

MOLECULAR PATHOLOGY OF ENDOMETRIAL HYPERPLASIA AND CARCINOMA

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In the Western World, endometrial cancer is the most common malignant tumor of the female genital tract. After an increase in the 70s that resulted from the unrestricted use of estrogen replacement therapy in postmenopausal women, the incidence rates became stable over the last two decades (10-20 per 100,000 person-years). Recently, the progressive use of tamoxifen - a non-steroidal estrogen agonist and antagonist - for the treatment of breast cancer has been associated with increased risk of endometrial cancer but there is not complete agreement among different studies. In this review, current knowledge about molecular pathology of endometrial carcinomas and their precursors will be presented.

TWO TYPES OF ENDOMETRIAL CARCINOMA

Over the past two decades, the tendency has been to classify endometrial carcinoma (EC)¹ into two different types: **Type I** tumors (about 80%) are **endometrioid carcinomas**, often preceded by complex and atypical hyperplasia and associated with estrogenic stimulation. They occur predominantly in pre or perimenopausal women and are associated with obesity, hyperlipidemia, anovulation, infertility, and late menopause. Typically, most endometrioid carcinomas are limited to the uterus and follow a favorable course. In contrast, **type II** tumors (about 10%) are **non-endometrioid** (largely papillary serous) **carcinomas**, arising occasionally in endometrial polyps or from precancerous lesions that develop in atrophic endometria (endometrial "intraepithelial" carcinoma)². Type II tumors are not associated with estrogen stimulation or hyperplasia, readily invade the myometrium and vascular spaces, and carry a high mortality rate. It has also been found that the molecular alterations involved in the development of endometrioid (type I) carcinomas are different from those of the non-endometrioid (type II) carcinomas³⁻⁴ (Table 1).

MOLECULAR PATHOLOGY

A dualistic model of endometrial carcinogenesis has been proposed.¹⁻³ According to this model, normal endometrial cells would transform into endometrioid carcinomas (EEC) through replication errors, so-called "microsatellite instability" (MI), and subsequent accumulation of mutations in oncogenes and tumor suppressor genes, whereas alterations of p53⁵ and loss of heterozygosity (LOH) on several chromosomes would drive the process of neoplastic transformation into the acquisition of a non-endometrioid carcinoma (NEEC) phenotype^{4, 5}.

There are several evidences in favor of this pathogenetic proposal; the majority of low grade EEC express estrogen receptors but not p53, and 25 to 30% of them exhibit MI. In contrast, most NEEC are negative or only weakly immunoreactive for estrogen receptors, strongly positive for p53 immunostaining, and do not exhibit MI⁶.

Although the dualistic model appears applicable to paradigmatic cases at both clinicopathological and molecular levels, exceptions occur. After all, there is a great overlapping in the clinical, pathological, immunohistochemical and molecular characteristics of the tumors. For instance, it has been shown that occasionally, NEEC may develop from preexisting EEC as a result of tumor progression. Obviously, these tumors may share the pathologic and molecular features of types I and II EC⁴.

Molecular alterations of endometrioid carcinomas of the endometrium (EEC)

Four main molecular alterations have been described in EEC: MI (25-30% of the cases)^{4, 7, 8}, PTEN mutations (37-61%)⁹⁻¹⁴, k-RAS mutations (10-30%)¹⁵⁻¹⁹, and beta-catenin mutations with nuclear protein accumulation (25-38%)²⁰. Although MI and PTEN or kRAS mutations may coexist in many cases, these three molecular alterations are not usually associated with beta-catenin abnormalities (Fig.1).

Microsatellite instability (MI)

MI was initially noted in colorectal cancers of patients with the hereditary non-polyposis colon cancer syndrome (HNPCC), but also in some sporadic colon cancers. EC is the second most common tumor found in HNPCC patients. MI has been demonstrated in 75% of EC associated with HNPCC, but also in 25-30% of sporadic EC^{4, 7, 8}.

MI occurs more frequently in EEC than in NEEC. To give further support to the hypothetical dualistic model of endometrial carcinogenesis, we investigated the presence of MI in 42 sporadic EC⁴. The results of our study supported the concept that MI is a common genetic abnormality in EC (28%), and appears to be more frequent in EEC (33%) than in NEEC (11%). However, the occasional detection of MI in NEEC, the lack of an inverse correlation between p53 and MI, and the frequent existence of tumors exhibiting mixed pathologic, immunohistochemical and molecular features of EEC and NEEC, indicate that individual tumors do not invariably follow the so-called dualistic model of endometrial carcinogenesis⁴.

