

## **Review**

# **Molecular pathology of endometrial carcinoma: Transcriptional signature in endometrioid tumors**

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**Summary.** A dualistic model, which has been established on a morphological basis and that differentiates type I endometrioid from type II non-endometrioid endometrial cancer, is widely accepted. Molecular genetics have provided us with data supporting the dualistic model of endometrial tumorigenesis and with some clues to speculate about the sequence of the molecular alterations defining the tumorigenesis pathways. In type I endometrioid endometrial cancer, PTEN gene silencing, microsatellite instability associated with defects in DNA mismatch repair genes, or mutations in the K-ras gene are the known major alterations defining the progression from normal endometrium to hyperplasia and then on to carcinoma. Recently, cDNA microarray technology for identifying the differences in gene expression patterns between the histological types of endometrial cancer have permitted the identification of differentially expressed genes that could help us to understand differences in the biology and the clinical outcome between histotypes. Genes involved in the mitotic checkpoint as a major mechanism of carcinogenesis in non-endometrioid endometrial cancer, or altered genes associated with the initial steps of myometrial infiltration in endometrioid endometrial cancer, represent examples of how useful large genetic screenings can be for understanding the tumorigenesis process and the future directions in the molecular pathogenesis of endometrial cancer.

**Key words:** Endometrioid endometrial carcinoma, Molecular pathology, Differential gene expression, RUNX1/AML1

## **Dualistic model for endometrial carcinogenesis**

The tumorigenesis of sporadic endometrial cancer, the most common gynecological malignancy encountered in western countries, is commonly explained on the basis of a dualistic model from both biological and clinical parameters (Lax and Kurman, 1997). Type-I endometrioid endometrial carcinomas (EECs) represent the majority of cases of sporadic endometrial cancer (70-80%), with unopposed oestrogen stimulation as the etiological factor associated with the development of the carcinoma (Potischman et al., 1996). High circulating levels of oestrogen, together with low levels of progesterone, can be related to obesity, anovulation, oestrogen-only hormone replacement therapy (HRT) (Weiderpass et al., 1999), polycystic ovary syndrome, nulliparity, or oestrogen-producing neoplasm. EECs usually develop in pre- and perimenopausal women, express oestrogen (ER) and progesterone (PR) receptors (Lax et al., 1998), and are associated with elevated levels of serum estradiol (Sherman et al., 1997). Histologically, most tumors are of endometrioid type and of low grade; they are frequently preceded by endometrial hyperplasia and overall, are characterized by a favorable prognosis.

In contrast to type-I EEC, about 10-20% of the cases of endometrial carcinoma, designated as type-II non-endometrioid carcinomas (NEECs), follow an oestrogen unrelated pathway and arise from a background of atrophic endometrium. They occur in post-menopausal women; ER and PR expression is usually negative or weakly positive, and serum levels of estradiol are not elevated. They are mainly papillary serous or clear-cell carcinomas of high cellular grade, frequently associated with endometrial intraepithelial carcinoma and are characterized by an aggressive clinical course, a greater propensity for early spreading, and poor prognosis (Oehler et al., 2003).

The validity of this dualistic model for endometrial cancer has persisted for more than twenty years

(Bokhman, 1983), and generally the distinction between these two tumor types is correctly established on a morphological basis (Burton and Wells, 1998). Nevertheless, some questions remain to be clarified. For example, it must be made clear whether or not atypical hyperplasia should be considered neoplastic; also, the fact that not all endometrial carcinomas fit into this dualistic model remains to be addressed, as well as the classification of those tumors showing mixed or overlapping morphological and immunohistochemical features of both type-I and type-II endometrial carcinomas. Indeed, it has been proposed that occasional serous carcinoma could develop through the dedifferentiation of a preexisting endometrioid carcinoma (Matias-Guiu et al., 2001).

### **Molecular genetics associated with endometrioid endometrial carcinoma**

Recently, molecular genetics have provided us with data supporting the dualistic model of endometrial tumorigenesis and with some clues to speculate about the sequence of the molecular alterations defining the tumorigenesis pathways for both type-I and type-II endometrial carcinomas.

