Novel molecular profiles of endometrial cancer—new light through old windows

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Abstract

Endometrial carcinoma (EC) is the most common gynecological malignancy in the western world. A widely accepted dualistic model, which has been established on a morphological basis, differentiates EC into two broad categories: Type I oestrogen-dependent adenocarcinoma with an endometrioid morphology and Type II non-oestrogen-dependent EC with a serous papillary or clear cell morphology.

Molecular genetic evidence indicates that endometrial carcinoma, as described in other malignancies, likely develops as the result of a stepwise accumulation of alterations in cellular regulatory pathways, such as oncogene activation and tumor suppressor gene inactivation, which lead to dysfunctional cell growth. These molecular alterations appear to be specific in Type I and Type II cancers.

In type I endometrioid endometrial cancer, PTEN gene silencing in conjunction with defects in DNA mismatch repair genes, as evidenced by the microsatellite instability phenotype, or mutations in the K-ras and/or β-catenin genes, are recognized major alterations, which define the progression of the normal endometrium to hyperplasia, to endometrial intraepithelial neoplasia, and then on to carcinoma. In contrast, Type II cancers show mutations of TP53 and Her-2/neu and seem to arise from a background of atrophic endometrium.

Nevertheless, despite the great effort made to establish a molecularly-based histological classification, the following issues must still be clarified: what triggers the tumor cells to invade the myometrium and what causes vascular or lymphatic dissemination, finally culminating in metastasis? RUNX1, a transcription factor, was recently identified as one of the most highly over-expressed genes in a microarray study of invasive endometrial carcinoma. Another candidate gene, which may be associated with an initial switch to myometrial infiltration, is the transcription factor ETV5/ERM. These studies, as well as those conducted for other genes possibly involved in the mitotic checkpoint as a major mechanism of carcinogenesis in non-endometrioid endometrial cancer, could help in understanding the differences in the biology and the clinical outcome among histological types.

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1. Introduction

Endometrial cancer (EC) is the most common gynecological malignancy encountered in western countries; the incidence is estimated at 15–20 per 100.000 women per year. Ninety percent of cases are sporadic, while the remaining 10% arise from a genetic background.
Based on clinico-pathological and molecular characteristics, our current understanding of the biology of uterine malignancies permits their dichotomy along two distinct pathways [1].

The first is the Type-I or oestrogen-dependent endometrioid endometrial carcinomas (EECs) (Fig. 1a), representing the majority of cases of sporadic endometrial cancer (approximately 80%) that arise in relatively younger pre- and post-menopausal women. They express oestrogen (ER) and progesterone (PR) receptors [2] and have a strong etiological association with either endogenous or exogenous, unopposed oestrogen exposure [3]. They usually are low grade with an endometrioid morphology (with variants in all pathways of Mullerian differentiation), and they are characterized by a favorable prognosis.

The second group, the Type II or non-endometrioid endometrial carcinomas (NEECs), is comprised of the high-grade papillary serous and clear cell carcinomas (Fig. 1b and c). These arise in relatively older women and are not usually preceded by a history of unopposed estrogen exposure, but rather from a background of atrophic endometrium. The latter tumors have an aggressive clinical course, a greater propensity for early spreading, and a worse prognosis than the more common endometrioid adenocarcinomas [4].

There is both epidemiological and molecular evidence to suggest that endometrial hyperplasia represents a true precursor lesion for endometrioid adenocarcinomas, with complex hyperplasia relying on a histological and genetic continuum between simple hyperplasia and carcinoma, whereas NEECs are frequently associated with endometrial intraepithelial carcinoma.

2. Molecular genetics associated with endometrioid endometrial carcinoma

2.1. DNA-mismatch repair genes and microsatellite instability

The progressive accumulation of alterations at microsatellite loci, so-called microsatellite instability (MSI), in important regulatory genes may promote carcinogenesis and is known to play an important role in sporadic colon cancers and in several non-colonic tumors. MSI is found in 17–25% of sporadic Type I ECs [5], but is rarely present in Type II tumors [6,7].

