Molecular Pathology of Endometrial Cancer

8

Xavier Matias-Guiu and Jaime Prat

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X. Matias-Guiu, MD, PhD
Department of Pathology, Hospitat Universitari Arnau De Vilanova, University of Lleida, Institute of Biomedical Research of Lleida (Irblleida), Lleida, Spain

J. Prat, MD, PhD, FRCPath (✉)
Department of Pathology, Hospital de la Santa Creu i Sant Pau, Institute of Biomedical Research (IIB Sant Pau), Autonomous University of Barcelona, Barcelona, Spain

Introduction

Overview of Endometrial Carcinoma

- In Western countries, endometrial carcinoma (EC) is the most common cancer of the female genital tract accounting for 10–20 per 100,000 person-years
- EC occurs in peri- and postmenopausal women, although it may also develop in premenopausal women, particularly in the setting of hyperestrogenism and hereditary nonpolyposis colon cancer (HNPCC) syndrome
- Etiological factors include unopposed estrogenic stimulation (anovulatory cycles, estrogen administration), obesity, tamoxifen treatment, or insulin resistance
- From a clinical viewpoint, EC falls into two different types (types I and II) (Tables 8.1 and 8.2)

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Table 8.1 Clinicopathological features of types I and II endometrial carcinomas

	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	Nonendometrioid
Behavior	Stable	Progressive

Table 8.2 Genetic alterations of endometrial carcinomas

Type I	Type II
Microsatellite instability	*TP53*
PTEN	LOH
KRAS	*p16*
Beta-catenin (*CTNNB1*)	E-cadherin
PIK3CA	*c-erb B2*
ARID1A	*STK15*

  - Type I tumors are low-grade and estrogen-related endometrioid endometrial carcinomas (EECs) that usually develop in perimenopausal women and coexist with or are preceded by endometrial hyperplasia
  - Type II tumors are high-grade nonendometrioid endometrial carcinomas (NEECs) (mainly serous and clear cell carcinomas), unrelated to estrogen stimulation, which may arise in endometrial polyps or from precancerous lesions that develop atrophic endometrium, and tend to occur in older women (Fig. 8.1)

- Whereas the vast majority of type I carcinomas are cured by hysterectomy, type II carcinomas are very aggressive tumors that require adjuvant therapy

Type I Pathology

- Type I tumors usually develop in perimenopausal women in the setting of hyperestrogenism, obesity, and diabetes. Pathologically, these tumors are EECs, variants of EECs, and mucinous carcinomas
- Although a proportion of EECs arise from endometrial hyperplasia, in many cases the nonneoplastic endometrium appears atrophic or weakly proliferative
- EECs are the most common histological type of EC (80%) (Fig. 8.1a)
  - EECs show a wide spectrum of morphological features including villoglandular pattern, squamous differentiation, secretory change, ciliated cells, or other appearances (sertoliform, microglandular, with small nonvillous papillae, mucin-rich, and oxyphilic type)
- Well-differentiated EEC contains complex glandular structures that resemble to those of the normal proliferative endometrium but are closely packed (back to back) (Fig. 8.1a)
  - Complexity of the glandular elements increases in high-grade tumors which may show gland fusion and cribriforming or may grow in sheets
- EECs with squamous differentiation account for 25–50% of EECs
  - The presence of squamous differentiation does not affect prognosis
- The villoglandular variant accounts for 15–30% of all EEC
  - These tumors are low-grade neoplasms composed of long, slender, delicate papillae with thin fibrovascular cores
  - Although tumors can be purely villoglandular, they often contain areas of typical EEC in the myoinvasive front
- The secretory variant of EEC, also called, secretory adenocarcinoma, is very uncommon. The tumor glands are composed of cells with large subnuclear vacuoles, similar to those of early secretory phase endometrium
  - Prognosis is similar to that of well-differentiated EEC
- Mucinous carcinoma of the endometrium accounts for less than 10% of all EC

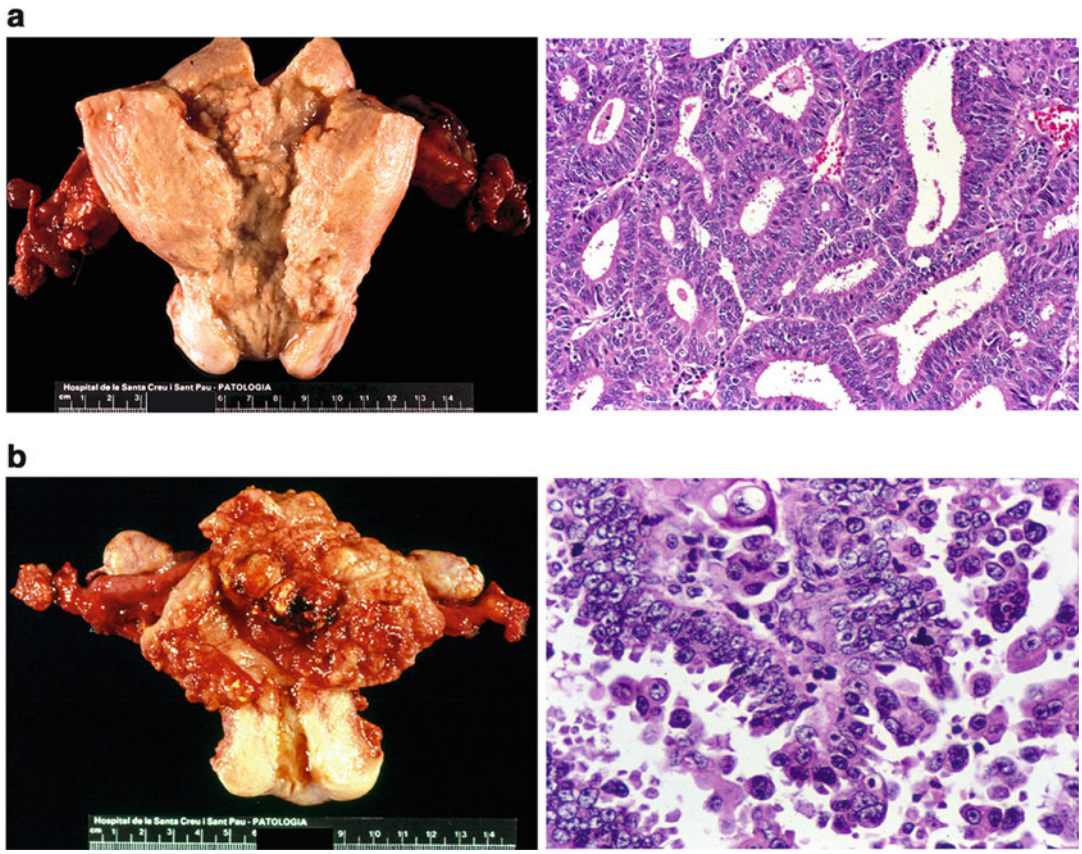

Fig. 8.1 (**a**) Endometrioid carcinoma. (*Upper left*) Polypoid tumor with only superficial myometrial invasion. (*Upper right*) Well-differentiated (grade 1) adenocarcinoma. (**b**) Nonendometrioid carcinoma. (*Lower left*) Large hemorrhagic and necrotic tumor with deep myometrial invasion. (*Lower right*) Serous carcinoma (grade 3) exhibiting stratification of anaplastic tumor cells and abnormal mitoses. (Reprinted from Diagnostic Histopathology, Catasus et al. (2009b), pp. 556–563, with permission from Elsevier)

  - These tumors share clinical features with EEC, which almost always contain mucin-producing cells
  - Mucinous carcinoma shows intracytoplasmatic mucin in at least 50% of the tumor cells

Type II Pathology

- Type II tumors (NEEC) are high-grade invasive serous and clear cell carcinomas
- Serous carcinoma, which is the prototype of NEEC, accounts for 5–10% of ECs
  - Histologically, it shows thick, fibrotic, or edematous papillae with prominent stratification of tumor cells and cellular budding
  - There are often anaplastic cells with large, eosinophilic cytoplasm (Fig. 8.1b)
  - The tumor usually invades the myometrium deeply and there is extensive lymphovascular space invasion
  - Multicentric involvement of other parts of the female genital tract and extrauterine spread may be found at the time of diagnosis
- NEECs are not preceded by endometrial hyperplasia
  - The "precursor" of serous carcinoma is thought to be the so-called *endometrial intraepithelial carcinoma* (SEIC). However, SEIC has metastatic potential.