Two major genetic alterations have been postulated as the initial hits for EEC: the silencing of the PTEN gene and microsatellite instability (MSI) at the short-tandem DNA sequence repeats distributed throughout the genome, which conforms the microsatellite loci. PTEN, a tumor suppressor gene that derives its name from its preserved tyrosine phosphatase domain and its sequence homology with the matrix protein tensin, is the most frequently altered gene in endometrial cancer and is almost exclusively restricted to EEC. Up to 80% of the cases of endometrioid carcinoma reveal a loss of expression, mainly due to mutations (Mutter et al., 2000b) and to a lesser extent, due to a loss of heterozygosity (LOH) (Simpkins et al., 1998). In addition, a subset of about 20% of those tumors with altered PTEN present promoter hypermethylation (Salvesen et al., 2001). It must be mentioned that the true significance of PTEN promoter methylation is under discussion, as some controversy exists in the literature regarding the possible interference of a processed PTEN pseudo-gene (psiPTEN) with PTEN (Zysman et al., 2002). psiPTEN is located on chromosome 9, and the genomic sequence has a 1.2 kb region with 98% homology for the entire coding region of PTEN. However, the high degree of homology could lead to erroneous interpretations, and since the frequency of PTEN suppression in some types of tumors exceeds that of PTEN mutations or deletions, it is very likely that epigenetic mechanisms, such as promoter hypermethylation, may account for its inactivation in some cases.

The PTEN protein has at least two biochemical functions: it has both lipid phosphatase and protein phosphatase activity. The lipid phosphatase activity of

PTEN has major implications in the PI3 kinase (PI3K) signal transduction pathway. It negatively controls PI3 phosphorylation, decreases intracellular PtdIns(3,4,5)P(3) levels, and affects the downstream Akt signal transduction pathway. Cell-cycle progression is arrested at G1/S, mediated at least partially through the up-regulation of the cyclin-dependent kinase inhibitor p27. In addition, agonist-induced apoptosis is mediated by PTEN through the up-regulation of pro-apoptotic machinery, involving caspases and BID, and the down-regulation of anti-apoptotic proteins, such as Bcl2. The protein phosphatase activity of PTEN is involved in the inhibition of focal adhesion formations, cell spreading and migration, as well as the inhibition of growth factor-stimulated MAPK signaling. Therefore, the combination of the losses of PTEN lipid and protein phosphatase activity may result in aberrant cell growth and an escape from apoptosis, as well as abnormal cell spreading and migration (Wu et al., 2003).

Concerning the other main genetic alterations associated with EEC, it has been proposed that the progressive accumulation of alterations induced by MSI on important regulatory genes may promote carcinogenesis. MSI has been described in 20-45% of the reported cases of endometrial carcinoma (MacDonald et al., 2000) and has been found with more frequency in EECs (33% versus 11% in NEECs; (Catasus et al., 1998)). A close relationship has been demonstrated between MSI and the defects in DNA mismatch repair genes (MSH-2, MLH-1, MSH6), which encode enzymes responsible for repairing nucleotide mismatches, insertions or deletions produced during DNA replication (Salvesen et al., 2001; Koul et al., 2002; Hardisson et al., 2003). The mismatch repair deficiencies underlying MSI seem to involve epigenetic mechanisms rather than mutations in DNA repair genes, as these are encountered with low frequency in 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 (Esteller et al., 1998; MacDonald et al., 2004) followed by the loss of expression of MSH2 and MSH6. Comparing the analysis of promoter hypermethylation patterns between type-I and type-II endometrial carcinomas has shown that EECs frequently have hypermethylated gene loci, especially at the promoters of the MLH1, APC and MGMT genes, whereas the NEECs were infrequently methylated (Risinger et al., 2003a).