Molecular consequences of MI in endometrioid carcinomas

It has been demonstrated that cancers showing MI, the so-called mutator phenotype, have mismatch repair deficiencies that result in the accumulations of mutations in repeated sequences in the coding mononucleotide repeats of some particular oncogenes and tumor suppressor genes. Transforming growth factor beta receptor type II (TGF- β RII), BAX, insulin-like growth factor II receptor (IGF RII), hMSH3, and hMSH6 are all putative targets of such phenomenon. These mutations are interpreted as secondary events in the mutator phenotype pathway in cancers with MI²¹⁻²⁵.

Our results indicate that frameshift mutations occurring at coding mononucleotide repeats in these putative target genes are quite frequent in MI positive EC; mutations in one or more of these microsatellites were detected in 16 of the 24 tumors (66.6%)²⁵. An interesting result of our study was the fact that the mutations were heterogeneously distributed in the tumors; they were found in some tumor areas but not in others. The heterogeneous distribution of the mutations suggests that they may be involved in tumor progression. The advantage of growth provided by each specific combination of

mutations in a particular area of the neoplasia could lead to its overgrowth in comparison with other tumor subclones.

In a previous report, we suggested that BAX frameshift mutations could play an important role in the progression of EC with MI²⁴. This speculation was based on the hypothesis that the presence of inactivating BAX mutations in tumors would explain the low frequency of p53 mutations in the neoplasias associated with MI, by relieving the selective pressure for p53 mutations during tumor progression. In the presence of BAX mutations, p53 mutations would not be necessary to inhibit BAX transactivation. To give further support to such hypothesis, we compared the pattern of mutations in the primary EC and their lymph node metastases²⁵. Interestingly, in two cases BAX mutations were found in the primary EC but not in their lymph node metastases, suggesting that the tumor subclones that exhibited BAX mutations were not responsible for the dissemination of the neoplasm. In contrast, IGF1R frameshift mutations were detected in three metastatic tumors, but only one of them also had the mutation in the corresponding primary neoplasm²⁵. The frequent finding of these mutations in the metastatic tumors gives support to the hypothesis that IGF1R mutations are related to tumor progression in EC with MI²⁵.

MI is secondary to DNA altered methylation

As mentioned above, MI was initially found in colorectal carcinomas from patients with the hereditary non-polyposis colon cancer, but also in some sporadic colon cancers. In these patients, germline and somatic mutations in the MSH2 and MLH1 genes have been detected in chromosomes 2p and 3p²⁶. However, the frequency of mismatch repair genes mutations in sporadic colonic, gastric or endometrial carcinomas with MI is very low, which suggests that other mechanisms of gene inactivation must be involved²⁷.

It has recently been described that MLH1 promoter hypermethylation may lead to loss of MLH1 expression and subsequent development of MI in EC. We have recently detected MLH-1 promoter hypermethylation in 11 of 12 of EC with MI (91%), but in none of the MI negative tumors. On the other hand, MLH-1 promoter hypermethylation was detected in 8 of 116 (7%) cases of endometrial hyperplasia, and it was almost exclusively restricted to atypical hyperplasias with coexisting carcinomas²⁸. These data suggest that hypermethylation of MLH-1 may be an early event in the pathogenesis of EEC, that precedes the development of MI²⁸.

The identification of CpG island methylation in several genes (p16, TSP-1, IGF-2, HIC-1 and MLH-1) in tumors with MI suggests that altered methylation may be a preliminary alteration in the development of the microsatellite mutator phenotype.

PTEN mutations

The tumor suppressor gene designated PTEN (phosphatase and tensin homologue deleted from chromosome 10), also called MMAC1 (mutated in multiple advanced cancers) is located on chromosome 10q23.3^{29, 30}. It is reasonable to think that the genes encoding protein phosphatases, like PTEN, act as tumor suppressor genes, since their proteins may counteract the effect of the proteins encoded for the protein kinase group of protooncogenes.

LOH at chromosome 10q23 occurs in 40% of EC. Somatic PTEN mutations are also common in EC and are almost exclusively restricted to EEC, occurring in 37-61% of the cases⁹⁻¹⁴. Several groups of investigators have found a concordance between MI status and PTEN mutations; the mutations occur in 60-86% of MI positive EEC, but in only 24-35% of the MI negative tumors. Such results have lead to the speculation that PTEN could be a likely candidate to be target for mutations in the MI positive EC. Recently, PTEN mutations have been detected in endometrial hyperplasias with and without atypia (19% and 21% respectively), both currently regarded as precursor lesions of EEC. Moreover, identical PTEN mutations have been detected in coexisting hyperplasias and MI positive EEC, which suggests that PTEN mutations are early events in the development of EEC^{13, 14}.