- Clear cell adenocarcinoma is also considered a type of NEEC
  - The endometrial tumor is similar to clear cell carcinomas of the ovary or cervix and comprises about 5% of all EC
  - Microscopically, clear cell adenocarcinomas are characterized by a variety of patterns such as solid, papillary, glandular, and tubulocystic
  - Tumor cells may exhibit a prominent clear appearance, with abundant glycogen, and a hobnail configuration

Distinction Between Type I and Type II

- Distinction between EEC and NEEC is usually done by microscopic examination
  - Differential diagnosis may be difficult and subjected to interobserver variation in some cases
  - Immunohistochemistry can be of help
- The typical immunohistochemical profile of EEC includes positive immunoreaction for cytokeratins, vimentin, and estrogen and progesterone receptors
- The typical immunohistochemical profile for serous carcinoma includes strong immunoreaction for p53 and p16
  - Whereas p53 is expressed in only 10–35% of EEC—usually in high-grade tumors—70–90% of serous carcinomas show p53 immunoreaction
  - Similarly, p16 shows patchy, weak to moderate staining in EEC but diffuse and strong immunoreaction in 95% of serous carcinomas
- Other potentially useful markers include beta-catenin, PTEN, and E-cadherin
  - Nuclear immunoreaction for beta-catenin and inactivation (lack of immunoreaction) of the PTEN tumor suppressor gene are seen in EEC
  - Lack of membranous immunoreaction for E-cadherin is seen in serous carcinomas
  - The squamous morules of complex atypical hyperplasia and well-differentiated adenocarcinoma typically show nuclear immunoreaction for beta-catenin

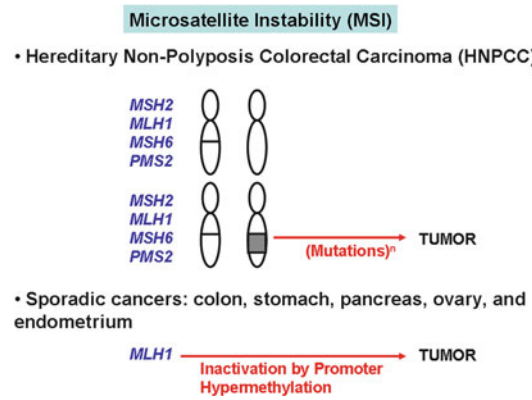

Fig. 8.2 Microsatellite instability in hereditary and sporadic endometrial carcinoma

  - Negative beta-catenin immunoreaction in curettings is frequently followed by the finding of invasive carcinoma (with *PTEN*, *RAS*, and *PIK3CA* mutations/alterations) in the corresponding hysterectomy specimen

- Recently, IMP2 has been proposed as a marker of serous carcinomas of the endometrium

Type I Molecular Features

- The molecular alterations involved in the development of EEC differ from those of NEEC. cDNA analysis has clearly shown that EEC and NEEC exhibit different gene expression profiles (Fig. 8.2)
- EEC show microsatellite instability (MI), and mutations in the *PTEN, KRAS, PIK3CA*, and beta-catenin genes
- NEECs exhibit alterations of *TP53*, loss of heterozygosity (LOH) on several chromosomes (chromosomal instability), as well as other molecular alterations (*STK15, p16*, E-cadherin, and *C-erbB2*)

Microsatellite Instability

- MI has been demonstrated in 75% of EC associated with hereditary nonpolyposis colon cancer (HNPCC) as well as in 25–30% of sporadic EC

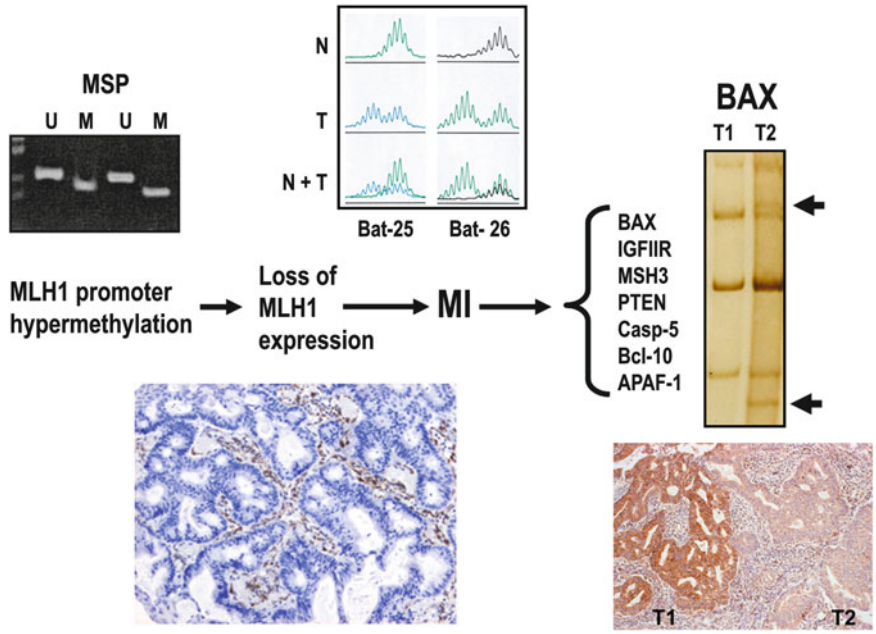

Fig. 8.3 *MLH1* inactivation by promoter hypermethylation is the most common cause of the MI phenotype in endometrial carcinoma. Progressive accumulation of alterations secondary to MI affects important regulatory genes, and promotes carcinogenesis. *BAX* somatic frameshift mutations are heterogeneously distributed throughout the tumor and provide selective growth advantage. (Reprinted from Diagnostic Histopathology, Catasus et al. (2009b), pp. 556–563, with permission from Elsevier)

- The MI-associated mismatch repair deficiency leads to accumulation of mutations in repetitive DNA sequences; i.e., microsatellites
- EC patients from HNPCC kindreds have an inherited germline mutation in *MLH-1, MSH-2, MSH-6*, or *PMS-2*; nevertheless, EC only develops after instauration of a deletion or mutation in the contralateral *MLH-1, MSH-2, MSH-6,* or *PMS-2* allele in endometrial cells (Fig. 8.2)
- In sporadic EC, MI occurs more frequently in EEC (30%) than in NEEC
  - In sporadic tumors, the main cause of mismatch repair deficiency is *MLH-1* inactivation by promoter hypermethylation
  - Abnormal methylation of *MLH-1* may also be detected in atypical hyperplasia, suggesting that it may be an early event in the pathogenesis of EEC that precedes the development of MI (Fig. 8.2)
- The prognostic significance of MI is controversial
  - There is convincing evidence for its association with adverse prognostic factors such as high histological grade
- Mutations in some repetitive mononucleotide tracts located within the coding sequence of some genes involved in cell proliferation, cell differentiation, DNA-repair, and apoptosis, such as *BAX, IGFIIR, hMSH3, hMSH6, MBD4, CHK-1, CASP-5, ATR, ATM, BML, RAD-50, BCL-10*, and *APAF-1*, are secondary events in EC with MI (Fig. 8.3)
- EEC is frequent in patients with HNPCC
  - Characteristic microscopical features of EEC arising in this setting are
    - Poor differentiation
    - Crohn-like lymphoid reaction
    - Lymphangioinvasive growth
    - Tumor infiltrating lymphocytes

- Immunoreaction for *MLH-1, MSH-2, PMS-2,* or *MSH-6* may be helpful in the evaluation of cases
- Occasionally, NEEC has been described in HNPCC patients, but these tumors usually show mixed areas, combining NEEC with EC

Phosphatase and Tensin Homolog *(PTEN)*

- *PTEN* is frequently abnormal in EC
  - LOH at chromosome 10q23 occurs in 40% of EC
  - Somatic *PTEN* mutations are common in EC
  - They are almost exclusively restricted to EEC and occur in 37–61% of cases (Fig. 8.4)
- Concordance between MI status and *PTEN* mutations suggests that *PTEN* could be a likely candidate to be targeted for mutations in the MI-positive EC
- *PTEN* mutations have been detected in endometrial hyperplasias with and without atypia (19% and 21%, respectively), both regarded as precursors of EEC
- Although the prognostic significance of *PTEN* mutations in EC is controversial, their association with favorable prognostic factors has been reported
  - It has been suggested that EECs with *PTEN* mutations have genomic instability, which is the rational for administrating PARP inhibitors
- In agreement with Knudson's two-hit proposal, LOH at 10q23 frequently coexists with somatic *PTEN* mutations
  - The coexistence of both alterations leads to activation of the PI3K/AKT pathway, which plays a key role in the regulation of cellular homeostasis (Fig. 8.5)

