude of this effect is minimal compared with the increased likelihood of random mutation conferred by increases in proliferative activity.

Less specific information is available regarding estrogen influence on cellular differentiation and growth. PAX2 is a paired-box gene known to be overexpressed in cancers of the kidney, prostate, breast, and ovary. PAX2 expression is increased by estrogens in neoplastic endometrial epithelium but not in normal endometrium, indicating that neoplastic tissues have an intrinsically altered estrogen response mechanism. Whether this is a cause or effect of malignant transformation is unclear. Endometrial cancer cell line expression of HOXB13, a member of the HOX gene family can be induced by estrogens and imparts invasive potential. Cables, a cyclin-dependent kinase binding protein that is upregulated by progesterone and down-regulated by estrogen in benign endometrium, is lost in the majority of endometrial cancers. Mice deficient in cables develop hyperplasia and cancer, and overexpression in cell lines slows proliferation.

Type I cancers are frequently preceded by pan-endometrial hormonally induced changes referred to as endometrial hyperplasia, from which a localized monoclonal population of genetically altered glands emerges as a discrete premalignant lesion, endometrial intraepithelial neoplasia (EIN). In the past, distinction between generalized hormonal field effects and actual premalignant lesions was obscured by imprecise diagnostic criteria and a nomenclature system that did not accurately segregate lesions by natural history. The common term “hyperplasia” was applied to all entities, with the assumption that nonatypical hyperplasia and atypical hyperplasia subgroups correspond to benign and premalignant. Diagnosis of atypia is itself poorly reproducible and architectural features were underutilized as relevant discriminators between hormonal field effects and premalignant disease. Improved resolution was possible with the advent of polymerase chain reaction–based clonal assays and relevant biomarkers that facilitated a molecular, rather than purely histopathologic, approach to precancer diagnosis. Careful correlation of genetically ascertained premalignant disease with histopathologic presentation and clinical cancer outcomes launched the molecular entity of EIN into a clinically relevant lesion that may be routinely diagnosed by pathologists and used for therapeutic intervention.

Somatic mutation of endometrial glandular cells is very common in normal endometrium, and occurs in advance of any discernable histopathologic changes. The term “latent precancer” has been applied to this condition to emphasize that endometrial cancer risk is not necessarily increased without additional genetic change and onset of morphologic change. This preclinical phase of disease is recognizable only through genetic analysis of mutant cells or identified by biomarkers (Fig 1), and may persist through several years of cyclical menstrual shedding. During that interval, systemic hormonal factors positively or negatively select for growth of these mutant cells. Inactivation of the PTEN gene through deletion and/or mutation and acquisition of microsatellite instability are two examples of such early genetic

Fig 1. PTEN inactivation is one of the earliest events in endometrial precancers. Loss of expression is detectable by immuno-histochemistry in scattered glands of normal-appearing proliferative endometrium (A), and carries forward to endometrial intraepithelial neoplasia (B) and carcinoma (C). In each panel, notice the absence of staining in the neoplastic glands; normal admixed glands and stroma serve as an internal positive control.

Hecht and Mutter

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events documented in otherwise normal appearing endometrium. The mutant cells in these cases participate in normal monthly endometrial regeneration, and may become widely dispersed by remodeling of the endometrial compartment. More work is needed to define the long-term behavior of latent phases of carcinogenesis.

Clinically evident premalignant lesions that can be diagnosed by a pathologist arise as localized clonal outgrowths of cytologically and architecturally altered endometrial glands that sequentially acquire additional somatic mutations leading to cancer (Fig 2). The premalignant lesions are referred to as EIN to distinguish them from the diffuse estrogen associated changes of benign endometrial hyperplasia. EIN is a histologically recognizable localized lesion composed of a clonal proliferation of glands and that usually carry one or several of the genetic abnormalities associated with endometrial carcinoma. Markers that have been informative in clonal evaluation of putative premalignant endometrial lesions include nonrandom X-chromosome inactivation (HUMARA assay) and clonal propagation of altered microsatellites. Identification of such monoclonal lesions, combined with demonstration of lineage continuity with subsequent carcinomas that occur in the same patient, provide a robust standard for molecular definition of precancers.

