Oncogene (2013) **32,** 403–413

www.nature.com/onc

REVIEW

Endometrial carcinoma: molecular alterations involved in tumor development and progression

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In the western world, endometrial carcinoma (EC) is the most common cancer of the female genital tract. The annual incidence has been estimated at 10–20 per 100 000 women. Two clinicopathological variants are recognized: the estrogen related (type I, endometrioid) and the non-estrogen related (type II, non-endometrioid).The clinicopathological differences are paralleled by specific genetic alterations, with type I showing microsatellite instability and mutations in *phosphatase and tensin homologue deleted on chromosome 10*, *PIK3CA*, *K-RAS* and *CTNNB1* (β-catenin), and type II exhibiting *TP53* mutations and chromosomal instability. Some non-endometrioid carcinomas probably arise from pre-existing endometrioid carcinomas as a result of tumor progression and, not surprisingly, some tumors exhibit combined or mixed features at the clinical, pathological and molecular levels. In EC, apoptosis resistance may have a role in tumor progression. Understanding pathogenesis at the molecular level is essential in identifying biomarkers for successful targeted therapies. In this review, the genetic changes of endometrial carcinogenesis are discussed in the light of the morphological features of the tumors and their precursors.

Oncogene (2013) **32,** 403–413; doi:10.1038/onc.2012.76; published online 19 March 2012

Keywords: endometrial carcinoma; genetics; microsatellite instability; molecular alterations; β-catenin; apoptosis; chromosomal instability

INTRODUCTION

In western countries, endometrial carcinoma (EC) is the most common cancer of the female genital tract. EC occurs in peri- and postmenopausal women, although it may also be present in premenopausal women, particularly in the setting of hyperestrogenism. From a clinical viewpoint, EC falls into two different types, so-called types I and II.[1] Type I tumors are low-grade and estrogen-related endometrioid endometrial carcinomas (EECs) that usually develop in perimenopausal women and coexist or are preceded by endometrial hyperplasia. In contrast, type II tumors are non-endometrioid endometrial carcinomas (NEECs). These aggressive tumors that occur in older women, are unrelated to estrogen stimulation and arise occasionally in endometrial polyps or from precancerous lesions in atrophic endometrium.

Over the last 15 years, knowledge about the molecular genetics of EC has increased notably. However, some issues remain to be elucidated and will be discussed in this review.

MOLECULAR FEATURES OF TYPES I AND II EC

It has been demonstrated that the molecular genetic alterations involved in the development of EEC (type I) differ from those of NEEC (type II).[2–4] First, complementary DNA (cDNA) analysis clearly shows that EEC and NEEC have different gene expression profiles. Moreover, whereas EEC shows microsatellite instability (MI) and mutations in the *phosphatase and tensin homologue deleted on chromosome 10* (*PTEN*), *PIK3CA*, *K-RAS* and β-catenin genes, NEEC have alterations of p53, loss of heterozygosity (LOH) on several chromosomes, as well as other molecular alterations (STK15, p16, E-cadherin and C-erbB2).

MI was initially noted in cancers of patients with the hereditary non-polyposis colorectal carcinoma (HNPCC), but also in sporadic colon cancers. EC is the second most common tumor found in HNPCC patients. MI is seen in 75% of EC associated with HNPCC, but also in 25–30% of sporadic EC.[5–8] HNPCC patients have an inherited germline mutation in *MLH1*, *MSH2*, *MSH6* or *PMS2*. MI occurs more frequently in EEC (30%) than in NEEC. In sporadic tumors, *MLH1* inactivation by promoter hypermethylation is the main cause of mismatch repair deficiency.[9] The MI-associated mismatch repair deficiency leads to the accumulation of mutations in coding and non-coding DNA sequences. Some small short-tandem repeats, like mononucleotide repeats, located within the coding sequence of some important genes; (*BAX*, *IGFIIR*, *MSH3*, *MSH6*, *MBD4*, *CHK1*, *CASP5*, *ATR*, *ATM*, *BML*, *RAD50*, *BCL10* and *APAF1*) are targets in the process of tumor progression of MI + EC. Mutations in these tracts are interpreted as secondary events in cancers with MI.[10,11]

The tumor-suppressor gene *PTEN* is frequently abnormal in EC.[12–14] LOH at chromosome 10q23 occurs in 40% of EC.[15] Somatic *PTEN* mutations are also common in EC, and they are almost exclusively restricted to EEC, occurring in 37–61% of them and lead to activation of the PI3K/AKT pathway. There are many evidences showing that EECs with mutations in *PTEN* have genomic instability.[16] For that reason, some authors have suggested to treat patients with PARP inhibitors.[17] Mutations in *PIK3CA* may contribute to the alteration of the phosphatidylinositol 3 kinase (PI3K)/AKT signaling pathway in EC.[18–20] PI3K is a

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Received 27 December 2011; revised 30 January 2012; accepted 1 February 2012; published online 19 March 2012

heterodimeric enzyme consisting of a catalytic subunit (p110) and a regulatory subunit (p85).The *PIK3CA* gene codes for the p110α catalytic subunit of PI3K. A high frequency of mutations in the *PIK3CA* gene has been reported recently in EC. Mutations are predominantly located in the helical (exon 9) and kinase (exon 20) domains, but they can occur also in exons 1 to 7.[21] *PIK3CA* mutations occur in 24–39% of the cases, and coexisted frequently with *PTEN* mutations. *PIK3CA* mutations, particularly in exon 20, have been associated with adverse prognostic factors such as high-grade and myometrial invasion. Although initially described in EEC, *PI3KCA* mutations also occur in NEEC, and also mixed EEC–NEEC.[22,23] Furthermore, gene expression profile differences in the PI3K–AKT signaling pathway identify two subgroups of high-grade EC with different molecular alterations (PI3K/AKT pathway versus p53 alterations), which may play distinct roles in endometrial carcinogenesis.[24] Moreover, mutations in *PIK3RI* (p85α), the inhibitory subunit of PI3K, have been detected in 43% of EEC, and 12% of NEEC.[25]

Among AKT targets, downstream effector mammalian target of rapamycin (mTOR) is of particular interest. mTOR inhibitors have been recently developed as potential anticancer agents. Tumors associated with PTEN inactivation, like EC, are particularly susceptible to the therapeutic effects of mTOR inhibitors. Pharmacological inhibition of mTOR in $PTEN^{+/-}$ mice has shown reduced neoplastic proliferation, tumor size and S6K activity.[26] Moreover, the use of dual PI3K–mTOR has been proposed as a targeted therapy, because they may target p110α, β and δ isoforms, mTORC1 and mTORC2.[27] These inhibitors are expected to be effective in cancers (like EC) with *PTEN* mutations, *PIK3CA* mutations and receptor tyrosine kinase-dependent activation.