Phosphatidylinositol-4,5-Bisphosphate 3-Kinase, Catalytic Subunit Alpha *(PIK3CA)*

- Mutations in *PIK3CA* (p110α) (alfa) contribute to the alteration of the *PI3K/AKT* signaling pathway in EC (Fig. 8.5)
  - They are predominantly located in the helical (exon 9) and kinase (exon 20) domains, but can also occur in exons 1–7
  - *PIK3CA* mutations are infrequent in endometrial hyperplasia

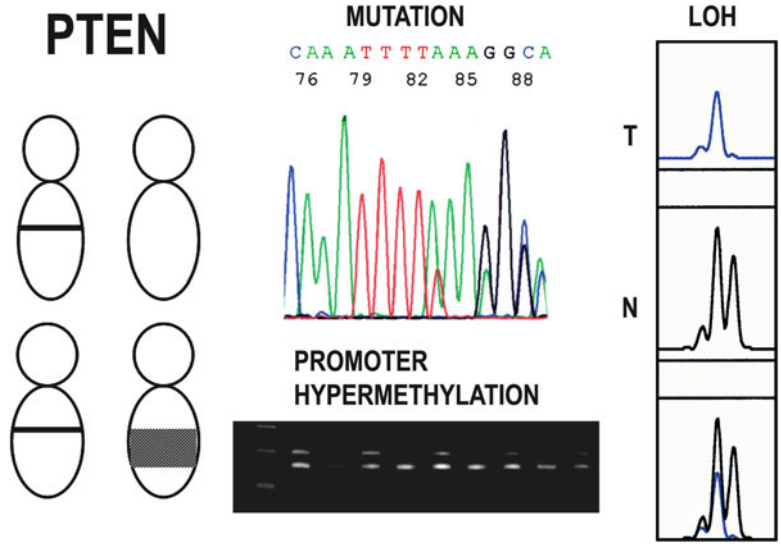

Fig. 8.4 *PTEN* inactivation may occur by several mechanisms such as mutation, LOH at 10q23, and promoter hypermethylation. (Reprinted from Diagnostic Histopathology, Catasus et al. (2009b), pp. 556–563, with permission from Elsevier)

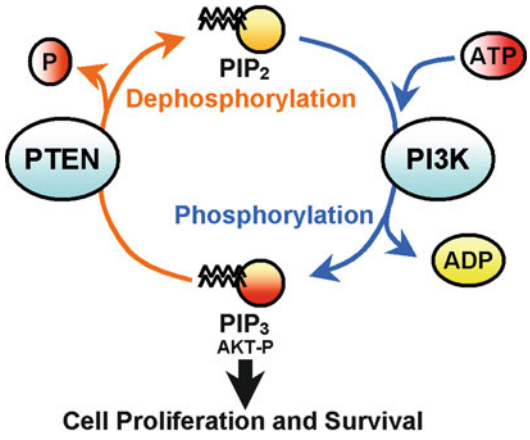

Fig. 8.5 PI3K/PTEN function. Phosphorylation of PI3K converts phosphatidylinositol biphosphate (PIP2) into phosphatidylinositol triphosphate (PIP3) promoting cell proliferation and survival. PTEN negatively regulates PI3K signaling by dephosphorylation of PIP3. (Reprinted from Pathology, Prat et al. (2007), with permission from Wolters Kluwer Health)

- *PIK3CA* mutations occur in 24–39% of the cases, and frequently coexist with *PTEN* mutations
  - *PIK3CA* mutations, particularly in exon 20, have been associated with adverse prognostic factors such as high histological grade and myometrial invasion Catasus et al. (2008)
  - Simultaneous alterations in both *PI3K/AKT* and the *TP53* pathways have a negative effect on prognosis and are associated with lower survival
- Although initially described in EEC, *PI3KCA* mutations also occur in NEEC, and also in mixed EEC–NEEC
- Gene expression profile differences in the *PI3K/AKT* signaling pathway identify two subgroups of high-grade endometrial carcinomas with different molecular alterations (PI3K/AKT pathway vs. p53 alterations) that may have distinct roles in endometrial carcinogenesis (Fig. 8.6) Catasus et al. (2010)
- Mutations in *PIK3RI* (p85α) (alfa), the inhibitory subunit of *PI3K*, have been detected in 43% of EEC, and 12% of NEEC
  - Distribution of *PIK3R1* mutations is nonrandom; most mutations are localized to the p85α-nSH2 (alfa) and -iSH2 domains that mediate binding to p110α (alfa)
- In EEC, *PIK3R1* mutations frequently coexist with *PTEN* and *KRAS* mutations but tend to be mutually exclusive with PIK3CA mutations

RAS–MAPK Pathway

- The RAS–RAF–MEK–ERK signaling pathway plays an important role in endometrial tumorigenesis (Fig. 8.7)
- The frequency of *KRAS* mutations in EC ranges between 10 and 30%
  - In some series, *KRAS* mutations are more frequent in EEC with microsatellite instability
- *RASSF1A* inactivation by promoter hypermethylation may contribute significantly to increase the activity of the RAS–RAF–MEK–ERK signaling pathway
- EC frequently shows inactivation of *SPRY-2* by promoter methylation
  - *SPRY2* is involved in the negative regulation of the FGFR pathway
  - Reduced SPRY2 immunoexpression is seen in almost 20% of EC, and is strongly associated with increased cell proliferation
- Somatic mutations in the receptor tyrosine kinase *FGFR2* have been recently found in 10–12% of EC, particularly in EEC (16%)
  - *FGFR2* mutations and *KRAS* mutations are mutually exclusive events

Beta-Catenin

- The beta-catenin gene (*CTNNB1*) maps to 3p21
  - Appears to be important in the function of both APC and E-cadherin
  - A component of the E-cadherin–catenin unit, important for cell differentiation and maintenance of the normal tissue architecture
  - Important in signal transduction
  - Increased cytoplasmic and nuclear levels result in transcriptional activation through the *LEF/Tcf* pathway