Abnormalities of DNA-mismatch repair genes were first detected in tumors of patients with hereditary non-polyposis colorectal carcinoma (HNPCC), where MSI arose from germ-line and somatic mutations in one of four DNA-mismatch repair genes. Most commonly, those genes are hMLH-1 and hMSH-2, which encode enzymes that are responsible for repairing nucleotide miscalts, insertions or deletions produced during DNA replication [8–10]. While a few germ-line mutations of hMLH-2 and hMLH6 have been described in EC [11,12], most mismatch repair deficiencies underlying MSI in EC seem to involve an epigenic inactivation or silencing mechanism, rather than mutations in DNA repair genes, as these are encountered with low frequency in sporadic cases of EC. Indeed, inactivation of MLH1 by the hypermethylation of normally unmethylated CpG islands in the promoter has been described as the most common cause of MSI in sporadic endometrioid carcinomas [13,14]. Similar hypermethylation has also been demonstrated in promoters of the APC and MGMT genes associated with Type I tumors. Microsatellite instability is an uncommon feature in high-grade endometrial carcinomas [15]. Other commonly implicated genes include the PTEN/MMAC1 tumor suppressor gene on chromosome 10q23, the inactivation of which is found in up to 50% of all endometrial cancers, and at an even higher frequency if the subset of tumors with microsatellite instability is considered.

2.2. Oncogenes

The K-ras gene encodes a small inner plasma, cellular membrane GTPase, which functions as a molecular switch during cell signaling and which is largely related to tumor growth and differentiation. Mutations of K-ras have been identified in 19–46% of ECs, with most of these cases involving point mutations at codon 12 [16].
Alterations of K-ras predominantly involve Type I tumors and have been reported in 10–30% of cases of endometrioid carcinoma, whereas they are almost absent in papillary serous and clear-cell carcinomas [17]. Constitutive activating mutations in K-ras have also been found to be more frequent in MSI-positive tumors [17], suggesting that both events may occur simultaneously before clonal expansion [18]. Mutations are also detected in endometrial hyperplasia at a similar rate to that observed in EC, suggesting that mutations in the ras gene may represent an early event in tumorigenesis within a subset of Type I ECs [19].

Studies have shown that the frequency of K-ras mutations rises progressively from simple through to complex hyperplasia and to carcinoma, and the presence of K-ras mutations in pre-malignant biopsy samples has been suggested as a marker of progression to malignancy. K-ras mutations have been shown to be associated with an adverse prognosis, independent of age or stage [20,21].

2.3. HER2/neu

The HER2/neu (also called erbB2) gene encodes a 185-kDa transmembrane receptor, tyrosine kinase, which is similar to the epidermal growth factor receptor (EGF-R). HER2/neu functions as a preferred partner for heterodimerization with members of the EGF-R family and, therefore, plays an important role in coordinating the complex ErbB signaling network that is responsible for regulating cell growth and differentiation [22].

The over-expression of Her-2/neu oncoprotein has been reported in 9–30% of all endometrial adenocarcinomas, and has been associated in some studies with an adverse prognostic outcome and has been linked to decreased overall survival [23,24].

2.4. Other oncogenes

The involvement of the above-mentioned oncogenes has been well established in EC. However, many additional proto-oncogenes are currently under investigation for their possible involvement in EC. C-myc, survivin, RUNX1, ETV5, and human telomerase reverse transcriptase (hTERT) have all been described in association with EC. c-myc amplification and over-expression is present in approximately 3–19% of ECs, and despite conflicting reports of links to tumor differentiation, myometrial invasion and lymph node metastases, a recent report has shown nuclear and cytoplasmic c-myc immunohistochemical staining to be an independent prognostic factor in EC [23,25,26].