The Rat Sarcoma Viral Oncogene Homolog (Ras)–Raf proto-oncogene serine/threonine-protein kinase (Raf)–Extracellular Signal-Regulated Kinase kinase (MEK)–Extracellular Signal-Regulated Kinase (ERK) signaling pathway plays an important role in EC. The frequency of *K-RAS* mutations in EC ranges between 10 and 30%.[28] *BRAF*, another member of the RAS–RAF–MEK–ERK pathways is very infrequently mutated in EC.[29] Recent studies have demonstrated that *RASSF1A* inactivation by promoter hypermethylation may contribute significantly to increased activity of the RAS–RAF–MEK–ERK signaling pathway.[30]

There are several evidences suggesting that the fibroblast growth factor (FGF) signaling pathway is important in EC. Recent studies have shown that EC presents frequent inactivation of SPRY-2, a protein that is involved in the negative regulation of the FGFR pathway.[31] Moreover, somatic mutations in *FGFR2*, identical to the germline mutations associated with craniosynostosis and skeletal dysplasia syndromes, have been recently detected in 10–12% of EC, particularly in EEC (16%).[32–34] FGFR-2 is of special interest, since it is a possible target for therapeutic approaches.

Mutations in exon 3 of β-catenin gene (*CTNNB1*) occur in 14 to 44% of EC,[35–37] and result in stabilization of the protein, cytoplasmic and nuclear accumulation, and participation in signal transduction and transcriptional activation through the formation of complexes with DNA binding proteins. They appear to be independent of the presence of MI, and the mutational status of *PTEN* and *K-RAS*. Mutations are homogeneously distributed in different areas of the tumors, which suggest that they do play a role in early steps of endometrial tumorigenesis. The presence of a cytoplasmic and nuclear β-catenin immunoreactivity in some ECs that did not show a mutation in *CTNNB1* suggests that alterations in other genes of the Wnt/β-catenin/LEF-1 pathway may be responsible for the stabilization and putative transcription activator role of β-catenin in these tumors.[38,39]

In contrast to EEC, NEEC show *P53* mutations (90%), inactivation of p16 (40%) and E-cadherin (80–90%), c-erbB2 amplification (30%), alterations in genes involved in the regulation of the mitotic spindle checkpoint (*STK15*) and LOH at multiple loci, reflecting the presence of chromosomal instability.[40–44] Although *P53* mutations occur in 90% of NEEC, they are only present in 10–20% of EEC, which are mostly grade 3 tumors.[45] Inactivation of the cell cycle regulator p16 is also more frequent in NEEC (40%) than in EEC (10%). The underlying mechanism is not clear, but probably involves deletion and promoter hypermethylation. Reduced expression of E-cadherin is frequent in EC, and may be caused by LOH or promoter hypermethylation. In fact, LOH at 16q22.1 is seen in almost 60% of NEEC, but only in 22% of EEC. C-erbB2 overexpression and amplification are also seen more frequently in NEEC (43 and 29%) than in EEC. However, the most typical molecular feature of NEEC is chromosomal instability. This phenomenon is characterized by the presence of widespread chromosomal gains and losses, which reflect the presence of aneuploidy. cDNA arrays have demonstrated that NEEC usually show upregulation of genes (*STK15*, *BUB1* and *CCNB2*) that are involved in the regulation of the mitotic spindle checkpoint. One of them, *STK15*, which is essential for chromosome segregation and centrosome functions, is frequently amplified in NEEC.

Among NEEC, clear cell carcinomas show specific features. Based on the similarities between ovarian and endometrial clear cell carcinoma, it has been suggested that both types of tumors may exhibit similar alterations, including mutations in *PIK3CA* and *PTEN*. Mutation of the *ARID1A* gene and loss of the corresponding protein BAF250a has recently been described as a frequent event in clear cell and endometrioid carcinomas of the ovary. In a recent study, these changes have been found in 29% of grade 1 or 2 and 39% of grade 3 EEC, 18% of uterine serous carcinomas and 26% of uterine clear cell carcinomas. In a different study, uterine low-grade EEC have also shown a relatively high-frequency loss of ARID1A expression (26%) and *ARID1A* mutations (40%).[46,47]

cDNA array studies have demonstrated that the expression profiling of EEC is different from that of NEEC. Some of the data obtained from these studies have helped in a better diagnosis and prognosis. In one study,[48] 191 genes exhibited a greater twofold differences between 19 EECs and 16 NEECs. One of the genes, trefoil factor 3 (*TFF3)* was significantly upregulated in EECs, while increased expression of folate-binding protein (*FOLR)* was seen in NEECs. Subsequent studies demonstrated that *TFF3* was highly expressed at gene and protein level in high-grade EECs, suggesting that *TFF3* could be a novel serum marker for early detection and/or monitoring EEC patients.[49] Moreover, over-expression of *FOLR* and mesothelin was found to be associated with NEEC,[50] and also with shortened progression-free survival in EC.[51] In a different study, a different expression profile was seen between EEC and NEEC, and the differences involved 66 genes. Interestingly, estrogen-regulated genes were upregulated in EEC, whereas NEEC showed increased expression of genes involved in the regulation of the mitotic spindle checkpoint.[52] A third study demonstrated differentially expression of 1055 genes between EECs and serous carcinomas. Genes that were differentially expressed were *IGF2*, *PTGS1*, p16, *TFF3*, *FOXA2* and *MSX2*.[53] A different study identified 315 genes that statistically differentiated EEC from NEEC.[54] Among the genes listed for EEC are *ras-related protein RAB14*, *α2-catenin*, *human transforming growth factor β3* and *ILGF1*. In contrast, *aldolase C* was one of the major discriminators for NEEC. In a different study, it was seen that the tumors (ovarian and uterine) with β-catenin alterations, showed common gene expression profile.[55] Recently, a low-density cDNA microarray approach identified five differentially expressed genes in EEC and NEEC, *NDC80, BUB1, FUT8, ANXA4* and *BBC3*.[56] Moreover, a different expression profile was also found between EC associated with MI and stable EC. Interestingly, two members of the secreted frizzled related protein family (*SFRP1* and *SFRP4*) were more frequently downregulated in EC with MI.[57]

Molecular profiling has also been evaluated in relationship with other prognostic parameters. A recent study with an array containing 492 genes was used to generate gene expression profiles in correlation with histologic type and grade, and stage.[58]

One cluster contained 38 genes that were upregulated in samples of the cluster representing the most advanced disease; and one of these genes was *CCNE2*. Gene expression profiling also has revealed that some genes (*Apolipoprotein E*) are differentially expressed in poorly differentiated tumors.[59] Our group has also identified, by c-DNA array studies, upregulation of *RUNX1/AML1* and *ERM/ETV5* in EC,[60] and suggested an implication of such genes in myometrial invasion. One study compared the expression profiles of similar histological subtypes of ovarian and ECs; and showed that clear cell carcinomas had a very similar profile, regardless of the organ of origin. In contrast, differences were seen when comparing endometrioid and serous carcinomas of ovarian and endometrial origin.