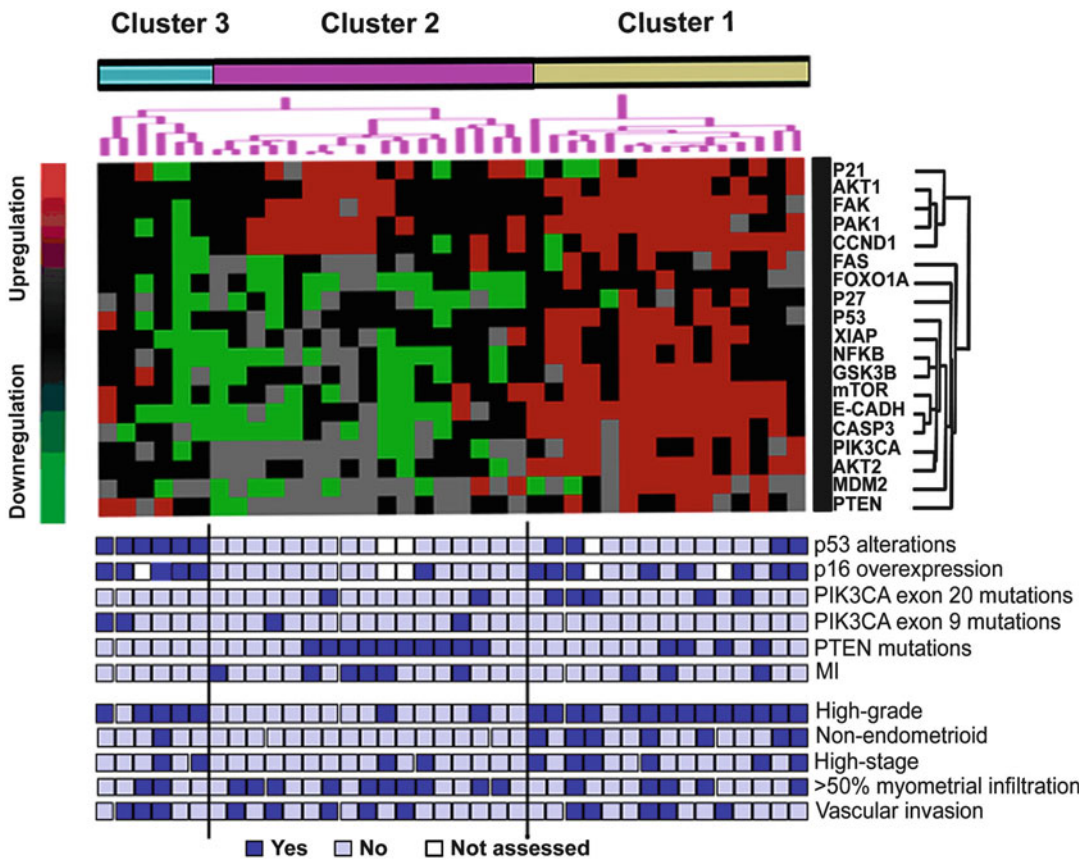

Fig. 8.6 Hierarchical clustering analysis of mRNA expression of 19 genes in 38 endometrial carcinomas. Upregulated and downregulated expression is indicated as *red* and *green cubes*, respectively. Genes that did not vary in their expression level are shown in black and genes with unsatisfactory results are labeled in gray. Enclosed in the clustering image, results of *TP53*, p16, PIK3CA, PTEN, and MI analysis as well as clinicopathological parameters, such as high-grade (grade 3), nonendometrioid, high-stage (stage 2 or higher), myometrial invasion (450%), and vascular invasion, are graphically represented for each case

- Mutations in exon 3 of *CTNNB1* occur in 14–44% of EC and result in stabilization of the protein, cytoplasmic, and nuclear accumulation (Fig. 8.8), and participation in signal transduction and transcriptional activation through the formation of complexes with DNA-binding proteins. *CTNNB1* mutations appear to be independent of MI and mutational status of *PTEN* and *KRAS*
- Although, there is a good correlation between *CTNNB1* mutations and beta-catenin nuclear immunoreaction, other genes of the *Wnt/beta-catenin/LEF-1* pathway may be responsible for the stabilization and putative transcription activator role of beta-catenin in EEC
- Beta-catenin alterations have been described in endometrial hyperplasias and grade 1 EECs that contain squamous metaplasia (morules)
  - They are typically absent in metastatic EC and help in the identification of synchronous independent primary EECs of uterus and ovary
- Prognostic significance of beta-catenin mutations in EC is controversial; however, tend to occur in tumors with favorable prognosis

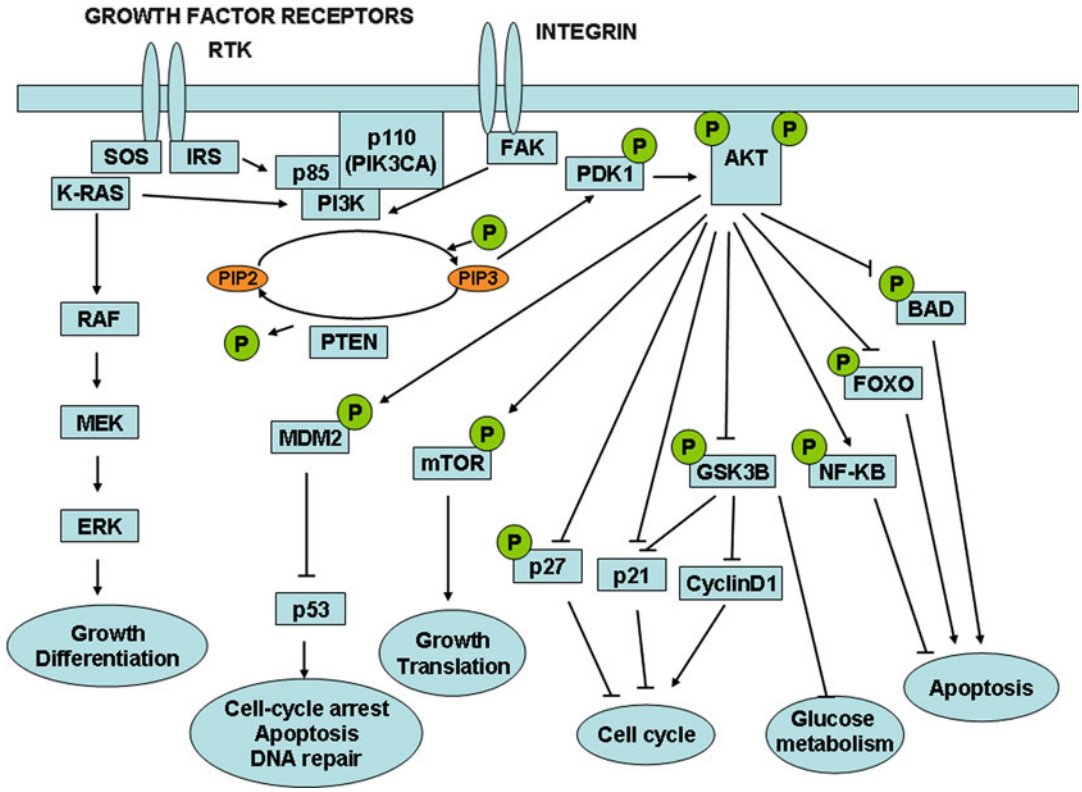

Fig. 8.7 PI3K-AKT, and RAS-MAPK signaling pathways (Reprinted from Diagnostic Histopathology, Catasus et al. (2009b), pp. 556–563, with permission from Elsevier)

Type II Molecular Features

TP53

- Whereas *TP53* mutations occur in over 90% of serous carcinomas, they are present in only 10–20% of EEC, mostly grade 3 tumors (Fig. 8.9)
  - *TP53* mutations are infrequent (<5%) in clear cell carcinomas
- P53 protein can induce apoptosis or prevent a cell from dividing if there is DNA damage
  - Mutation of the *TP53* gene diminishes the cell's ability to repair DNA damage before entry to S-phase, leading to a greater chance that mutations will be fixed in the genome and passed to successive generations of cells

Other Alterations

- Inactivation of the cell cycle regulator *p16* is also more frequent in NEEC (40%) than in EEC (10%)
  - Although the underlying mechanism is unclear, it probably involves deletion and promoter hypermethylation
- Reduced expression of E-cadherin is frequent in EC, and may be caused by LOH or promoter hypermethylation
  - LOH at 16q22.1 is seen in almost 60% of NEEC but only in 22% of EEC
- *C-erbB2* overexpression and amplification are seen more frequently in NEEC (43%) than in EEC (29%)
- NEEC shows chromosomal instability, widespread chromosomal gains and losses, and aneuploidy