In our recent study, PTEN mutations were detected in 18 of 38 tumors (47.3%); which falls into the range of previous studies (32-55%). PTEN mutations were more frequently found in EEC (51.5%) than in NEEC (20%). Moreover, PTEN mutations were detected more commonly in MI positive tumors (66.6%), than in MI negative neoplasms (34.8%)¹³.

Interestingly, PTEN mutations were detected in short coding mononucleotide repeats (A)5 and (A)6 in 4 of the 10 (40%) EC with MI¹⁴. Such frameshift mutations may have the same significance than the mutations that occur in BAX, TGF- β RII, IGFIIR, MSH3, or MSH6. We could then hypothesize that PTEN (A)5 and (A)6 mutations may be secondary to deficiencies in mismatch repair that lead to the development of MI¹³ and so, explaining the high frequency of PTEN. In fact, mutations at the (A)5 and (A)6 short mononucleotide tracts have been previously detected in EC with MI.

k-RAS mutations

k-RAS mutations have been identified in 10-30% of EC compared to approximately 40-50% of colon carcinomas¹⁵⁻¹⁹. Although some authors have failed to demonstrate a correlation between k-RAS mutations and stage, grade, depth of invasion, age or clinical outcome in EC, others have described associations between k-RAS mutations and the presence of coexistent EH, lymph node metastases, and clinical outcome in postmenopausal patients above 60 years. Also, some authors have reported an almost complete absence of k-RAS mutations in papillary serous and clear cell carcinomas of the endometrium.

In our series of 58 EC, k-ras mutations occurred in 11 (18.9%) carcinomas, all of them EEC¹⁹. We found a higher frequency of k-RAS mutations in MI positive carcinomas (6/14, 42.8%) than in MI negative tumors (5/44, 11.3%), what seems to indicate that, at least in our series, kRAS mutations are common in EC with the microsatellite mutator phenotype¹⁹.

The evidence that altered methylation in several genes occurs in MI positive carcinomas has lead to the hypothesis that MI is just a secondary alteration triggered by an abnormal hypermethylation of hMLH-1. The finding of methylation-related GC \rightarrow AT transitions in EC with MI, and their low frequency in MI- tumors, provides some basis to explain the occurrence of kRAS mutations in MI positive EEC¹⁹.

Beta-catenin alterations

β -catenin, a component of the cadherin-mediated cell adhesion system, plays an important role in the Wnt signaling pathway. β -catenin functions as a downstream transcriptional activator by forming a

complex with transduction factors, such as the leukocyte enhancing factor (LEF) and T-cell factor (TCF). The adenomatous polyposis coli (APC) protein regulates β -catenin levels by cooperating with glycogen synthase kinase-3 β (GSK-3 β) via phosphorylation of serine/threonine residues coded on exon 3 of the β -catenin gene (*CTNNB1*) and followed by degradation of β -catenin through the ubiquitin-proteasome pathway.

Activation of the APC/ β -catenin/Tcf pathway has been implicated in several human neoplasias including endometrial and ovarian carcinomas with an endometrioid phenotype. Mutations in *CTNNB1* and others genes involved in the same pathway are associated with abnormal nuclear β -catenin accumulation. The incidence of *CTNNB1* mutations in EEC ranges from 25-38%. Several groups have described mutations of *CTNNB1* gene with intranuclear accumulation of β -catenin in a significant number of endometrial cancer cases, without an evident relationship with MI or *PTEN* mutations^{20,31,32}. The mutations are evenly distributed through different areas of the tumors, which suggest that they play a role in the early steps of endometrial tumorigenesis.

CTNNB1 mutations are located within the glycogen synthase kinase-3 β consensus site of exon 3 (Ser, and Thr) together with other residues which probably alter recognition sequences or tertiary protein structure and inhibiting its phosphorylation. However, the presence of nuclear β -catenin immunostaining in carcinomas which did not show *CTNNB1* mutations suggests that there may be alterations in others genes which contribute to up-regulation of β -catenin/Tcf-associated transcriptional activity. Increased β -catenin expression caused by inactivating mutations in the GSK-3 β phosphorylation sites of β -catenin or in APC results in the accumulation of β -catenin in the nucleus and the uncontrolled activation of target gene expression such as matrix metalloproteinase-7 (MMP-7), cyclin D1, Connexin 43, ITF2, c-myc, PPAR- δ which may have an important role in tumor progression^{33,34}.