We have recently performed a cDNA microarray analysis to identify new genes and pathways involved in endometrial carcinogenesis. We used cDNA microarrays containing 6386 different genes to analyze gene expression profiles in 24 EECs and 8 normal endometria (NE) samples. After supervised analysis of the microarray data, there was an at least twofold difference in expression between EEC and NE in 159 genes (adjusted *P*-values <0.07, unadjusted *P*-values <0.001), including, among others, tumor-suppressor genes (*SMAD4* and *WT1*), genes involved in endometrial homeostasis (*IGF1, IGF2*), immune modulation (*CD74, LCN2, IL2, IL5R* and *IL7R*), the Wnt pathway (*WNT5A* and *DVL2*), mismatch repair (PMS1), cell signaling (*FGFR1, PGFRA, PLXNB1, KSR, LYN, FYN, PRKCD, STAT3* and *STAT12*), components of the extracellular matrix (*SPARC, COL5A1, COL5A2, COL5A1, COL6A3* and *COL15A1*) and genes involved in extracellular matrix remodeling (*MMP9* and *MMP19*). To validate the quality of our array data, a subset of genes (*IGF1, IGF2, WNT5A, WT1, PMS1, PRKCD* and *FGFR1*) differentially expressed in normal endometrium and EECs were examined in all samples using semi-quantitative reverse transcriptase–PCR and also in an independent series of EECs obtaining similar results, with the only exception was *PRKCD*, which just failed to reach significance, with a *P*-value of 0.079 (Table 1; Figure 1). In addition, CD74 and Smad4, were immunohistochemically analyzed in a tissue microarray containing 190 ECs, including both EEC and NEEC, in order to further validate microarray data and to gain insights into the role of these proteins in EC (Table 2). Immunohistochemical analysis demonstrated that *CD74*, which was not expressed in normal endometrium, was expressed in 123 (80.4%) tumors and was associated with the endometrioid phenotype and lower grade. Finally, Smad4 expression was reduced in 75 (57.3%) tumors, including 17 (13%) cases with complete absence of Smad4 expression in neoplastic epithelial cells (Figure 2).

ECs not fitting within the dualistic (type I versus type II) model

The classification of EC into two groups (type I and type II) is artificial and too rigid and the dualistic model needs to be challenged (Figure 3). In daily practice, pathologists are faced with tumors showing combined or hybrid morphologic and molecular characteristics (often endometrioid and serous tumors). Furthermore, even if serous and clear cell carcinomas have been classified within the same category of tumors as they are more aggressive than EC, recent studies have shown that these are in fact distinct tumor types, as they exhibit different clinical, immunohistochemical and molecular features. When an EC has an admixture of EEC and EC, with the minor component representing at least 10% of the neoplasm, the tumor should be classified as a *mixed carcinoma*. Based on molecular analysis, it has been suggested that the NEEC component originates as a result of tumor progression from a pre-existing EEC, because frequently these tumors retain the molecular alterations of typical EEC. There are some tumors that exhibit overlapping and intermediate features between EEC and NEEC and fail to show two distinct components. The term '*EC with ambiguous features*' has been proposed for these carcinomas.[61] Their clinical and molecular features should be defined better. Moreover, the overlap between EEC and NEEC is also seen when we look for differences in the frequency of the main molecular alteration. The typical molecular alterations of EEC are occasionally seen in NEEC, while the typical molecular features of NEEC are also detected in EEC.

Occasionally, undifferentiated carcinomas are associated with well- or moderately differentiated EEC. The term *dedifferentiated carcinoma* has been used to designate such special type of tumor. Several groups of investigators have reported that the EECs with a tendency to develop a high-grade, undifferentiated carcinoma present MI as a frequent molecular genetic alteration.[62] However, MI is not seen in all cases and other, have *P53* mutations.

Malignant mixed müllerian tumors (MMMT), (carcinosarconas or sarcomatoid carcinomas), are uncommon uterine neoplasms. They are composed by a bifasic pattern, with epithelial malignant elements and a sarcomatoid component. It has recently suggested from the molecular point of view that MMMT should be regarded as metaplastic carcinomas.[63–65] Like sarcomatoid carcinomas of other locations, carcinosarcomas probably develop through epithelial-to-mesenchymal transition (EMT) in EC. Although the transient occurrence of the EMT phenomenon is important for myometrial invasion in conventional EC, MMMT show permanent expression of EMT leading to repression of epithelial markers (E-cadherin) and increased expression of mesenchymal markers including proteins involved in skeletal muscle development. All of these molecular changes are responsible for the appearance of the sarcomatous areas as well as the presence of heterologous differentiation. It has been recently seen that MMMT show *HMGA2* overexpression, and a microRNA signature typical of EMT.[66,67]

MYOMETRIAL INVASION

Deep myometrial invasion is an important prognostic factor. It correlates with high-grade components, vascular invasion and lymph node metastasis. EMT has recently been recognized as an important mechanism in invasion and metastasis. Different transcriptional repressors of E-cadherin have been identified, including the zinc-finger factors Snail and Slug, the two handed factors SIP-1 (Zeb-2) and EF1 (Zeb-1), and the bHLH factors E12/E47 and Twist. Snail expression is increased and correlates inversely with E-cadherin inmunoreactivity in metastatic EC but not in the corresponding primary tumors.[68] Nevertheless, a significant negative correlation between E-cadherin decrease and Snail expression has also been found in primary EC. Furthermore, high Twist expression has been shown in invasive EC. Our group[69] has recently compared samples from the surface area and the myoinvasive front of EC in order to investigate whether the EMT program is activated in early stages of EC. We found increases in *SLUG*, *ZEB1* and *HMGA2* mRNA expression in the myoinvasive front of tumor samples, indicating the role of these transcriptional factors in endometrial tumor progression and invasion. Increase in Snail and Twist expression occurred concomitantly with decrease in E-cadherin expression at the myoinvasive front of early stage EC. For better understanding of the potential role of EMT in the genesis and development of ECC, an *in vitro* scenario mimicking this process was developed using IK V600E transformed EC cells. The overexpression of the *BRAF* missense mutation V600E leads to a persistent activation of ERK1/2 and increase in Snail protein levels as demonstrated by immunofluorescence and western blot analysis.[69]