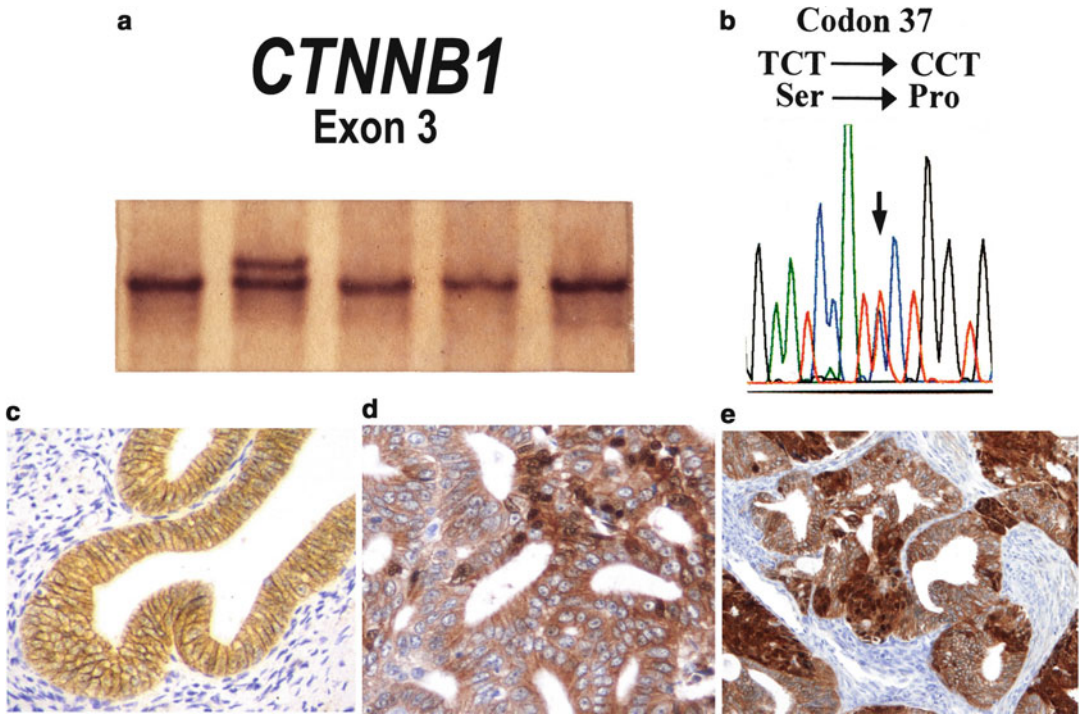

Fig. 8.8 (**a**) CTNNB1 (b-catenin gene) mutations shown by SSCP with abnormal extra band and (**b**) corresponding partial representative nucleotide sequence demonstrating a missense mutation in exon 3. Different patterns of b-catenin immunostaining in endometrioid carcinoma; (**c**) membranous immunoreaction, (**d**) membranous immunostaining with occasional positive nuclei, and (**e**) membranous and nuclear immunostaining in squamous morules. (Reprinted from Pathology, Prat et al. (2007), with permission from Wolters Kluwer Health)

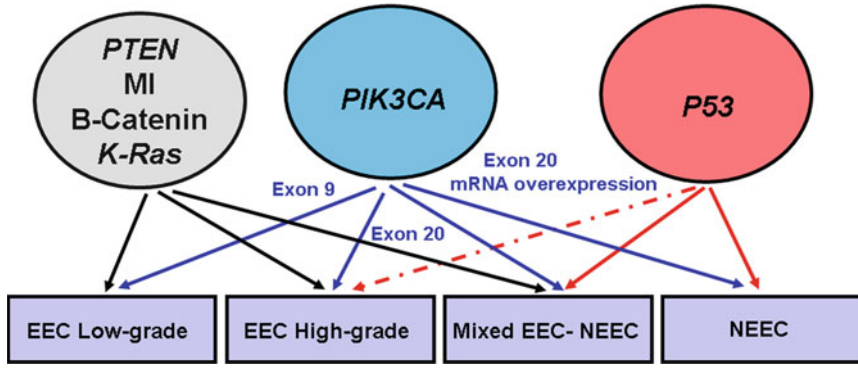

Fig. 8.9 MI, and *PTEN*, *PIK3CA*, *KRAS*, *CTNNB1* (beta-catenin), and *TP53* mutations are the most common molecular genetic alterations in endometrial carcinomas; *EEC* endometrioid endometrial carcinoma; *NEEC* nonendometrioid endometrial carcinoma. (Reprinted from Diagnostic Histopathology, Catasus et al. (2009b), pp. 556–563, with permission from Elsevier)

- cDNA arrays have demonstrated that NEECs usually show overexpression of genes (*STK-15, BUB1, CCNB2*) involved in the regulation of the mitotic spindle checkpoint
  - One of these genes, *STK-15*, essential for chromosome segregation and centrosome functions, is frequently amplified in NEEC (60%)
- New biomarkers of serous carcinoma are EpCAM, claudin-3, and claudin-4 receptors, serum amyloid A, folate-binding protein, mesothelin, LRP-1, and IMP2
- Clear cell carcinomas (NEECs) show specific features including lack of *TP53* alterations and, possibly, mutations in *PIK3CA* and *PTEN*, as well as immunoreactivity for hepatocyte nuclear factor (HNF1β) (beta)
- Mutation of the AT-rich interactive domain-containing protein 1A (*ARID1A*) gene and loss of the corresponding protein BAF250a have recently been described as a frequent event (almost 50% of cases) in clear cell and endometrioid carcinomas of the ovary
  - This mutation has also been found in 29% of grade 1 or 2, and 39% of grade 3, endometrioid carcinomas of the endometrium; 18% of uterine serous carcinomas, and 26% of uterine clear cell carcinomas
  - Uterine low-grade endometrioid carcinomas frequently exhibit loss of *ARID1A* expression (26%)

Molecular Features of Tumors not Fitting in the Dualistic Model

- Classification of EC into type I and type II is artificial and the dualistic model has recently been challenged
  - In daily practice, pathologists are faced with ECs showing combined or hybrid morphologic and molecular characteristics (often EEC and serous carcinomas) (Fig. 8.10)
  - Furthermore, even though serous and clear cell carcinomas have been classified within the same category of tumors (NEECs), recent studies have shown that these are in fact distinct tumor types that exhibit different clinical, immunohistochemical, and molecular features

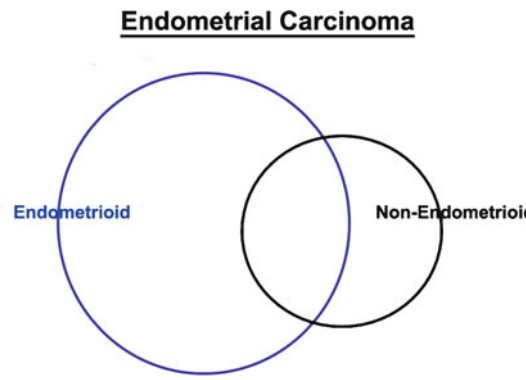

Fig. 8.10 A significant number of EC show combined morphologic and molecular features of EEC and NEEC

Mixed Endometrioid–Nonendometrioid Adenocarcinomas

- ECs showing an admixture of EEC and NEEC (serous and/or clear cell carcinomas) with the minor component representing at least 10% of the neoplasm are classified as a mixed carcinomas and prognosis depends on the proportion of the most aggressive component
- It has been suggested that, in mixed carcinomas, the NEEC component develops as a result of tumor progression—through *TP53* and *PIK3CA* mutations—from a preexisting EEC, since these tumors frequently retain the molecular alterations of typical EEC (Fig. 8.11)
  - This hypothesis would explain not only the existence of mixed EEC–NEEC, but also the presence of MI, as well as alterations in *PTEN, KRAS*, or beta-catenin, in NEEC
- Mixed EEC–NEEC may exhibit overlapping features with EEC (mixed EEC–NEEC morphology, early age at presentation, evidence of estrogen stimulation or preexisting hyperplasia, coexistence of *TP53* mutations, and MI or *PTEN* mutations)

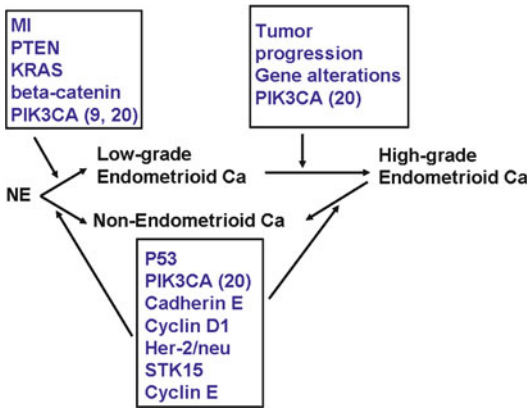

Fig. 8.11 Pathogenesis of endometrial carcinoma: an alternative to the dualistic model. Ca, carcinoma; NE, normal endometrium. (Reprinted from Diagnostic Histopathology, Catasus et al. (2009b), pp. 556–563, with permission from Elsevier)

Endometrioid Carcinomas with Ambiguous Features

- Some ECs exhibit overlapping or intermediate features between EEC and NEEC
  - In these tumors, it is not possible to delineate two different components; i.e., one of EEC, and another of NEEC
  - The term "EC with ambiguous features" has been proposed for such cases
- Although the features of these tumors need to be better defined, the term EC with ambiguous features can be applied to ECs that exhibit architectural pattern typical of low-grade EEC, but high-grade nuclear features, as seen in NEEC
  - These tumors tend to behave more aggressively than conventional EEC and occasionally show the molecular alterations of NEEC
- Another controversial issue is the gray zone between high-grade (predominantly solid) EEC, and NECC
  - Differential diagnosis between these two tumor types is difficult
  - Some high-grade EECs exhibit the typical molecular alterations of NEEC, such as *TP53* mutations