Comparison of the extent and range of genomic damage between premalignant EIN and malignant carcinoma phases indicates a greater cumulative mutational load in cancers, a feature that must contribute to their differing morphology and behavior. For example, while 55% of EIN lesions have demonstrable PTEN inactivating events the proportion rises to 83% in those cancers which follow an EIN diagnosis. Similarly, for those lesions with microsatellite instability, the burden of altered microsatellite alleles increases between EIN and carcinoma.

Based on similarities between the length of DNA microsatellites in adenocarcinoma and associated EIN in hysterectomy specimens, Faquin et al constructed philogenetic trees linking clonal foci of EIN to surface cancer and invasive cancer. These findings were confirmed in a larger series of hysterectomy specimens. Although it is convenient to label PTEN and microsatellite alterations as “early” events, these genetic targets may in fact be altered in the latent, premalignant, or malignant disease phases.

Accurate diagnosis of type 1 endometrial precancers has presented a major challenge to pathologists. The WHO endometrial hyperplasia schema captures many precancers in the atypical hyperplasia subgroup, but is a poorly reproducible system, which, because of its vintage, is missing diagnostic elements that have only become clear in the last 15 years. For example, the clonal origin of precancers is reflected in an initially localizing topographic distribution and a need to establish size thresholds for diagnosis, and morphometric studies have precisely quantified diagnostic architectural changes.

The application of computer based morphometry has been successful at further improving diagnostic reproducibility of precursor diagnosis. Biopsy lesions that progress to carcinoma can be discriminated from those unlikely to progress using a series of discrete measurable variables. A recent meta-analysis combining the cumulative outcome prediction experience of the D-score (Fig 3) shows that patients with a D-score less than 1 have an overall 89-fold increased cancer risk than those with a D-score more than 1. Even if one excludes concurrent cancers, those diagnosed within 12 months of EIN, cancer risk over the next two decades is 45-fold elevated.

Molecular, histologic, and clinical outcome definitions of premalignant disease have merged into a unified EIN concept. This was possible through a study which showed that those high cancer risk lesions identified through molecular analysis (monoclonal lesions with lineage continuity to subsequent carcinoma) and morphometric analysis (D-Score) are essentially identical.

In order to develop histologic criteria that could be reproducibly recognized by a practicing pathologist, a “type” collection of EIN

**Fig 2.** Initial genetic changes, including PTEN inactivation and microsatellite instability may occur in the absence of a change in histomorphology. Endometrial intraepithelial neoplasia arises through clonal expansion of the mutant cells and is characterized by altered cytology and architecture. The combination and order of additional mutations leading to invasive carcinoma differs between patients, but may include changes in K-ras and β-catenin. Nongenetic factors such as sex hormone exposures may act as positive (estrogens) or negative (progestins) selectors of mutant clones.
lesions complete with photomicrographs and associated specialized studies was created.109 Computerized morphometric analysis of these and other samples with respect to clinical follow-up have defined architectural and cytologic features of endometrial precancers; a detailed description and examples are available at www.endometrium.org. Application of these criteria has been shown to be reproducible, and reliable in predicting concurrent cancer and the presence of monoclonal growth.

Pathologists applying EIN diagnostic criteria subjectively, are able to recognize some lesions as low risk that would be scored as high risk by morphometry alone.110 While all morphometrically low-risk endometria (D-score > 1) are consistently recognized by pathologists as non-EIN, morphometrically high-risk endometria (D-score < 1), comprise an admixture of subjectively benign endometria and EIN. Pathologists are able to recognize a subset of endometria such as normal secretory endometrium as low risk, whereas these benign patterns demonstrate morphometric characteristics which mimic those seen in EIN. Cases that progress to adenocarcinoma are mutually recognized as high risk by both D-score (D-score < 1) and subjective (EIN) classification, emphasizing the need for the human element in diagnosis to ensure diagnostic specificity.