Interestingly, mutations in the PTEN gene have been described to occur with greater frequency in those tumors with MSI (60-86%) than in those without (24-35%); this suggests that PTEN could be a target for mutations in a deficient DNA repair context. The association of PTEN inactivation with MSI and methylation and the low expression of MLH1 seems to lay the foundations for the initial steps towards endometrioid endometrial tumorigenesis (Salvesen et al., 2004). Moreover, endometrial precancers (e.g.,

endometrial intraepithelial neoplasia) have been postulated to share common genetic alterations with EEC, including PTEN mutations and MSI. Mutations of the PTEN tumor suppressor gene have been identified in histologically normal-appearing endometria exposed to oestrogen and 18-55% of the cases of endometrial precancers (Latta and Chapman, 2002). In another study, Mutter et al., reported 55% of PTEN mutations in precancers, while no normal endometria showed PTEN mutations. While most precancers and cancers presented a mutation in only one PTEN allele, endometrioid endometrial adenocarcinomas showed the complete loss of PTEN protein expression in 61% of the cases, and 97% showed at least some diminution in expression. Thus, the loss of PTEN function may be an early event in endometrial tumorigenesis, occurring in response to known endocrine risk factors (Mutter et al., 2000b). And, in fact, PTEN is highly expressed in the proliferative, high oestrogen phase of the normal menstrual cycle, suggesting some kind of oestrogen modulation. Moreover, the stromal and epithelial compartments contribute to the hormone-driven changes in endometrial PTEN expression and infer that abnormal hormonal conditions may, in turn, disrupt normal patterns of PTEN expression in this tissue (Mutter et al., 2000c). Finally, Campbell et al., proposed oestrogen receptors to be central elements in the survival pathway of PI3Kinase/AKT and implied that an inactivated PTEN could have an increase in activated AKT; they concluded that inactivating mutations would, therefore, make endometrium more sensitive to stimulation by oestrogens (Campbell et al., 2001).

Minor genetic alterations in endometrioid endometrial carcinoma include mutations in the K-Ras oncogene, described in 10-30% of the reported cases of endometrioid carcinoma, while they are almost absent in papillary serous and clear-cell carcinomas (Lax et al., 2000). K-Ras encodes for a small inner plasma cellular membrane GTPase, functioning as a molecular switch during cell signaling, and it is largely related to tumor growth and differentiation. GTPase activating proteins (GAPs) terminate K-Ras signaling by stimulating intrinsic GTPase activity; oncogenic K-Ras mutants are resistant to GAPs and are constitutively activated. Among the well-studied K-Ras effectors are the serine-threonine kinases of the Raf family and their downstream target, the mitogen-activated protein kinase (MAPK) cascade, playing a major role in the mitogenic action of oncogenic K-Ras. MAPK activation results in phosphorylation and the activation of transcription factors, such as c-Jun, c-Myc and c-Fos, causing the enhanced transcription of genes that are associated with cell proliferation. K-Ras also binds directly to the p110 subunit of PI3K, up-regulates lipid kinase activity, and can activate the anti-apoptotic PKB/Akt pathway (Hancock, 2003). Constitutive activating mutations in K-Ras have been found more frequently in MSI tumors, suggesting that both events may occur simultaneously before clonal expansion (Lagarda et al., 2001). In

keeping with the same line of evidence, the presence of K-Ras mutations in 16% of the cases of endometrial hyperplasia indicates that K-Ras mutations may represent an early event within a subset of endometrial carcinomas (Sasaki et al., 1993).

The frequency of mutations in the  $\beta$ -catenin gene, crucial in cell-cell adhesion through a complex with E-cadherin and in cell signaling as a member of the Wnt pathway, is around 20% and is restricted to endometrioid carcinomas (Machin et al., 2002). Mutations in  $\beta$ -catenin lead to the accumulation of cytoplasmic  $\beta$ -catenin. In the absence of a Wnt signal, the phosphorylation of  $\beta$ -catenin binding sites in APC by glycogen synthase kinase-3 (GSK-3) promotes the APC/ $\beta$ -catenin association, which ultimately leads to the rapid reduction of the cytoplasmic pool of  $\beta$ -catenin by its degradation through the ubiquitin-proteasome pathway. Upon stimulation of the Wnt pathway, the stability of  $\beta$ -catenin is enhanced. The increase in  $\beta$ -catenin concentration leads to its migration to the nucleus, where it binds to and activates members of the TCF/LEF-1 family of transcription factors (T cell factor/lymphocyte enhancing factor). The function of  $\beta$ -catenin in endometrioid tumorigenesis is still unknown; no correlation to MSI, K-Ras or PTEN mutations have been found, suggesting that the Wnt pathway may play an independent role in endometrial cancer (Palacios et al., 2001).