The over-expression of hTERT is involved in EC development, particularly in conjunction with tamoxifen, by causing the activation of telomerase and the subsequent telomere maintenance and potential cell immortalization; however, the regulation of hTERT is known to be partly dependent on gene products previously linked to EC (e.g., c-myc). Further studies are needed to establish its exact role [27]. The same goes for the inhibitor of the apoptosis protein survivin, whose over-expression has been associated with a higher clinical grade and stage, but whose definitive role in tumorigenesis has yet to be established [28]. None of these three oncogenes has been studied in relation to specific prevalence in type I or type II tumors.

ETV5/ERM (Ets-related protein) is a transcription factor of the ETS family and a divergent member of the winged helix-turn-helix super-family. ETV5 binds to sequences containing the consensus pentanucleotide 5′-CGGA(AT)-3′. ETV5 is a proto-oncogene that plays a role in the progression of breast cancer, functions as an adaptor molecule in the interactions of adhesion receptors and intracellular tyrosine kinases, and is required for spermatogonial stem cell self-renewal [29,30].

ETV5 has been proposed to play a role during the early events of endometrial tumorigenesis and could be associated with an initial switch to myometrial infiltration. Furthermore, tissue array immunohistochemistry has shown that this up-regulation correlated with the process of tumorigenesis from normal atrophic endometrium to simple and complex hyperplasia and then, on to carcinoma [31,32].

RUNX1/AML1 (runt-related transcription factor 1/acute myeloid leukemia 1) belongs to the RUNX gene family of transcription factors that bind DNA as components of the core-binding factor (CBF) complex, in partnership with the CBβ cofactor. This complex activates and represses the transcription of key regulators of the growth, survival and differentiation pathways.

The RUNX genes have been found to function as both tumor suppressors and dominant oncogenes in a context-dependent manner. The RUNX genes are closely related and are essential for hematopoesis, osteogenesis and neurogenesis, but are also important for other developmental processes [34].

The possibility that RUNX1 over-expression plays an oncogenic role outside the hematopoietic system has been indicated by its recent identification as one of the most highly over-expressed genes in a microarray study of invasive endometrial carcinoma [31]. A strong positive correlation between RUNX1/AML1 and p21WAF1/CIP1 was found, especially in stage IC carcinomas, which infiltrated more than 50% of the myometrium. Thus, it could be hypothesized that p21WAF1/CIP1, a target of p53-mediated growth arrest, and RUNX1/AML1 interact during the initial steps of tumor dissemination in EEC through TGFβ/SMAD-mediated myometrial infiltration and/or through a shift in the balance of the cell growth/cell differentiation toward invasive, rather than proliferative, phenotypes [35].

2.5. Tumor suppressor genes

The PTEN (phosphatase and tensin homolog deleted on chromosome ten) gene is a tumor suppressor gene localized...
on chromosome 10 (10q23-24) whose name derives from its preserved tyrosine phosphatase domain and its sequence homology with the matrix protein tensin [36].

The PTEN gene encodes a 403 amino acid protein, which acts as a lipid phosphatase that helps to modulate cell signal transduction pathways by acting on phospholipid phosphatidylinositol-(3,4,5)-triphosphate (PIP3), a second messenger produced after growth factors bind to cell surface membrane receptors. Decreased activity or loss-of-function mutations of PTEN and constitutive activation lead to constitutive activation of multiple signaling pathways, including the PI3K/Akt pathway, which affects cell proliferation, apoptosis and migration [37,38].

PTEN has also been shown to control p53 protein levels and transcriptional activity [39]. A number of tumors (e.g., glioblastoma multiforme, prostate carcinoma, etc.) have been shown to have acquire abnormalities of the PTEN gene. Up to 80% of cases of endometrioid carcinoma reveal a loss of expression, mainly due to mutations [40], and to a lesser extent, to a loss of heterozygosity (LOH) [41].