For the most part, the dichotomous classification of endometrial cancers holds up to genetic analysis and the histologic subtypes are underscored by systematic changes in a limited set of genes. Combined molecular, histomorphometric, and clinical outcome analysis of premalignant lesions, has provided a clearer multidisciplinary definition of endometrial precancers, known as EIN. Genetic and endocrine disease mechanisms have been integrated into a multistep model for oncogenesis in which hormonal exposures act as selection factors for mutated endometrial cells. Of particular interest is the newly defined preclinical phase of “latent” precancers in which mutant cells capable of responding to the modulating influences of hormones have been shown to commonly occur in “normal” endometrium. This is a potentially exciting target for preventive therapy.

Although the model in Figure 2 is appealing, it is incomplete. We have described the role of several candidate genes active in this progression scenario, but not all genes are effective in each case. Clearly, there are other primary events that drive progression. These could be abnormalities of genes elsewhere in the regulatory pathways represented by the exemplars described here, or perhaps entirely new pathways that have yet to be discovered. Finally, the heterogeneity of endometrioid cancers has not been fully explained.
Molecular Biology of Endometrial Cancer

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GLOSSARY

**β-catenin**: Originally identified as a component of cell-cell adhesion complexes composed of cadherins and actin, β-catenin has now been shown to be a downstream signaling molecule in the Wnt signaling pathway.

**EIN (endometrial intraepithelial neoplasia)**: EIN is a premalignant lesion of the endometrium composed of a localized monoclonal population of genetically altered glands. The presence of EIN on biopsy indicates a 30% chance of concurrent type I (endometrioid) endometrial cancer and a 45 fold increased risk for developing cancer 1 or more years later.

**K-ras**: The gene that encodes K-Ras, a protein that is a member of the small GTPase superfamily, in which a single amino acid substitution results in an activating mutation. Alternative splicing gives rise to variants encoding two isoforms that differ in the C-terminal region.

**LOH (loss of heterozygosity)**: A situation where one chromosome has a normal allele of a gene and one chromosome has a mutant or deleted allele.

**Mismatch repair**: One of four major pathways of DNA repair in mammalian cells. Mismatch repair recognizes and corrects errors in DNA replication leading to single base-pair mismatches or insertions/deletions in small repetitive tracts of DNA known as microsatellites.

**MSI (microsatellite instability)**: Microsatellites are repeating units of 1-4 DNA base pairs that are distributed widely throughout the genome and have a high degree of repeat length variation in the population. Their length remains stable with cell division and inheritance so they may be used as molecular markers of cell lineage, in population genetic studies or paternity testing. Defects in the genes involved in DNA mismatch repair result in genomic instability that may be detected as MSI, an alteration in the length of the microsatellites from cell to cell.

**Non-random X-chromosome inactivation (HUMARA assay)**: Tumors arise from a single genetically altered cell. Consequently, detection of monoclonality, a genetic similarity among the cells in a tumor mass, can be used to distinguish neoplastic from polyclonal or hyperplastic lesions. Clonality can be established based on X-chromosome inactivation analysis. During early embryonic development of mammalian female embryos, one of the two X-chromosomes in each cell is randomly deactivated by the methylation of deoxycytosine residues. Once established, genetic imprinting retains X-chromosome inactivation through all subsequent cell divisions. The cells in a clonal tumor all show inactivation of the same copy of the X-chromosome. One common assay detects this inactivation by examining inactivation of the human androgen receptor (HUMARA) on the X-chromosome.

**Null**: Complete loss of function phenotype. This term is used in reference to the mutation or inactivation of specific genes, resulting in complete absence of protein expression.

**p53**: p53 is a tumor suppressor gene. The normal function of p53 is to act as a transcriptional activator of genes with a p53-binding site and an inhibitor of genes lacking a p53 binding site. Expression of high levels of wild-type p53 is associated with cell cycle arrest and apoptosis through induction of P21Waf1/Cip1 and hMdm2. Mutations in p53 are seen in many tumors as well as in the Li-Fraumeni–inherited cancer syndrome.

**PTEN (phosphatase and tensin homolog)**: PTEN is a tumor suppressor gene with a gamut of regulatory activities. The gene product is a multifunctional molecule. The predominant activity identified for PTEN is its lipid phosphatase activity that converts inositol trisphosphates into inositol bisphosphates, thus inhibiting survival and proliferative pathways that are activated by inositol trisphosphates. PTEN acts to maintain arrest in the G1 phase of the cell cycle and enable apoptosis through an AKT-dependent mechanism.