Mutations in the tumor suppressor gene p53 are detected in about 20% of the reported cases of high grade endometrioid carcinoma, while hyperplasia or low grade carcinomas are characterized by a lack of signal (Lax et al., 2000). In addition, no concurrence with PTEN mutations has been described (Koul et al., 2002). In contrast, p53 mutations occur in up to 90% of the cases of serous carcinoma, a scenario close to that described for the Her2/neu (c-erbB2) oncogene coding for a transmembrane receptor tyrosine kinase involved in cell signaling. This has been reported in 10-30% of all ECs and in up to 80% of reported uterine serous papillary malignancies (Esteller et al., 1995; Saffari et al., 1995; Santin et al., 2002). Both alterations are associated with high grade, advanced disease and poor prognosis, and they characterize an alternative progression model for serous carcinomas, where microsatellite instability is a rare event, as are PTEN and K-Ras mutations (Lax, 2004).

In summary, a progression model for the development of endometrioid carcinoma has been proposed based on genetic alterations already present in atypical hyperplasia, on the increase in the nature and prevalence of these genetic alterations in well differentiated carcinomas when compared to atypical hyperplasia, and on a higher number of chromosomal aberrations in endometrioid lesions than in hyperplasias (Lax, 2004) (Fig. 1). First, the genetic background can provide us with information about the endometrial carcinoma susceptibility, especially information on those high-penetrance genes (i.e., DNA mismatch repair genes), and also the status of low-penetrance genes

associated with the oestrogen metabolism (Schneider et al., 1984; Esteller et al., 1997b,c). This hypothesis proposes a progression from simple to complex hyperplasia as a reactive process due to hyper-oestrogenism (Mutter et al., 2000a), 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 (Sun et al., 2002). 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 (Esteller et al., 1997a). Both alterations, together with the methylation of the MLH1 promoter, coexist in atypical hyperplasia adjacent to endometrioid carcinoma (Duggan et al., 1994; Levine et al., 1998; Esteller et al., 1999; Kanaya et al., 2003). Mutations in p53 and the amplification and over-expression of HER2/neu characterize late events during progression and the dedifferentiation of endometrioid carcinoma (Rolitsky et al., 1999; Lax et al., 2000). Alternatively, these later molecular alterations might define early events in *de novo*, occurring in poorly differentiated endometrioid carcinomas and those serous carcinomas developing from endometrioid carcinomas, based on findings from mixed endometrioid and serous carcinomas (Matias-Guiu et al., 2001).

#### Future directions in the molecular pathogenesis of endometrial cancer

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 unrecognized pathways. Very recently, cDNA microarray technology for identifying differences in gene expression patterns between histological types of EC permitted the identification of differentially expressed genes that could help us to understand differences in the biology and clinical outcome of the different histotypes. A pioneer article from Mutter et al., compared the gene expression patterns of normal and malignant endometrial tissues (Mutter et al., 2001). Hormonally responsive genes, selected by comparison from proliferative and secretory subsets of normal endometrium, provided them with a list of fifty genes that discriminated between normal and malignant groups, with diminished expression levels in the cancers. Moreover, tumors resembled proliferative more than secretory endometria, and neoplastic transformation was accompanied by a predominant loss of the activity of many genes constitutively expressed in normal source tissues and an absence of expression profiles, which characterize the anti-tumorigenic progesterone response (Mutter et al., 2001).

High throughput cDNA microarray technology has

been used since then in order to characterize genes or groups of genes that could cluster the different histotypes among endometrial cancers separately, in addition to providing new routes to explore for the comprehension of endometrial tumorigenesis. Risinger and co-workers described, in an unsupervised analysis, 191 differentially expressed genes in endometrioid and non-endometrioid endometrial carcinomas. Among them, differences in 24 transcripts permitted them to distinguish the serous from the endometrioid carcinomas. Moreover, cDNA microarray data identified additional pathways that might be important in the development of endometrial cancer, suggesting multiple avenues for investigation, such as gene silencing mediated by epigenetic mechanisms, deregulation of growth factor pathways due to inappropriate epithelial-stromal interactions, or impaired cell adhesion through altered expression of the intestinal trefoil protein TFF3 (Risinger et al., 2003b). In another assay, to further elucidate the molecular events involved in endometrial carcinogenesis, Cao and co-workers were able to differentiate between endometrioid and serous carcinomas, in an unsupervised cluster analysis. Supervised analysis of the two defined groups led to the identification of 315 genes that statistically differentiated type-I from type-II endometrial carcinomas. In addition, to corroborate the altered expression of known markers, the authors described novel alterations relevant to cell cycle, cell adhesion, signal transduction, apoptosis and tumor progression (Cao et al., 2004).