Undifferentiated Carcinoma and Dedifferentiated Carcinoma

- The World Health Organization (WHO) defines undifferentiated carcinoma (UC) of the endometrium as an epithelial tumor that fails to show evidence of either glandular or squamous differentiation
  - UC represents 1–10% of ECs
- UC is characterized by a monotonous proliferation of medium-sized, epithelial cells growing in solid sheets
  - The tumor cells have enlarged nuclei with prominent nucleoli
  - UC is associated with poor prognosis in the vast majority of the cases
    - Over 50% of cases present with advanced stage disease and 75% of patients die of tumors
- Occasionally, UC arises from preexisting well- or moderately differentiated EEC
  - The term *dedifferentiated carcinoma* (DDC) has been used to designate this type of EC
  - Prognosis of DDC is identical to that of UC
  - In DDC, the undifferentiated component is regarded as a result of tumor progression from the coexisting low-grade EEC
- Microsatellite instability is the predominant molecular feature of DDC
  - *TP53* mutations may also be found

Malignant Mixed Müllerian Tumors

- Malignant mixed müllerian tumors (MMMT), also called uterine carcinosarcomas, represent less than 5% of ECs
  - Composed of a biphasic pattern, with malignant epithelial elements and a sarcomatous component
  - Epithelial elements have usually the features of NEEC, but may also have the appearance of EEC
  - Sarcomatous component may be either homologous or heterologous

- Even if MMMT is currently classified as ECs, it should be regarded as a special type since it is associated with worse prognosis than that of the ordinary EC
  - The metastatic pattern of MMMT supports the theory of a neoplasia driven by the epithelial component
    - Myometrial infiltration, lymphovascular involvement, and metastases display more often the epithelial elements than the sarcomatous components of the tumor
- Immunohistochemical and molecular genetics studies support the clonal nature of the two—epithelial and mesenchymal—components in MMMT, supporting the hypothesis that they represent in fact metaplastic (sarcomatoid) carcinomas
  - Expression of epithelial markers in the sarcomatous components occurs in a large proportion of cases
- MMMT cell lines are capable of differentiating into epithelial, mesenchymal, or both components
  - Chromosome X inactivation studies, LOH, and gene mutation analyses all have shown that the epithelial and mesenchymal elements share common genetic alterations
- MMMT probably occurs through epithelial to mesenchymal transition (EMT) in ECs
  - EMT is a process of cellular trans-differentiation in which epithelial cells lose polarity and cell–cell contacts, reorganize their cytoskeleton, and acquire expression of mesenchymal phenotype
- MMMT show expression of genes that repress epithelial markers (E-cadherin) and enhance expression of mesenchymal markers, including proteins involved in skeletal muscle development
  - MMMT have revealed a microRNA signature typical of EMT Castilla et al. (2011)

cDNA Array Results

- cDNA array studies have demonstrated that the expression profiling of EEC differs from that of NEEC
  - In one study, 191 genes exhibited greater than twofold differences between 10 EECs and 16 NEECs
    - One of the genes, *TFF3*, was significantly upregulated in EECs, while increased expression of *FOLR* was seen in NEECs
- In another study, different expression profile involving 66 genes was seen in EEC and NEEC
  - Estrogen-regulated genes were upregulated in EEC
  - NEEC showed increased expression of genes involved in the regulation of the mitotic spindle checkpoint
- Differential expression of 1,055 genes between EECs and serous carcinomas was seen in another investigation
  - Genes upregulated in serous carcinomas were *IGF2, PTGS1*, and *p16*
  - Genes upregulated in EEC included *TFF3, FOXA2*, and *MSX2*
- Another analysis identified 315 genes that statistically distinguished EEC from NEEC
- ECs with microsatellite instability and stable ECs also have different gene expression profiles
  - Two members of the secreted frizzled related protein family (SFRP1 and SFRP4) are downregulated more frequently in EC with microsatellite instability
- Ovarian and uterine tumors with beta-catenin alterations show similar gene expression profile
- The gene expression profiles of similar histological subtypes of ovarian and endometrial carcinomas show that clear cell carcinomas have similar profile regardless of the organ of origin
  - Differences were seen when comparing endometrioid and serous carcinomas of ovarian and endometrial origin

Molecular Alterations in Myometrial Invasion

Overview on Myometrial Invasion

- Deep myometrial invasion is an important prognostic factor of EC

  - Usually correlates with high histological grade, vascular invasion, cervical involvement, and lymph node metastasis
  - Associated with high risk of recurrence
- EEC may exhibit various patterns of myometrial invasion, including diffuse infiltration or expansile-type invasion
  - A distinctive pattern of myometrial invasion designated *microcystic, elongated, and fragmented* (MELF) change shows glands lined by attenuated epithelium with luminal neutrophilic infiltrate resembling endothelium
  - MELF most likely represents EMT and is highlighted by the low-molecular-weight cytokeratin CK19

Epithelial to Mesenchymal Transition

- EMT is a process whereby epithelial cells lose polarity and cell–cell contacts, undergo remodeling of the cytoskeleton, acquire migratory abilities and a mesenchymal-like gene expression program
- Although EMT is a well-known mechanism for interconversions between epithelium and mesenchyme during embryonic development, EMT has recently been recognized as an important phenomenon that participates in invasion and metastasis
- EMT can be induced by different signals and pathways, such as those mediated by TGFβ (beta), tyrosine kinase receptors, and/or Wnt, depending on the specific cellular context
  - Activation of one or more of these pathways frequently converges in a group of transcription factors such as *SNAIL1, SLUG, ZEB1, ZEB2, E47, E2-2*, and *TWIST*, most of them capable of repressing E-cadherin, a master regulator of cell adhesion and polarity
- SNAIL protein expression is increased, and correlates inversely with E-cadherin immunoreactivity, in metastatic EC but not in the corresponding primary tumors
- By protein microarray analysis, a significantly negative correlation between E-cadherin protein decrease and SNAIL expression has also been demonstrated in primary EEC
  - High TWIST expression occurs in invasive EC and affects patient survival
- Comparison between EC samples from the most superficial tumor and the myoinvasive front has shown increases in *SNAIL, SLUG, HMGA2*, and *TWIST* mRNA expression, and decrease in E-cadherin expression, at the myoinvasive front Montserrat et al. (2011)
- EMT features are particularly evident in EECs exhibiting MELF pattern of myometrial invasion at the invasive front

ETS Transcription Factors

- ETS transcription factors activate matrix-degrading proteases and are related to EMT
  - Upregulation of ERM/ETV5, an ETS transcription factor, is associated with early myometrial invasion and correlates with increased matrix metalloproteinase (MMP)-2
- Higher expression of matrix metalloproteinases (MMP-2, MMP-9) in EC is associated with invasive and aggressive behavior in NEEC
  - Increases of MMP-7 have been seen, as a result of beta-catenin nuclear accumulation, in EC with *CTNNB1* mutations
- Transcription factor RUNX1/AML1 is upregulated in EC during invasion
  - A cooperative role of ERM/ETV5 and RUNX1/AML1 has been proposed

Proteomics

- Proteomic analysis shows differential protein expression in superficial and invasive areas of EC
  - Some of the proteins expressed in the invasive front, like Fascin1, have been associated with promotion of the acquisition of migratory and invasive phenotypes
- Different enzymes involved in oxidative stress (ROS), such as SOD1 and BLVRB, are preferentially expressed at the myoinvasive front
  - In the initial stages of EMT and cell migration, ROS is generated and targets downstream molecules which trigger tumor metastasis

Apoptosis–Resistance

Overview of Apoptosis

- Cell death is a phenomenon that commits irreversibly to loss of cellular functions
  - Morphologically, cells that undergo apoptosis frequently show cell volume shrinking, chromatin condensation, nuclear fragmentation, and plasma membrane blebbing
- Commonly, cell death occurring during menstrual phase has been associated to a hormone-dependent ischemic-derived necrosis
  - Several evidences point to apoptosis as another phenomenon with important implications in endometrium tissue remodeling by removing cells from functional layer
- Deregulation of apoptosis plays an important role in development and progression of cancer
  - Cells resistant to apoptosis are likely to have survival advantage, escape the immune surveillance, and may also be resistant to therapy
- Apoptosis can be initiated by two main mechanisms
  - Intrinsic pathway—originated in the mitochondria
  - Extrinsic pathway—triggered by the activation of death receptors in the cell surface (Fig. 8.12)