Also related to EMT, Ets transcription factors have been associated with the activation of matrix-degrading proteases.[70,71] Upregulation of *ERM/ETV5*, a member of the Ets transcription factors, has been recently associated with initial steps of myometrial invasion in EC, in correlation with increased matrix metalloproteinase (MMP) 2. Higher expression of MMPs (MMP2, MMP9) in EC, has been recently associated with invasive and aggressive behavior in NEEC. Moreover, increased MMP7

Table 1. Selected expressed genes found to be significantly associated ($P < 0.05$) between normal endometrium (N) and endometrioid endometrial carcinoma (T)

Gene symbol	*Fold (N/T)*	*Fold (T/N)*	*Acc. number, description*
Immune response:			
IL13RA1		2.0	AA478570, interleukin 13 receptor, alpha 1
ITGAL		2.0	R48796, integrin, alpha L (antigen CD11A (p180), lymphocyte function-associated antigen 1; alpha polypeptide)
LTB		2.1	AI351740, lymphotoxin beta (tumor necrosis factor (TNF) superfamily, member 3)
NFKBIA		2.1	W55872, nuclear factor of kappa light polypeptide gene enhancer in B-cell inhibitor, alpha
NFKBIE		2.1	AA953975, nuclear factor of kappa light polypeptide gene enhancer in B-cell inhibitor, epsilon
IL5RA		2.1	AI381503, interleukin 5 receptor, alpha
SCYD1		2.1	R66139, small inducible cytokine subfamily D (Cys-X3-Cys), member 1 (fractalkine, neurotactin)
IL7R		2.1	AA487121, interleukin 7 receptor
IGHG3		2.2	AA663981, immunoglobulin heavy constant gamma 3 (G3 m marker)
Clone_N168K		2.6	g1847166, homo sapiens isolate donor N clone N168K immunoglobulin kappa light chain variable region mRNA
IFI30		2.7	AA630800, interferon, gamma-inducible protein 30
CD74		2.9	g712300, CD74 antigen (invariant polypeptide of major histocompatibility complex, class II antigen associated)
HLA-DRB3		3.4	g1350342, major histocompatibility complex, class II, DR beta 3
HS3ST1		3.5	T55714, heparan sulfate (glucosamine) 3-O-sulfotransferase 1
Ig-partial_cds		3.9	g1791068, human immunoglobulin heavy chain variable region (V4-31) gene, partial cds
Ig_Partial_cds		4.7	g6927383, human rearranged immunoglobulin heavy chain mRNA, partial cds
MALT1		5.4	AA826328, mucosa-associated lymphoid tissue lymphoma translocation gene 1
LCN2		6.2	AA401137, lipocalin 2 (oncogene 24p3)
IL2		9.2	g2466805, Interleukin 2
SDF1	2.0		AA447115, stromal cell-derived factor 1
IL6ST	3.2		AA406546, interleukin 6 signal transducer (gp130, oncostatin M receptor)
Cell cycle/proliferation/differentiation:			
FGFR1		2.0	AA281189, fibroblast growth factor receptor 1 (fms-related tyrosine kinase 2, Pfeiffer syndrome)
RBBP7		2.0	AA995351, retinoblastoma-binding protein 7
TNFSF13		2.1	AA041396, tumor necrosis factor (ligand) superfamily, member 13
IGF1		4.5	N67876, insulin-like growth factor 1 (somatomedin C)
BST2		5.6	AA485371, bone marrow stromal cell antigen 2
EGR1	2.0		AA486628, early growth response 1
SMAP	2.1		AA481621, thyroid hormone receptor co-activating protein
SIX2	2.1		g2816419, Sine oculis homeobox (*Drosophila*) homolog 2
SUPT6H	2.1		R85545, suppressor of Ty (S.cerevisiae) 6 homolog
FHL1	2.1		AA456394, four and a half LIM domains 1
AIP-1	2.1		W19461, Abl-interactor 12 (SH3-containing protein)
VEGFC	2.3		H07991, vascular endothelial growth factor C
EDG2	2.6		AA193405, endothelial differentiation, lysophosphatidic acid G-protein-coupled receptor, 2
PDGFRA	4.9		H23235, platelet-derived growth factor receptor, alpha polypeptide
CUL3	5.2		R27581, cullin 3
WNT5A	6.4		W49672, wingless-type MMTV integration site family, member 5A
IGF2	9.1		H59614, insulin-like growth factor 2 (somatomedin A)
Cell death:			
HIG2		2.3	AI343669, hypoxia-inducible protein 2
NME3		2.7	AA398218, non-metastatic cells 3, protein expressed in
CARD12	2.1		AA443290, caspase recruitment domain protein 12
ANXA5	2.8		AA451895, annexin A5
DNA repair:			
PMS1	2.1		AA504838, postmeiotic segregation increased (*S. cerevisiae*) 1
Transcription related:			
ATF5		2.1	AA496253, activating transcription factor 5
CEBPB		2.1	H26183, CCAAT/enhancer-binding protein (C/EBP), beta
BTF3	2.0		AA609731, basic transcription factor 3
MADH4	2.0		AA456439, MAD (mothers against decapentaplegic, *Drosophila*) homolog 4 (Smad4)
PLAGL1	2.0		AA463297, pleiomorphic adenoma gene-like 1
SMARCA5	2.0		AA416971, SWI/SNF related, matrix associated, actin-dependent regulator of chromatin, subfamily a, member 5
ZHX1	2.1		N50828, zinc-fingers and homeoboxes 1
JUN	2.3		W96155, v-jun avian sarcoma virus 17 oncogene homolog
SON	2.4		AA431848, DNA-binding protein
PBX3	2.6		W48726, pre-B-cell leukemia transcription factor 3
MAF	2.6		AA043501, v-maf musculoaponeurotic fibrosarcoma (avian) oncogene homolog
MYC	2.6		W87741, v-myc avian myelocytomatosis viral oncogene homolog
GTF3A	2.7		AA456147, general transcription factor IIIA
RUVBL1	2.8		AI023590, RuvB (*E. coli* homolog)-like 1
ZNF6	2.9		AA928817, zinc-finger protein 6 (CMPX1)
WT1	3.3		AA130187, Wilms tumor 1