Taking one more step forward concerning cDNA microarrays and the mechanisms underlying endometrial tumorigenesis, Moreno-Bueno and co-workers described a 2-fold difference in expression between EEC and NEEC in 66 genes during a supervised analysis of cDNA microarray data containing 6386 different genes. The 31 up-regulated genes in the EECs included genes known to be hormonally regulated during the menstrual cycle and important in endometrial homeostasis. Conversely, of the 35 over-expressed genes in the NEECs, three genes, *STK15*, *BUB1*, and *CCNB2*, were involved in the regulation of the mitotic spindle checkpoint; and, *STK15* amplification/overexpression, associated with aneuploidy and an aggressive phenotype, lead to the hypothesis that alterations in the mitotic checkpoint may be a major mechanism of carcinogenesis in NEECs (Moreno-Bueno et al., 2003).

In our group, we focused on the identification of new genes that could trigger transformation in endometrioid endometrial cancer (Planaguma et al., 2004). For this purpose, we analyzed the differential gene expression profile of tumoral and non-tumoral endometrial specimens using cDNA array hybridization. Among the 53 genes whose expression was found to be altered in EEC, 47 cDNA sequences were identified as known genes and were classified into seven functional categories: 9% were involved in cell cycle regulation and cellular proliferation (i.e., *DNAJA2*, *RPS4X*); 17% were involved in transcriptional regulation (i.e.,



SNAPC1, TCEB3); 21% were involved in signaling (i.e., ADD3, NPTX1); 6% were involved in protein traffic (i.e., NCALD); 8% were involved in the immune response (i.e., SIAT1); 9% corresponded to membrane proteins (i.e., MS4A6); and finally, 19% of the genes were involved in the cellular metabolism (i.e., BTN2A1). The top ten genes both up-regulated and down-regulated in endometrioid endometrial cancer are listed in Table 1. One of the most highly up-regulated genes corresponded to the acute myeloid leukemia proto-oncogene RUNX1/AML1 (runt-related transcription factor 1/acute myeloid leukaemia 1). Real-time quantitative PCR validated RUNX1/AML1 up-regulation in EEC and demonstrated a specific and significantly stronger up-regulation in those tumor stages associated with myometrial infiltration. Furthermore, tissue array immunohistochemistry showed that this up-regulation correlated to the process of tumorigenesis from normal atrophic endometrium to simple and complex hyperplasia and then, on to carcinoma (Fig. 2).

Our results lead us to propose that RUNX1/AML1 may play a role in the early events of endometrial tumorigenesis associated with myometrial infiltration. As a DNA-binding transcription factor, RUNX1/AML1 could be involved in the regulation of the expression of genes involved in EEC. RUNX family members have been implicated in transcriptional activation by acting as organizing factors at the promoters and enhancers of target genes, where they associate with co-factors and other DNA-binding transcription factors which are required for gene regulation (Mao et al., 1999). Alternatively, RUNX proteins are potent repressors of transcription in a cell type-specific manner, resulting in either temporal transcriptional repression or irreversible epigenetic silencing (Taniuchi and Littman, 2004). In

this context, RUNX1 has been described to form stable complexes with co-repressors, such as histone deacetylases and histone methyltransferases (Durst and Hiebert, 2004). Moreover, the kinetics of RUNX1/AML1 expression, which show a peak at early myometrial invasion stages, led us to speculate that the up-regulation of RUNX1/AML1 in EEC might be associated with promoter methylation as a mechanism for the transcriptional silencing of genes involved in the first steps of tumor invasion of adjacent tissue. Finally, RUNX1/AML1 gene translocations are not excluded from being involved in EEC.