Interestingly, higher rates of mutations in the PTEN gene have been described to occur in MSI tumors (60–86%) compared to tumors without MSI (24–35%). This suggests that PTEN could be a target for mutations in a deficient DNA repair context [42]. They are also well documented in endometrial hyperplasia with and without atypia [43,44]. Given the role of endometrial hyperplasia as the putative precursor of Type I tumors, PTEN mutations are presumed to play an early role, although probably not a determining step, in tumorogenesis. Recent studies have documented a case of synchronous PTEN-positive/MSI-negative hyperplasia and PTEN-positive/MSI-negative carcinoma, and they have proposed that PTEN mutations could precede microsatellite instability; however, this remains controversial [43,45].

The association of PTEN inactivation with MSI and methylation and the low expression of MLH1 seem to lay the foundations for the initial steps towards endometrioid endometrial tumorogenesis [46]. Moreover, endometrial pre-cancers (e.g., endometrial intraepithelial neoplasia) have been postulated to share common genetic alterations with EEC, including PTEN mutations and MSI.

The catenins are a family of structurally related cytoplasmic proteins, which have been classified as alpha (α), beta (β), and gamma (γ), according to their electrophoretic mobility. The β-catenin gene is located on chromosome 3p21 and encodes an 88 kDa protein [47,48].

β-Catenin is a multi-functional protein that plays an essential role in the E-cadherin-mediated anchoring and organization of the cytoskeleton and acts as a downstream transcriptional activator in the Wnt signaling transduction pathway. Cellular β-catenin levels are tightly regulated by a multi-protein complex comprised of serine/threonine kinase GSK3β, the APC (adenomatous polyposis coli) tumor suppressor gene product, and axin, which facilitates phosphorylation and subsequent degradation of the β-catenin protein [49]. In the latter role, β-catenin has been implicated in a wide variety of malignancies including those of the endometrium [50]. Excessive β-catenin protein is usually removed rapidly from the cytoplasmic pool by the ubiquitin-proteasome pathway (so-called ubiquitination). However, the mutated gene product resists degradation and can accumulate in the cytoplasm and the cell nucleus with constitutive target gene activity.

Abnormalities of tumor suppressor genes, such as the APC gene or mutations in β-catenin, have been shown to cause dysregulation of β-catenin degradation, leading to a cytoplasmic accumulation of the protein, which is then followed by translocation to the nucleus. The accumulation of β-catenin has been demonstrated by immunohistochemistry, and several studies have analyzed series of EC for nuclear accumulation, which has been found to be significantly more common in tumors of endometrioid morphology (31–47%) than in the non-endometrioid forms (0–3%); [51,52]. It has been suggested that the discordance of gene mutation and the nuclear accumulation rates could be attributable to abnormalities in other Wnt proteins (e.g., APC, γ-catenin), but the function of β-catenin in endometrioid tumorogenesis remains unclear. No correlations to MSI, K-Ras or PTEN mutations have been found, suggesting that the Wnt pathway could play an independent role in endometrial cancer [53]. Because nuclear accumulation has also been demonstrated in atypical hyperplasia, it has been suggested that β-catenin abnormalities could arise relatively early in the development of Type I EC [54,55].

The TP53 tumor suppressor gene located on chromosome 17 codes for a nuclear protein, which plays an important role in preventing the propagation of cells with damaged DNA. After DNA damage, nuclear p53 accumulates and, through p21, causes cell cycle arrest by inhibiting cyclin-D1 phosphorylation of the Rb gene and by promoting apoptosis [56]. Abnormalities of TP53 have been well described in various malignancies, and germ-line mutations are associated with the Li-Fraumeni syndrome. The mutant p53 protein is non-functional but resists degradation, and it accumulates in the cell, thereby acting as a dominant negative inhibitor of the wild-type p53. The accumulated mutant protein can be demonstrated immunohistochemically. Mutations in the p53 gene are a frequent and characteristic finding in Type II serous tumors with positive immunohistochemistry reported in 71–85% of tumors [57,58]. It has been shown that p53 abrogation occurs relatively early in the evolution of these tumors, and over-expression is present in 75% of endometrial intraepithelial carcinomas (EICs), which is considered to be the putative precursor lesions of papillary serous cancers [59,60]. In contrast, p53 is mutated in only about a third of endometrioid adenocarcinomas [61,62]. When stratified by histological grade, grades 1 and 2 tumors show positive staining at much lower rates than high grade (grade 3) tumors. Only a minor proportion of endometriai...
hyperplasias, with complex hyperplasias show positive staining, demonstrating a higher frequency of abnormalities than simple hyperplasias. This suggests, in contrast to serous tumors, that p53 occurs as a late molecular event in Type I tumors [17].