Table 1 (*Continued*)

Gene symbol	*Fold (N/T)*	*Fold (T/N)*	*Acc. number, description*
KLF4	3.6		H45711, Kruppel-like factor 4 (gut)
STAT3	3.8		AA399410, signal transducer and activator of transcription 3 (acute-phase response factor)
EIF5	4.6		AA669443, eukaryotic translation initiation factor 5
Cell signaling:			
PRKCD		2.0	H11054, protein kinase C, delta
LYN		2.1	R83837, v-yes-1 Yamaguchi sarcoma viral-related oncogene homolog
KSR1		2.1	H88143, kinase suppressor of ras
VAV1		2.1	T65770, Vav 1 oncogene
PLXNB1		2.2	AA496565, Plexin B1
PPFIA4		3.0	AI653137, protein tyrosine phosphatase, receptor type, f polypeptide (PTPRF), interacting protein (liprin), alpha 4
RAN	2.0		AA456636, member RAS oncogene family
FYN	2.1		N22980, FYN oncogene related to SRC, FGR, YES
STATI2	2.1		R23241, STAT-induced STAT inhibitor-2
GJA1	2.1		AA487623, gap junction protein, alpha 1, 43kD (connexin 43)
PIM2	2.2		AA863383, Pim-2 oncogene
DVL2	2.4		R39405, dishevelled 2 (homologous to *Drosophila* dsh)
SGK	2.4		AA486082, serum/glucocorticoid regulated kinase
TNFSF11	2.5		AA504211, tumor necrosis factor (ligand) superfamily, member 11
RAB31	2.6		AA449333, RAB31, member RAS oncogene family
PIM1	3.7		N63635, Pim-1 oncogene
Adhesion, and extracellular matrix:			
SELL		2.1	H00756, selectin L (lymphocyte adhesion molecule 1)
CHL1		2.2	H15267, cell adhesion molecule with homology to L1CAM (close homologue of L1)
MMP9		3.4	AA425227, matrix metalloproteinase 9 (gelatinase B, 92 kD gelatinase, 92 kD type IV collagenase)
COL15A1	2.1		AA464342, collagen, type XV, alpha 1
MMP19	2.1		AI361112, matrix metalloproteinase 19
CTNND1	2.1		AA024656, catenin (cadherin-associated protein), delta 1
CALD1	2.3		AA447737, caldesmon 1
COL5A2	3.2		AA461456, collagen, type V, alpha 2
COL1A2	3.2		AA490172, collagen, type I, alpha 2
ITM2B	3.4		AA453275, integral membrane protein 2B
COL5A1	3.4		R75635, collagen, type V, alpha 1
CDH11	3.5		H96738, cadherin 11, type 2, OB-cadherin (osteoblast)
CORO2B	3.6		N92783, coronin, actin-binding protein, 2B
COL6A3	3.6		R62603, collagen, type VI, alpha 3
CSPG2	3.6		AA101875, chondroitin sulfate proteoglycan 2 (versican)
SPARC	3.7		g839914, secreted protein, acidic, cysteine-rich (osteonectin)
CNTNAP1	18.1		AA028905, contactin-associated protein 1
Basic cellular function/miscellaneous:			
CHD3		2.0	AA454980, chromodomain helicase-DNA binding protein 3
KIAA0677		2.0	AA620458, KIAA0677 gene product
EST		2.0	N23708, ESTs
EST		2.0	AA678087, ESTs
EVPL		2.0	AA029418, Envoplakin
FLJ23231		2.0	T97601, hypothetical protein FLJ23231
EST		2.0	AA682558, ESTs
EST		2.0	g1030258, ESTs
HD		2.1	T64094, Huntingtin (Huntington disease)
AGPAT2		2.1	AA938623, 1-acylglycerol-3-phosphate O-acyltransferase 2 (lysophosphatidic acid acyltransferase, beta)
KIAA0485		2.1	AI685539, KIAA0485 protein
FLJ21935		2.1	AA143726, hypothetical protein FLJ21935
AK1		2.1	AA775325, adenylate kinase 1
TBCD		2.1	AI668870, tubulin-specific chaperone d
KIAA1856		2.1	H40023, KIAA1856 protein
SULT1A3		2.1	AA398458, sulfotransferase family, cytosolic, 1A, phenol-preferring, member 3
Clone_mcg53-54		2.1	g6925032, homo sapiens clone mcg53-54 immunoglobulin lambda light chain variable region 4a mRNA, partial cds
UBD		2.3	N49629, diubiquitin
EST		2.4	AA699824, ESTs
EST		2.4	AA678361, ESTs
clone_ASPBLL54		2.4	g6923541, homo sapiens clone ASPBLL54 immunoglobulin lambda light chain VJ region mRNA, partial cds
FLJ20940		3.4	N34316, hypothetical protein FLJ20940
cDNA_2010107G23		3.4	AA452165, homo sapiens, Similar to RIKEN cDNA 2010107G23 gene, clone MGC:9596 IMAGE:3896656, mRNA, complete cds
EST		3.5	H50747, ESTs
Clone_MGC_17279		4.6	N90491, clone MGC:17279 IMAGE:4212772, mRNA, complete cds
FEZ2	2.0		AA043280, fasciculation and elongation protein zeta 2 (zygin II)

Table 1 (*Continued*)