Members of the Bcl-2 Family

- Members of the *Bcl-2* family of genes are abnormal in EC
  - Compared to normal tissue, there is upregulation of *Bcl-xL* and *Bcl-2*, which is important for development of metastasis
- Pathways that control Bcl-2 expression like the PI3K/AKT pathway are abnormal in EC
  - Other "noncanonical" molecular events, such as NF-κB pathway, which plays an important role in EEC tumorigenesis, correlate with strong immunoreactivity for Bcl-xL (Fig. 8.12)
- In normal endometrium, both BAX and BAK are modulated during menstrual cycle and reach their higher levels in apoptotic epithelial

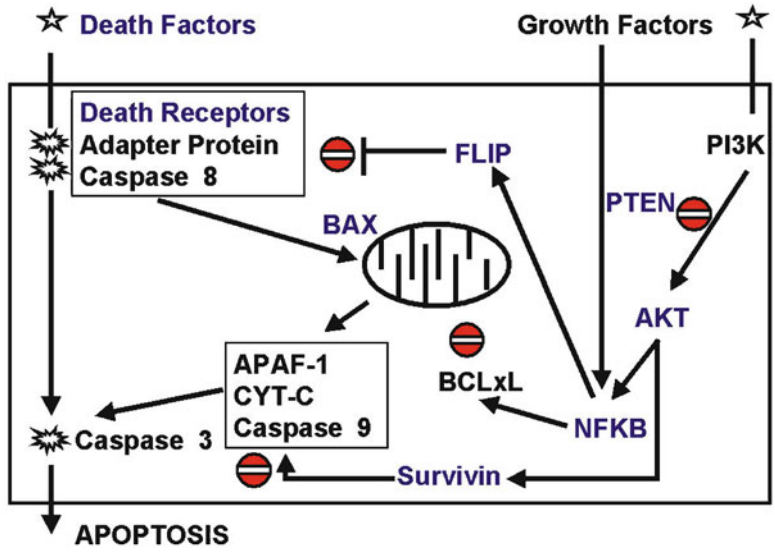

Fig. 8.12 Apoptosis: intrinsic (mitochondrial), and extrinsic (death receptor-initiated) pathways. (Reprinted from Pathology, Prat et al. (2007), with permission from Wolters Kluwer Health)

cells at menstruation, indicating a potentially ovary-derived hormone-dependent regulation
  - Lower to undetectable Bcl-2 protein levels are detected in late secretory through menstrual phases suggesting a direct relationship between anti-apoptotic Bcl-2 proteins and BAX-like proapoptotic members in decision of cell fate
- *BAX* is a target gene for mutations in EEC with microsatellite instability and may have a role in resistance to apoptosis in these tumors

Extrinsic Pathway

- One of the most important regulators of death receptor signaling is *FLIP*, which shares high homology to caspase-8 but lacks proteolytical activity (Fig. 8.12)
  - Transfection of EC cell lines with *FLIP* siRNA results in a marked decrease in cell viability after *TRAIL* exposition
  - In EEC, *FLIP* may be regulated by a cellular complex containing casein kinase 2 and kinase suppressor of RAS1 (CK2–KSR1)
  - CK2 beta regulatory subunit is overexpressed in EC and regulates cell proliferation
  - The kinase suppressor of RAS1 (KSR1) is considered a scaffold protein that regulates the intensity and duration of the MAP kinase pathway
  - KSR1 interacts with different kinases of the Raf/MEK/ERK signaling pathway to enhance its activation
  - KSR1 expression is increased in EC Llobet et al. (2011)

Resistance to Hypoxia and Radiation Therapy

- EC is treated by surgery and adjuvant radiation
  - Recurrence postradiotherapy is usually associated with poor prognosis and increased risk of metastases
  - These tumors are treated by chemotherapy (doxorubicine, cisplatin, paclitaxel)
  - Understanding the molecular and genetic mechanisms underlying resistance to either radio and/or chemotherapy is crucial for the establishment of new therapeutic targets that improve outcome in EEC
- Ionizing radiation (IR) causes lesions at the DNA level, known as DNA single-strand breaks and double-strand breaks (DSBs)
  - IR can rapidly prevent DNA replication by activation of cell cycle checkpoints to avoid formation of toxic DNA replication lesions
  - The main signaling molecules involved in the DNA damage response are the serine threonine kinases ATM and ATR
    - ATM responds primarily to DNA DSBs
    - ATR acts mainly in response to replication fork stalling
    - Ionizing radiation also activates ATR
- In response to DSB, ATM and DNA-protein kinases phosphorylate H2AX. γ-H2AX plays an essential role in the repair process regulating the recruitment of repair factors, such as Nbs1, 53BP1, and BRCA1, to foci located at DSB sites
- Unrepaired DNA damage is measured by H2AX phosphorylation (γ-H2AX), and γ-H2AX is used as a standard marker of unrepaired double-strand DNA damage
- Tumor hypoxia renders tumors more resistant to IR treatment
  - By reacting with the radiation-created broken ends of DNA, oxygen fixes the damage and thus enhances radiation-induced cell death
  - The "oxygen enhancement effect" renders oxygenated cells three times more radiosensitive than hypoxic cells
  - Under hypoxic conditions, the oxygen enhancement effect is lost, and cells become more radio resistant
- Low PR expression is associated with recurrence
  - PR expression in ECs from patients carrying one specific DNA polymorphism (so-called PROGINS allele) is predictive of the risk of recurrence

- *MLH-1* promoter methylation and decreased MLH-1/MSH-2 expression are not predictive of recurrence in stage I EC
  - De novo *MLH-1* promoter methylation is occasionally found during EC progression in patients receiving radiation therapy
- p53 overexpression is significantly predictive of recurrence in EC
  - It does not correlate with *TP53* mutations
- Absence of E-cadherin expression predicts distant metastasis but not local recurrence
  - Nuclear immunoreaction of beta-catenin does not predict recurrence
- Immunohistochemical comparison of postradiation recurrent tumor and primary EC reveals increased nuclear expression of beta-catenin in the recurrent tumors
- HIF-1α (alfa) is another candidate molecule for conferring radio resistance to EC cells
  - HIF-1 is the most important mediator of hypoxia and controls the expression of over 100 genes
  - Compared to levels in primary EC, HIF-1α (alfa) expression increases in postradiation recurrences
  - HIF-1α (alfa) controls classical NF-κβ (beta) activation pathway and survival under hypoxia through RelA (p65) nuclear accumulation
  - Possibly, HIF-1α (alfa) expression results in increased radio resistance

Stem Cells

- Somatic stem cells (SSC) are undifferentiated cells present in most adult tissues
  - SSC are defined by their functions: high proliferative potential, self-renewal, and differentiation into one or more lineages
  - Another property of SSC is the retention of a DNA synthesis label (bromodeoxyuridine—BrdU) for prolonged periods of time
  - The Hoechst dye exclusion is a good method for the identification and isolation of stem cells in adult tissues
  - A side population (SP) can be identified by dual-wavelength flow cytometry after incubating the target cells with the DNA-binding dye Hoechst 33342 (Fig. 8.13) Cervello et al. (2011)
- Cancer stem cells (CSC) have been defined in analogy to SSC as cells that have the capacity to self-renew
  - Undergo divisions that allow the generation of more identical CSC and give rise to the variety of more differentiated cells found in the tumor

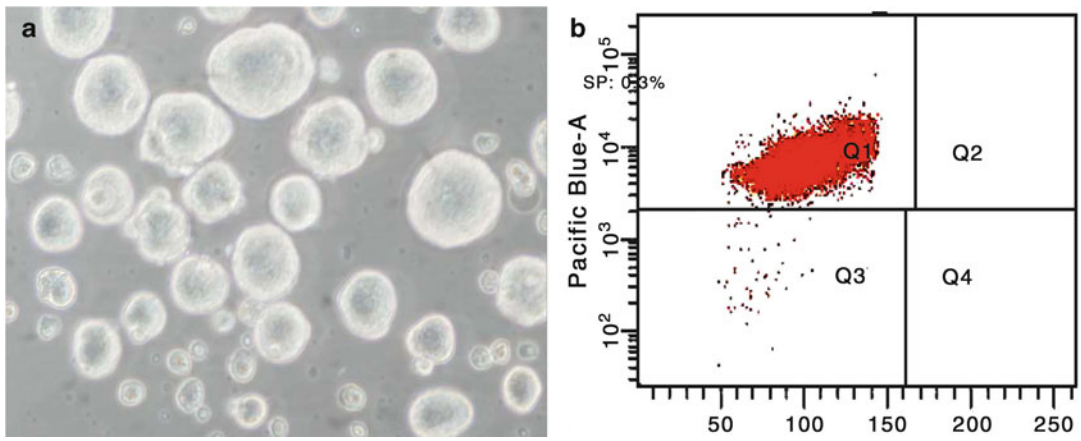

Fig. 8.13 The endometrial carcinoma cell line Ishikawa shows cancer stem cell features. Ishikawa cells can grow as floating spheres (**a**). This cell line contains a side population that excludes Hoescht stain. Q1, stained region; Q3, unstained region, side population (**b**). (Reprinted from International Journal of Gynecological Pathology, Cervello et al. (2011), with permission from Wolters Kluwer Health)