2.6. Steroid receptor genes

Oestrogen receptors ERα, ERβ and progesterone receptors (PR-A, PR-B) belong to the steroid/thyroid hormone nuclear receptor super-family. They are ligand-activated transcription factors involved in hormone-mediated signaling, hormone-mediated inhibition of gene expression, and cellular proliferation and differentiation in various target tissues. They are translocated from the plasma membrane to the nucleus and are mediated by MAP kinase activation. They bind as a homo- or heterodimer with ESR2 to oestrogen-responsive elements (ERE), or they interact with other transcription factors such as AP-1 or NF-κB [63].

The predominant subtype in the uterus is ERα. Increased cell proliferation and exaggerated response to oestrogen in ERβ was found in an ERα knockout model, suggesting that ERβ could play a role in modulating ERα function and that it consequently could have an anti-proliferative function [59,60]. An imbalance in ERα and ERβ expression, therefore, could signify the crucial, critical step in oestrogen-dependent tumorigenesis.

ERα mRNA and protein expression are reported to decrease in stages from normal or grade I to grade 3 tumor lesions. In contrast, ERβ expression is not altered, suggesting a shift to a decreased ERα/ERβ ratio [64,65]. The significance of the relative expression of both ER subtypes in EC remains to be clarified.

Variant proteins originating from transcriptional splicing errors have been described for ERα and ERβ. For example, an ERα exon 5 splice variant (Δ5 ERα) has not been detected in normal endometrium but has been found at significantly increased levels in endometrial carcinomas, when compared with endometrial hyperplasias [66]. Δ5 ERα has been shown to be able to constitutively activate the transcription of ER-dependent genes in the absence of hormone [67,68]. This could, therefore, provide endometrial tumor cells with a growth advantage, potentially leading to uncontrolled proliferation.

Progesterone acting through the PR is the physiological negative regulator of the oestrogen action in the endometrium. The expression of PR in endometrial glands is under the control of oestrogen and progesterone, where oestrogen induces PR synthesis and progesterone downregulates the expression of its own receptor. The receptor exists in two distinct isoforms: PR-A and PR-B, which are identical except for an additional 164 amino acids in the N-terminus of PR-B. PR-A acts as a transcriptional repressor and PR-B as an activator [69].

The major role of PR-A in the endometrium is thought to be the down-regulation of the oestrogen action through prevention of ERα transactivation. In contrast, PR-B acts as an endometrial oestrogen-agonist. PR-A is therefore believed to be essential for the inhibition of oestrogen-induced endometrial proliferation, partly by limiting PR-B effects. The importance of the PR-A/PR-B ratio has been indicated in a report on the aberrant ratios of PR isoforms in endometrial hyperplasias and EC. Only one PR isoform is commonly found in endometrial cancers, and the expression of a single PR isoform is associated with a higher clinical grade, pointing to a relationship between the loss of PR isoform expression and a poor prognosis. The disruption of relative PR isoform expression has also been observed in cases of complex atypical hyperplasia. Early alterations in the ratio of PR-A/PR-B may therefore precede and/or be implicated in the development of EC [70]. The importance of a balanced PR-A/PR-B ratio has been further underlined by a recently described functional polymorphism in the promoter of PR. This polymorphism resulted in the increased transcription of PR-B and an altered PR-A/PR-B ratio and was associated with an increased risk for EC [71].