Gene symbol	*Fold (N/T)*	*Fold (T/N)*	*Acc. number, description*
EST	2.0		W52340, EST, weakly similar to A60764 Ig gamma-3 chain C region, form LAT
HRB2	2.0		W52273, HIV-1 rev binding protein 2
CYR61	2.0		AA777187, cysteine-rich, angiogenic inducer, 61
EST	2.0		g1102475, ESTs
FLJ22128	2.1		N34358, homo sapiens cDNA: FLJ22128 fis, clone HEP19543
HSPC207	2.1		H99997, hypothetical protein
KIAA1938	2.1		AA777448, KIAA1938 protein
KIAA1224	2.1		AA443116, KIAA1224 protein
EST	2.1		R33303, ESTs, weakly similar to I38022 hypothetical protein
ARIH1	2.1		AA188416, Ariadne (*Drosophila*) homolog, ubiquitin-conjugating enzyme E2-binding protein, 1
FLJ23249	2.1		AA625756, homo sapiens cDNA: FLJ23249 fis, clone COL04196
GNG11	2.1		AA999901, guanine nucleotide binding protein 11
CYP1B1	2.1		AA448157, cytochrome P450, subfamily I (dioxin-inducible), polypeptide 1 (glaucoma 3, primary infantile)
KIAA0468	2.1		AA167273, KIAA0468 gene product
FLJ11658	2.1		R99080, homo sapiens cDNA FLJ11658 fis, clone HEMBA1004577
FLJ14368	2.1		AA131421, Homo sapiens cDNA FLJ14368 fis, clone HEMBA1001122
FLJ12815	2.2		AA046679, homo sapiens cDNA FLJ12815 fis, clone NT2RP2002546
HIBADH	2.3		N77326, 3-hydroxyisobutyrate dehydrogenase
CAV1	2.4		AA055835, caveolin 1, caveolae protein, 22kD
LHFPL2	2.4		AA863469, lipoma HMGIC fusion partner-like 2
EIF3S10	2.4		AA916914, eukaryotic translation initiation factor 3, subunit 10 (theta, 150/170kD)
PRO2032	2.5		AA287122, hypothetical protein PRO2032
THBD	2.5		H59861, thrombomodulin
FLJ20783	2.7		AA126862, hypothetical protein FLJ20783
Clone_MGC_5564)	2.7		H05769, homo sapiens, clone MGC:5564, mRNA, complete cds
CLIC4	2.9		AA634261, chloride intracellular channel 4
DKFZp761K1423	3.3		AA460826, hypothetical protein DKFZp761K1423
DKFZP434J214	3.7		AA707871, DKFZP434J214 protein
D2S448	4.3		AI356709, melanoma associated gene
KIAA1474	4.7		N70608, KIAA1474 protein
AD036	4.7		AA496988, AD036 protein
FLJ22059	4.9		N36421, hypothetical protein FLJ22059
ALDH1A2	14.1		AA447978, aldehyde dehydrogenase 1 family, member A2
DKFZp761M0223	16.3		AA404249, homo sapiens mRNA; cDNA DKFZp761M0223 (from clone DKFZp761M0223)

expression has been seen as a result of β-catenin nuclear accumulation, in EC with *CTNNB1* mutations. Transcription factor *RUNX1/AML1* has been found to be upregulated in EC during invasion; and a cooperative role of ERM/ETV5 and RUNX1/AML1 during early steps of myometrial invasion has been proposed.[72]

EECs with myometrial invasion show higher number of CD163-tumor macrophages and greater microvessel density than EECs without myometrial invasion.[73] In carcinomas confined to the corpus uteri (stage I), expression of hypoxia-inducible factor 1α subunit (HIF-1A) is associated with deep myoinvasion (stage IC).[73] Also, high-grade EECs have more macrophage infiltrates and microvessels than low-grade tumors. These findings suggest that enhanced tumor angiogenesis, triggered by stromal macrophages regulates the progression of EEC.

A proteomic approach has been recently taken to characterize specific components of the invasive front or reactive stroma by comparing the invasive area of a tumor with pure tumor and normal tissue from the same patients.[74] Some of these proteins have been already described as specific of the invasive tumor front, like Fascin1 in colorectal cancer, with a transient upregulation that promotes the acquisition of migratory and invasive phenotypes that lead to metastasis. Of interest resulted the identification of different enzymes involved in oxidative stress, as SOD1 or BLVRB. Reactive oxygen species (ROS) have been recently proposed to be involved in tumor metastasis. ROS is generated and ROS targets downstream molecules to trigger tumor metastasis, especially in the initial stage that includes EMT and cell migration.

APOPTOSIS RESISTANCE IN EC

Deregulation of apoptosis plays an important role in development and progression of cancer. The lack of response to such stimuli can originate a survival advantage, and the expansion of a population of neoplastic cells. Moreover, cells resistant to apoptosis are likely to escape the immune surveillance, but they may be also resistant to therapy.

Several of the molecular abnormalities that have been detected in EC may be associated with apoptosis deregulation. EEC show a high frequency of mutations in *PTEN*, which lead to constitutively active Akt, which in turn suppresses apoptosis triggered by various stimuli. Moreover, the recent evidence that nuclear factor (NF)-kB activation is frequent in EC[75] may explain the presence of apoptosis resistance by activation of target genes such as FLIP and Bcl-XL. P53 alterations, which are characteristic of NEEC, may also occur in EEC and they may have an impact in apoptosis at several different levels. Also, members of the Bcl-2 family of genes are abnormal in EC. In EC, divergent observations have been reported with respect to Bcl-2 and Bcl-xL. Some authors have found upregulated Bcl-xL and Bcl-2 in EC compared with normal tissue and have also been reported to be involved in development of metastases. However, others described high Bcl-2 levels in initial hyperplasia but decreased expression of Bcl-2 in EC thereby indicating a restrictive role for Bcl-2 in initial steps of EC development. Many pathways can control Bcl-2 expression and typical EC molecular alterations such as those involved in exacerbated PI3K/AKT signaling could trigger Bcl-2 family members overexpression.

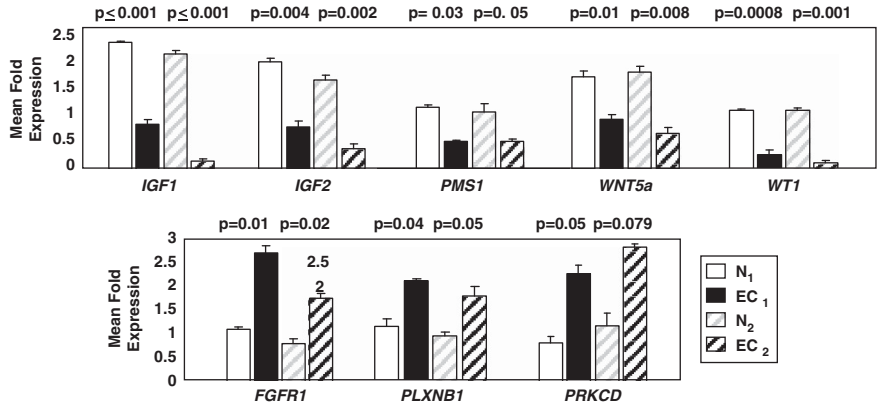

Figure 1. Validation data of selected genes in the samples used in the analysis of cDNA arrays (series 1) and in another independent series (series 2). Mean fold expression of all studied genes in EEC and semiquantitative reverse transcriptase–PCR. Significant differences between EEC and NE for each of the analyzed genes are indicated at the top of the graph.