- An epithelial and stromal SP has been identified in human endometrium
  - The mean percentage of SP cells in the epithelial fraction is 0.21% during the menstrual phase, 0.15% during the proliferative phase, and 0.02% in the secretory phase
  - Currently, there are no markers for endometrial epithelial stem cells and they cannot be distinguished from their mature progeny
- SP cells have been identified in both human primary ECs and some cell lines
  - In vitro studies showed that AN3CA-SP cells proliferate slower than the corresponding non-SP fraction
    - Cells showed long-term self-renewal properties and accumulated in the G1 phase of the cell cycle, suggesting they are in a dormant quiescent state
  - AN3CA-SP cells are resistant to paclitaxel treatment compared to the non-SP subset
    - No differences were observed with cisplatin
- Investigations with Hec-1 SP cells revealed long-term proliferating and self-renewal capacity in vitro
  - These cells initiate larger tumors than the non-SP population and, more important, they undergo EMT during tumor development
- To date, only one surface marker, the CD133/1 epitope, has been proposed for identification and isolation of endometrial CSC
  - CD133+ cells have been isolated from Ishikawa, Hec1-A, RL-95, AN3CA, MFE280, and MFE296 cell lines
  - The ratio of positive cells differs between lines, ranging from 0.38% to 15.5%
  - CD133+ cells showed higher proliferative potential and tumorigenicity than the negative subset
  - There is no conclusive evidence that CD133 is the universal marker for endometrial CSC

Targeted Therapies

- The importance of the PI3K/PTEN/AKT survival pathway in EC raises the possibility that PI3K inhibitors, such as Wortmannin and derivatives, may be used as potential anticancer agents
  - Decrease of Akt phosphorylation and increased apoptosis are seen in mutated *PTEN* human endometrial cancer cells in the presence of PI3K inhibitor
- ECs with *PTEN* mutations show a high level of genetic instability similar to the one seen in breast and ovarian cancers with *BRCA-1* and *BRCA-2* alterations
  - PARP inhibitors are used in the treatment of patients with *PTEN*-mutated Ecs (Table 8.3)
- mTOR is the downstream effector of AKT
  - Upon activation, mTOR-Raptor activates S6K and inhibits 4EBP1 to accelerate mRNA translation
  - Tumors associated with *PTEN* inactivation are particularly susceptible to the therapeutic effects of mTOR inhibitors (Table 8.3)
  - Several mTOR inhibitors are available for clinical trials: CCI-779 (temsirolimus), RAD001 (everolimus), and AP23573
  - Pharmacological inhibition of mTOR by CCI-779 in $PTEN^{+/-}$ mice has shown reduced neoplastic proliferation, tumor size, and S6K activity
- Dual PI3K–mTOR inhibitors may also be used as a targeted therapy in EC
  - The p110 subunits of PI3K and mTOR share similar structures
  - The dual PI3K–mTOR inhibitors may target p110α (alfa), β (beta), and δ (delta) isoforms, mTORC1 and mTORC2
  - BEZ235, a dual PI3K and mTOR inhibitor, suppresses cell growth in EC cell lines, especially in cells with *PIK3CA* and/or *PTEN* mutations

Table 8.3 Endometrial carcinoma (targeted therapies)

Target	Predictive marker
mTor (temsirolimus, everolimus, deferolimus)	*PTEN* loss, *PIK3CA, KRAS* mutations?
HER2 (trastuzumab)	*c-erbB2* amplification
EGFR (Erlotinib)	EGFR expression
VEGF (bevacizumab/ thalidomide)	VEGFR expression?
Tyrosine kinases (sorafenib/ sunitinib)	NFkB, MCL-1, FLIP?
PARP	*PTEN* loss
FGFR2	*FGFR2* mutations

- EGFR, which is highly expressed in normal endometrium, is also overexpressed in EC—a finding associated with poor prognosis
  - In these cases, GW572016 (Lapatinib) has been used as a single agent (Table 8.3)
- Medroxiprogesterone acetate is frequently used in EC patients
  - Two large GOG trials evaluating oral progestins in these patients showed an overall response rate or 15–25% with median progression-free survival of less than 4 months and overall survival of less than 11 months
- Proteasome inhibitors trigger cell growth arrest or apoptosis on several tumors
  - Bortezomib causes cell death by blocking NF-κB activity in some tumors
  - In EC, proteasome inhibitors induce cell death by increasing NF-κB transcriptional activity
- Sorafenib (BAY 43-9006, Nexavar) is a potent tyrosine kinase inhibitor with antiproliferative and antiangiogenic activities
  - Although originally described as a B- and c-RAF kinase inhibitor, it has also shown activity against vascular endothelial growth factor receptor 2 (VEGFR2), platelet-derived growth factor receptor, FLT3, RET, and c-Kit
  - Sorafenib sensitizes EC cells to TRAIL-induced apoptosis by downregulating FLIP and Mcl-1 (Table 8.3) Ortega et al. (2008)
- Histone acetylation is one of the mechanisms involved in the epigenetic control of gene expression
  - Histone deacetylase inhibitors (HDACI) are promising anticancer drugs
  - HDACI cause derepression of genes whose reactivation would promote an antiproliferative effect
  - Examples of genes upregulated by HDACI are *p21, TRAIL-R2, p19ARF, Bmf*, and *Rap1*
  - Paradoxically, HDACI cause downregulation of important genes such as thymidylate synthetase, *Bcr-Abl* and *c-Myc*
  - HDACI have growth inhibitory effect on EC cell lines, by decreasing the proportion of cells in S phase, and increasing the proportion of cells in the G_0–G_1 and or G_2–M phases of the cell cycle
  - HDACI upregulates *p21*, *p27*, and E-cadherin, and downregulates Bcl-2, and cyclin D1 and cyclin D2
  - The growth-suppressor effects seem to be irrespective of the *TP53* gene status

Summary of Keypoints

- There are two clinicopathologic variants of endometrial carcinomas (endometrioid and nonendometrioid) that show specific molecular features and different gene expression profiles
- The main molecular features of endometrioid carcinomas are: microsatellite instability and mutations of *PTEN, PIK3CA, KRAS*, and beta-catenin genes
  - Although the clinical and prognostic relevance of each of these alterations has not been fully elucidated, mutations in *PTEN* and beta-catenin gene seem to be associated with a favorable outcome
- In HNPCC patients, microsatellite instability analysis and immunoreactivity of mismatch repair proteins are important to confirm the diagnosis of hereditary endometrial carcinoma
- The main features of nonendometrioid carcinomas are:
  - *TP53* mutations
  - Inactivation of *p16* and E-cadherin, *c-erbB2* amplification
  - Alterations in genes involved in the regulation of the mitotic spindle checkpoint (*STK-15*)
  - LOH at multiple loci indicating chromosomal instability
- Some nonendometrioid carcinomas probably arise from preexisting endometrioid carcinomas
  - This is the most likely reason why some tumors exhibit combined or mixed features at the clinical, pathological, and molecular levels
- Some EC do not fit in the dualistic (type I vs. type II) model
  - Mixed endometrioid–nonendometrioid tumors (*TP53*)
  - Dedifferentiated carcinomas (microsatellite instability)
  - Malignant mixed müllerian tumors (epithelial to mesenchymal transition)

- EMT, Ets transcription factors, and enzymes involved in oxidative stress may have a role in myometrial invasion
- In endometrial carcinoma, apoptosis–resistance may play a role in tumor progression (FLIP under CK2 and KSR1 regulation)
- Molecular pathology is important for identifying biomarkers as predictive factors for success in targeted therapies
  - Candidate pathways are PI3K, mTOR, EGFR, apoptosis, and histone acetylation

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