Progesterone exposure diminishes endometrial cancer risk, due to the interruption of continued oestrogen stimulation of the endometrium. Endometrial hyperplasia, caused by unopposed oestrogenic stimulation, can be reversed by treatment with progestins. Progestin treatment also provides protection against the stimulatory effects of oestrogenic treatments; i.e., hormone replacement therapy (HRT), using combinations of oestrogens and progestins, yields a lower risk of endometrial carcinoma than treatments associated with oestrogen alone. Progestational agents are the most common type of hormone treatment for endometrial hyperplasia and endometrial cancer.

3. Conclusions

The dualistic model for endometrial cancer was first recognized over 20 years ago [72], and in general, the discrimination between endometrioid adenocarcinomas and high-grade papillary serous and clear cell carcinomas has been well established on a morphological basis [73,74].
A Dualistic Model for Endometrial Tumorigenesis

In summary, a progression model for the development of endometrioid carcinoma has been proposed, based on genetic alterations already present in atypical hyperplasias, on the increase in the nature and prevalence of these genetic alterations in well-differentiated carcinomas when compared to atypical hyperplasias, and on a higher number of chromosomal aberrations in endometrioid lesions than in hyperplasias [75] (see Table 1). First, a genetic background can provide us with information about endometrial carcinoma susceptibility, especially with information on those high-penetration genes (i.e., DNA mismatch repair genes), and also with information on the status of low-penetration genes associated with the oestrogen metabolism [76–78]. This hypothesis proposes the progression from simple to complex hyperplasia as a reactive process due to hyper-oestrogenism [79], while monoclonality associated with PTEN and K-Ras mutations, as well as the appearance of microsatellite instability, seem to define the progression from atypical hyperplasia to endometrioid carcinoma [80]. It has been demonstrated that the detection of clonality in endometrial biopsy samples obtained by pipelle is a useful application for the early diagnosis of endometrial cancer [81]. Both alterations, together with the methylation of the MLH1 promoter, coexist in atypical hyperplasia adjacent to endometrioid carcinoma [43,82–84]. Mutations in p53 and the amplification and over-expression of HER2/neu characterize late events during progression and the dedifferentiation of endometrioid carcinoma [17,85]. Alternatively, based on findings from mixed endometrioid and serous carcinomas [86], these later molecular alterations could define early events in de novo, occurring in poorly-differentiated endometrioid carcinomas and those serous carcinomas developing from endometrioid carcinomas. Despite the great effort made to unravel the molecular alterations associated with endometrial tumorigenesis and the clearly demonstrated usefulness of these alterations in understanding the molecular pathogenesis of endometrial carcinomas, tumors lacking the MSI phenotype or mutations in any of the above-mentioned genes suggest the existence of still unrecognized pathways.

Nevertheless, not all endometrial carcinomas fit into these two pathways; some tumors show mixed or overlapping morphological and immunohistochemical features of both Type-I and Type-II endometrial carcinomas. Thus, it has been proposed that occasional serous carcinomas could develop through the dedifferentiation of pre-existing endometrioid carcinomas [86].

Nevertheless, despite the great effort made to establish a molecularly-based histological classification, some points remain to be clarified. What triggers the tumor cells to invade the myometrium, and what causes vascular or lymphatic dissemination, finally culminating in metastasis? RUNX1, a transcription factor recently identified as one of the most highly over-expressed genes in a microarray study of invasive endometrial carcinoma, and ETV5/ERM, which may be associated with an initial switch to myometrial infiltration and whose up-regulation correlated to the process of tumorigenesis from normal atrophic endometrium to simple and complex hyperplasia and then, on to carcinoma, are good candidate genes meriting further characterization. These studies, as well as those conducted on other genes involved in the mitotic checkpoint as a major mechanism of carcinogenesis in non-endometrioid endometrial cancer, could help to understand the differences in the biology and the clinical outcome among the histotypes.

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