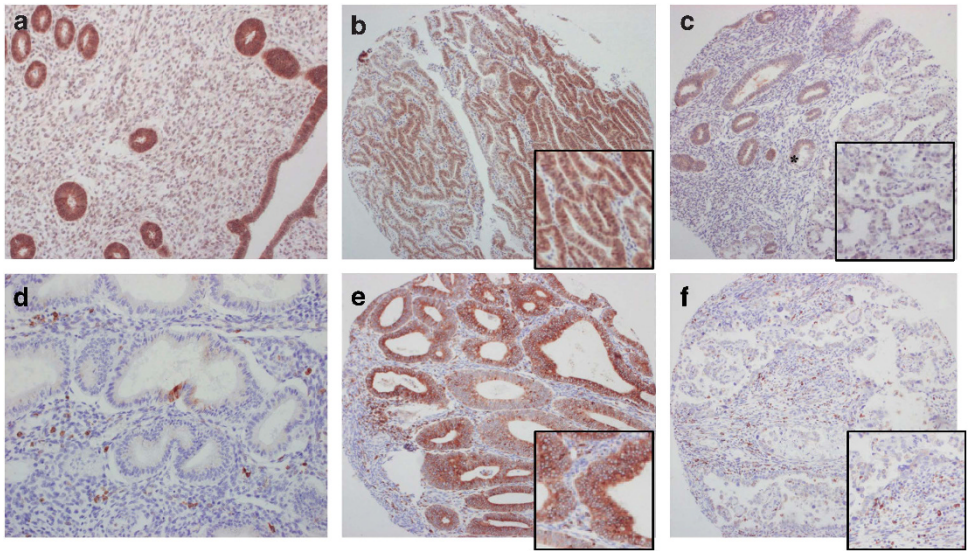

Figure 2. Immunohistochemical analysis of Smad4 (**a**–**c**), CD74 (**d**–**f**) in a tissue microarray containing 190 ECs. (**a**) Smad 4 is expressed in the nuclei and cytoplasms of both epithelial and stromal cells in this normal proliferative endometrium. (**b**) A well-differentiated EEC showing intense smad4 expression. (**c**) Absence of smad4 expression in a NEEC. Note the transition of smad4 expression in a gland containing both normal and neoplastic cells (asterisk). (**d**) CD74 expression is seen is inflammatory cells in the stroma of a normal secretory endometrium. Focal staining of some epithelial cells is also observed. (**e**) Extensive CD74 cytoplasmic expression in a well-differentiated EEC. (**f**) Absence of CD74 expression in a NEEC. Note the positivity of inflammatory cells scattered in the stroma.

Resistance to extrinsic apoptotic pathway represents an excellent acquired phenotype for progression of different types of tumor malignances. One of the most important regulators of death receptor signaling is FLIP.[76] A direct evidence of the role of FLIP in TRAIL apoptosis resistance on EC cells is provided by treatment with specific small interfering RNA targeting FLIP. Transfection of EC cell lines with FLIP small interfering RNA produces a marked decrease in cell viability after TRAIL exposition. This is accompanied by activation of both caspase-8 and caspase-3 suggesting activation of the extrinsic pathway. Moreover, in EEC FLIP can be transcriptionally regulated by casein kinase-2 (CK2), a Ser/Thr kinase implicated in development and progression of many neoplasias. This data further points to CK2 as an important modulator of TRAIL sensitivity.[67,68] In fact, CK2 β regulatory subunit has been found overexpressed in EC compared with normal tissue and to regulate cell proliferation and anchorage-independent cell growth. Recent studies have shown that FLIP may be regulated by a cellular complex composed by CK2–BRAF–KSR1.[77–80] This is interesting because that will connect apoptosis resistance with the RAS–RAF–MEK–ERK signaling pathway.

The kinase suppressor of RAS 1 (KSR1) is considered a scaffold protein that interacts and regulates the intensity and duration of mitogen-activated protein kinase pathway. KSR1 can interact with different kinases of the RAS–RAF–MEK–ERK signaling pathway to enhance its activation. KSR1 is critical for Ras-induced transformation by active forms of Ras both *in vitro* and *in vivo*. KSR1 regulation of RAS–RAF–MEK–ERK has also been involved in modulation of apoptotic response to death receptors. It has been recently demonstrated that the expression of KSR1 is increased in EC suggesting a possible role in endometrial carcinogenesis. Inhibition of KSR1 expression by lentiviral delivered short hairpin RNA in ECCs resulted in a marked reduction of both proliferation and anchorage-independent cell growth properties of ECCs. Interestingly, inhibition of KSR1 expression sensitized resistant ECC lines to both TRAIL- and Fas-induced apoptosis by a mechanism dependent on downregulation of FLIP.

Apoptosis may be also subjected to targeted therapy. Proteasome inhibitors are currently used as chemotherapeutic drugs because of their ability to trigger cell growth arrest or apoptosis on several tumors. In many different types of tumor cells, Bortezomib and other proteasome inhibitors cause cell death by blocking NF-kB activity. However, in EC, proteasome inhibitors induce cell death, but, instead of blocking NF-kB, they increase its transcriptional activity. Proteasome-inhibitor induced cell death was accompanied by activation of caspases and apoptotic nuclear morphology.[81] Sorafenib was originally described as an inhibitor of b- and c-RAF kinase, but has also activity against several receptor tyrosine kinases, including vascular endothelial growth factor receptor 2, platelet-derived growth factor receptor, FLT3, Ret and c-Kit. There is recent evidence showing that Sorafenib sensitize EC cells to TRAIL-induced apoptosis, by downregulating FLIP, and Mcl-1.[77]

Table 2. Relationship between SMAD4, and CD74 expression and clinicopathological features in endometrial carcinoma

	SMAD 4 downregulation	*CD74 overexpression*
	n/n_t (%)	
Type:		
EEC	56/99 (56.6)	103/119 (86.6)
NEEC	19/32 (59.4)	20/34 (58.8)
	$P = 0.780$	$P \leqslant 0.001$
EEC		
FIGO grade:	19/36 (52.8)	41/43 (95.3)
G1 ($n = 36$)	19/36 (52.8)	41/45 (91.1)
G2 ($n = 36$)	18/29 (42.9)	20/32 (62.5)
G3 ($n = 29$)	$P = 0.697$	$P \leqslant 0.001$
Stage:		
I	32/65 (49.2)	71/79 (51.4)
II	57/11 (45.5)	11/14 (78.6)
III-IV	3/ 7 (42.9)	5/8 (62.5)
	$P = 0.932$	$P = 0.069$
NEEC stage:		
I	4/12 (33.3)	7/13 (53.8)
II	5/ 7 (71.4)	4/7 (57.1)
III-IV	9/10 (90.0)	7/10 (70.0)
	$P = 0.020$	$P = 0.724$

Abbreviations: EEC, endometrioid endometrial carcinoma; *n*, positive cases number; NEEC, non-endometrioid endometrial carcinoma; n_t, total evaluate cases.