We thus propose a role for RUNX1/AML1 during the early events of endometrial tumorigenesis, which may be associated with an initial switch to myometrial infiltration. E-Cadherin expression was also positively correlated to myometrial invasion (Mell et al., 2004). Moreover, disturbances of the TGF beta RII and SMAD4 expression, as well as the localization of SMADs, may be important in the infiltration of the myometrial wall by type I endometrial carcinomas (Pięstrzeniewicz-Ulanska et al., 2004). Finally, the myometrial invasion of endometrial cancers involved an increase in gelatinase activity, regulated to some extent by TGF-beta 1 in an autocrine or a paracrine fashion (Yabushita et al., 2000) (Fig. 1).

## Conclusion

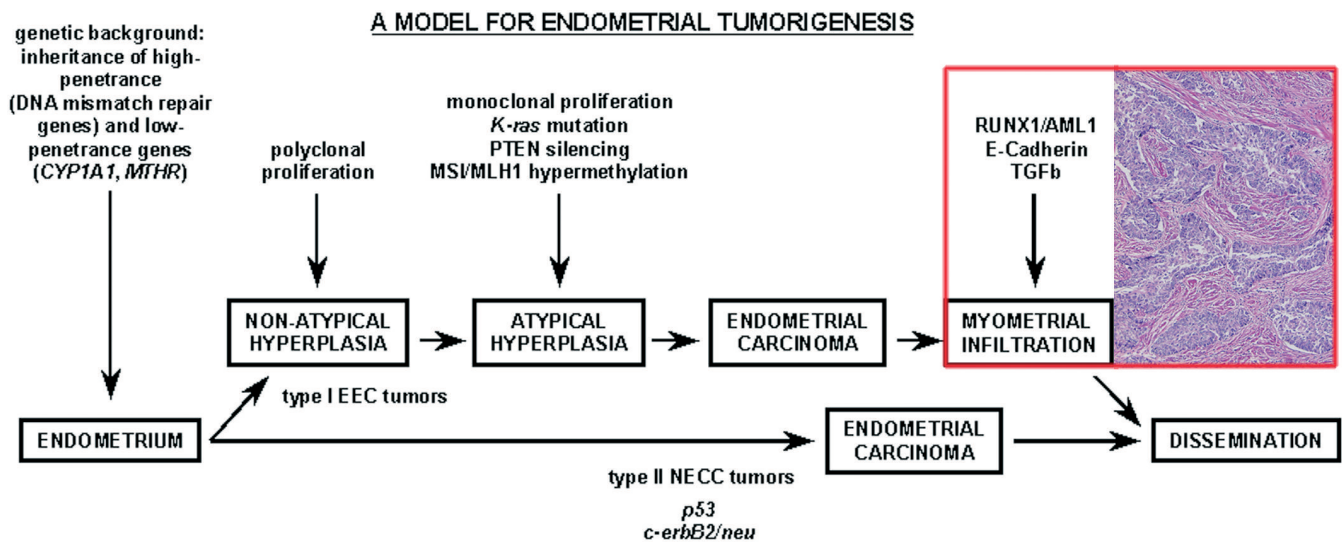
Nowadays, and mainly thanks to the early appearance of symptoms such as post-menopausal metrorrhagia, 80% of the cases of EEC are diagnosed at stage I FIGO (International Federation of Gynecology and Obstetrics) with good response to surgical treatment. Stage I FIGO is comprised of three sub-types determined