Summary and prospectives (Fig.2)

Putting everything together, we may hypothesize that altered methylation is an initial alteration in EEC. MLH-1 promoter hypermethylation causes mismatch repair deficiencies that produce the phenomenon of MI, and a stepwise progressive process of accumulation of mutations at coding mononucleotide repeat microsatellites in some particular oncogenes and tumor suppressor genes such as BAX, IGFIIR, MSH3, or MSH6. PTEN mutations would occur early in the process of the acquisition of the fully developed microsatellite mutator phenotype. The frequent occurrence of frameshift mutations at PTEN (A)5 and (A)6 would provide some basis to explain the close association between PTEN and MI. On the other hand, the detection of PTEN mutations in a significant percentage of MI negative EC, may also indicate that PTEN may participate in the process of endometrial carcinogenesis by following molecular pathways independent from the microsatellite mutator phenotype. The frequent occurrence of methylation-related transitions in k-RAS also provides some basis to explain the common coexistence of k-RAS mutations and MI. Finally, beta-catenin seems to play a role in EEC independently from MI.

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TABLE 1: The two types of Endometrial Carcinoma

	Type I	Type II
Age	Pre- and Perimenopausal	Postmenopausal
Unopposed Estrogen	Present	Absent
Hyperplasia-Precursor	Present	Absent
Grade	Low	High
Myometrial Invasion	Minimal	Deep
Histologic Type	Endometrioid	Non-Endometrioid
Behavior	Stable	Progressive
Genetic alterations	PTEN, MI, β -catenin	P53 mutations, LOH