RESISTANCE TO HYPOXIA AND TO IONIZING RADIATION TREATMENT

EC are treated by means of surgery with additional radiation. Although a high percentage of patients with EC present a favorable outcome, treatment fails in those with either advanced stage or high histological grade EC. Post radiotherapy recurrences are usually of poor prognosis, as they are usually associated with increased risk of metastases. They are treated by chemotherapy. Understanding the molecular and genetic mechanisms underlying either radio and or chemotherapy treatment resistance is of crucial importance for the establishment of new therapeutic targets, in order to improve the outcome of EEC.

Among the mechanisms in radioresistance, tumor hypoxia has been demonstrated to render tumors more resistant to ionizing radiation treatment. By reacting with the radiation-created broken ends of DNA, the oxygen fixes the damage and thus enhances radiation induced cell death. This phenomenon is known as the oxygen enhancement effect, which could render oxygenated cells three times more radiosensitive than hypoxic cells. Under hypoxic conditions, the oxygen enhancement effect is lost, and cells become more radioresistant (Figure 4). Although the absence of oxygen is the major factor inducing radioresistance under hypoxic conditions, there are increasing evidences showing that signaling pathways activated under hypoxia may modulate cancer cells radioresistance.

Some investigators have addressed the molecular mechanisms involved in resistance to radiotherapy in EC. PR expression and polymorphisms in the gene coding for PR seem to play an important role.[82] Defective mismatch repair has also been looked at in this setting. In one study,[83] *MLH1* promoter methylation and decreased MLH1/MSH2 expression were not predictive of recurrence in stage I EC, but '*de novo*' *MLH1* promoter methylation was occasionally detected during tumor progression in patients receiving radiation therapy. In another study,[84] alterations in the *P53*-suppressor gene were assessed in a series of patients with ECs with and without recurrences. Finally, three components of the Wnt pathway (APC, β-catenin and E-cadherin) were evaluated in a small series of patients with stage I EC in correlation with development of recurrence.[85] Important information has been obtained regarding the mechanisms of resistance to radiation, by comparing by immunohistochemistry tissue microarrays from post-radiation recurrences of EC with a group of primary EC.[86] Results have revealed that post-radiation recurrences exhibited increased expression β-catenin. In recent work, it has been showed that hypoxia-induced β-catenin nuclear translocation in EC Ishikawa and HEC-1A cell lines. Moreover, hypoxia induced

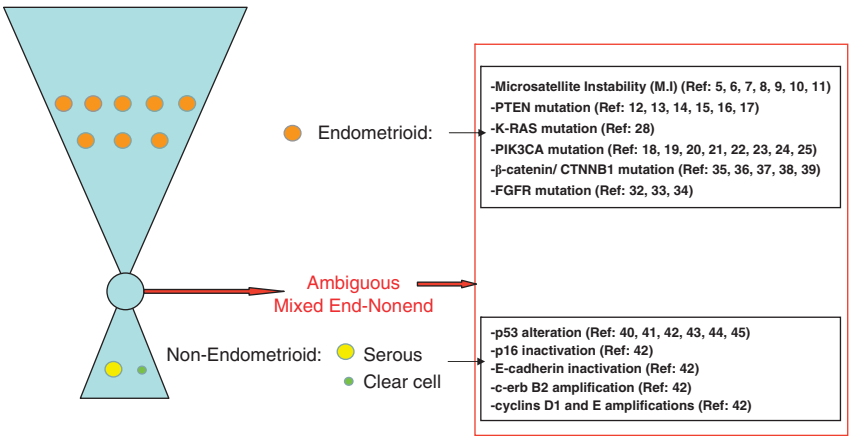

Figure 3. There are many different types of EC; type I, endometrioid carcinoma, type II, non-endometrioid carcinoma, but also tumors showing mixed and overlapping features.

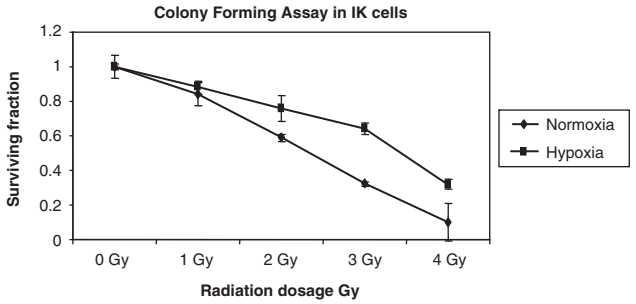

Figure 4. Hypoxia induces resistance to radiotherapeutic treatment: Ishikawa (EC cell line) cells were seeded onto six-well plates. Cells were either exposed to hypoxic conditions (1% O_2) or maintained under normoxia (21% O_2) for 6 h, and then irradiated at the indicated doses. Cells were then cultured for additional 14 days to allow colony formation. After staining with MTT, the colony numbers were counted. The graphic shows how the presence of oxygen enhances sensitivity to ionizing radiation therapy.

an increase in TCF-4 reporter (Wnt reporter) activity in both EC cell lines previously cited.

HIF-1α is another candidate that could be involved in conferring radioresistance to EC cells. HIF-1 is the most important mediator of hypoxia, as it controls the expression of >100 genes. It has been recently shown that HIF-1α expression is increased in post-radiation recurrences compared with primary EC, and that HIF-1α controlled classical NF-κB activation pathway and survival under hypoxia through RelA (p65) nuclear accumulation.[87] Moreover, in addition to the reported classical NF-kB activation pathway under hypoxia, we have found that the alternative NF-κB pathway is also activated under hypoxic conditions through HIF-1α-independent pathway. Although IKKα and IKKβ kinases control RelA(p65) and p100 accumulation, p52 processing under hypoxia is exclusively IKKα dependent. Both classical and alternative NF-κB pathways enhanced EC cell survival under low oxygen tension. These results may have a clinical application, as targeting the signaling pathways on which hypoxic cell survival depends, may enable oncologists to overcome tumor progression and resistance to therapy.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This work was supported by grants from FIS PI100922, Fundación Mutua Madrileña AP75732010, FIS PI080410, 2009SGR794, RD06/0020/1034, RD06/0020/0013, RD06/0020/0058, RD06/0020/0015, Fundación Asociación Española contra el Cancer and programa de intensificación de la investigación, Instituto Carlos III. AY holds a postdoctoral fellowship from Programa Juan de la Cierva, Ministerio de Ciencia e Innovación (JCI-2008-1969).

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