**Table 1.** Top ten up- and down-regulated genes in endometrioid endometrial cancer from (Planaguma et al., 2004).

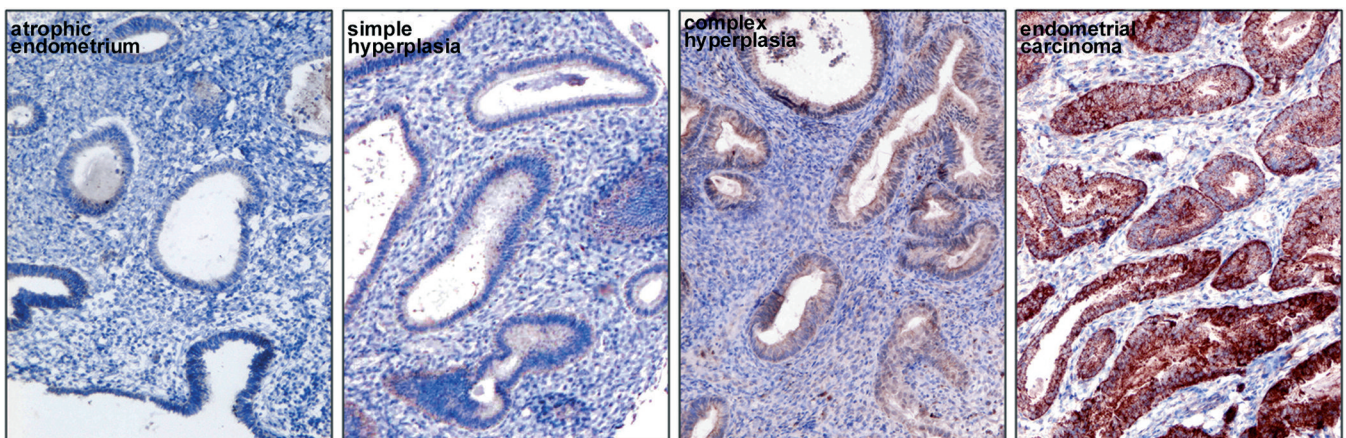
Up-regulated genes in endometrioid endometrial cancer
Ets variant gene 5 (ets-related molecule)
Runt-related transcription factor 1 (acute myeloid leukemia 1; aml1 oncogene) (RUNX1), transcript variant 1
Protein geranylgeranyltransferase type I, beta subunit (PGGT1B)
Membrane glycoprotein (POM121)
Granzyme A (granzyme 1, cytotoxic T-lymphocyte-associated serine esterase 3)
COP9 constitutive photomorphogenic homolog subunit 2
Ciliary neurotrophic factor (CNTF)
Zinc finger, BED domain containing 1 (ZBED1)
Glucosidase, beta; acid (includes glucosylceramidase)
Fucosidase, alpha-L- 1, tissue
Down-regulated genes in endometrioid endometrial cancer
Membrane-spanning 4-domains, subfamily A, member 6A (MS4A6A)
NADH dehydrogenase (ubiquinone) 1 alpha subcomplex 2 (NDUFA2)
DnaJ (Hsp40) homolog, subfamily A, member 2
Pancreatic zymogen granule membrane associated protein GP2 beta form
Inositol hexaphosphate kinase 1 (IHPK1)
Mouse mammary tumor virus receptor homolog 1 (MTVR1)
Prodynorphin (PDYN)
Neuronal pentraxin 1 (NPTX1)
Retinoic acid receptor responder (tazarotene induced) 1, transcript variant 1
ERO1-like (S. cerevisiae) (ERO1L)

by the extent of affected endometria (stage IA), and myometrial infiltration below or above 50% (stages IB and IC, respectively), and stage IC correlating to more undifferentiated tumors, lymph-vascular invasion and 10-20% lymph node involvement. This latter group corresponds to those patients with a higher risk of recurrence among stage I carcinomas and who receive adjuvant treatment after surgery, almost exclusively based on radiotherapy. In this context, the characterization of RUNX1/AML1, during the initial steps towards myometrial infiltration in EEC, represents an example of the usefulness of large genetic screenings

in the understanding of the tumorigenesis process from the early steps of invasion. By combining cDNA microarray large screenings with the microdissection of tumor areas corresponding to the superficial non-infiltrating zones and tumor areas corresponding to the invasion front, at all EEC stages I through III, a differential gene expression profile associated with endometrioid endometrial cancer focused on invasion should be the result. Ideally, this should culminate in the validation of new markers for the further stratification of endometrial cancer sub-types, in order to improve prognostic impact and provide us with new targets for



**Fig. 1.** A Model for Endometrial Tumorigenesis. A model for endometrial tumorigenesis through two divergent pathways and the genetic alterations involved in each step. Highlighted in red are genes associated with myometrial infiltration, together with a representative hematoxylin-eosin picture of an infiltrating stage IC endometrioid endometrial carcinoma.



**Fig. 2.** Representative examples of RUNX1/AML1 immunohistochemistry intensity gradation among different types of samples correlating to tumorigenesis, from atrophic endometria to simple and complex hyperplasias and then, on to carcinoma. x 400.

therapy.

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