Modified from Bockman¹

Endometrioid Carcinoma

Molecular Alterations

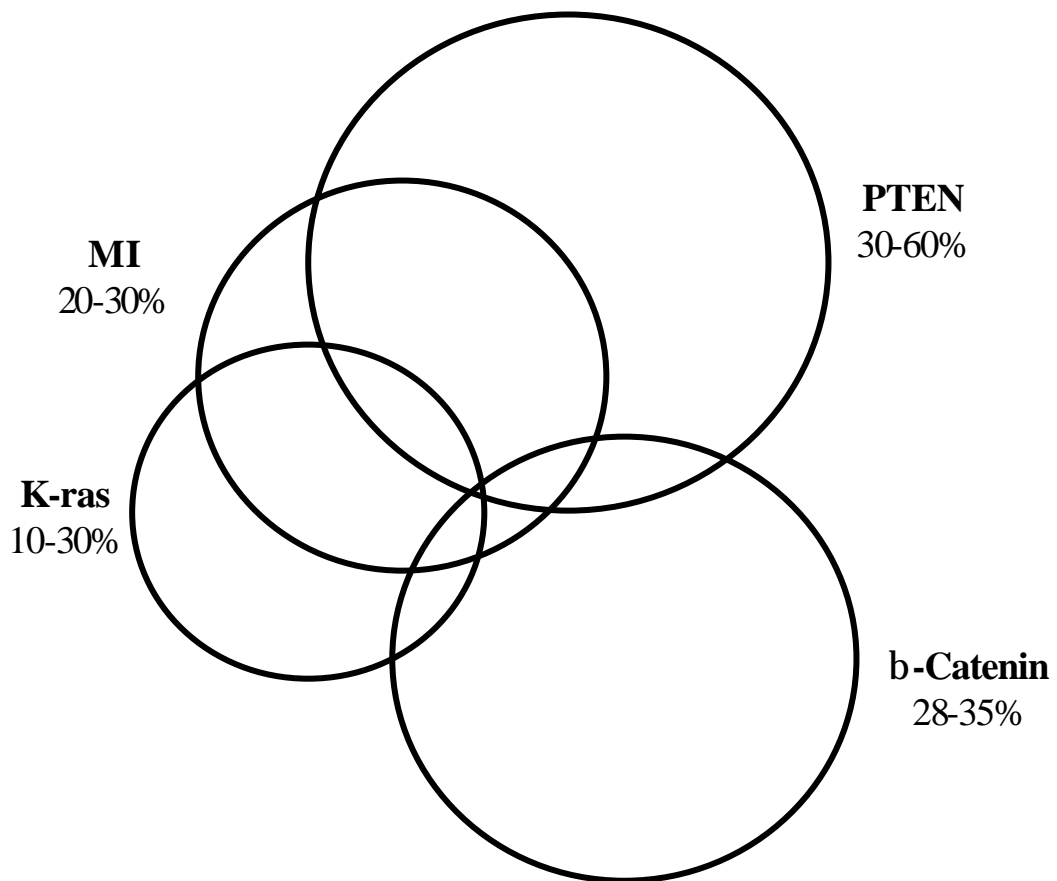


Fig. 1

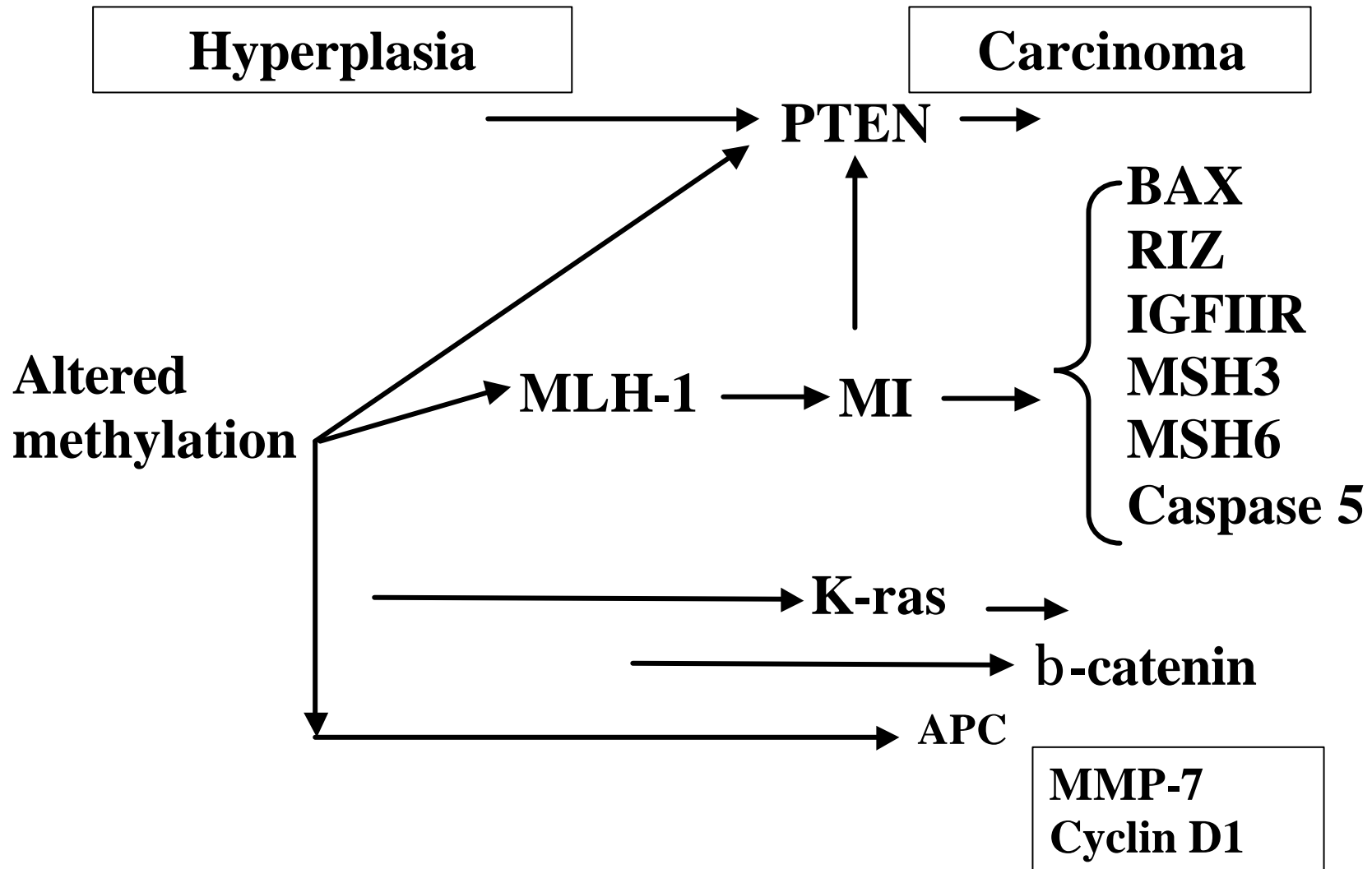


Fig. 2: From Hyperplasia to Endometrioid Carcinoma. Pathogenetic proposal