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Advances in Brief

Differential Gene Expression Profile in Endometrioid and Nonendometrioid Endometrial Carcinoma: *STK15* Is Frequently Overexpressed and Amplified in Nonendometrioid Carcinomas[1]

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Abstract

Endometrial carcinoma (EC) comprises at least two types of cancer: endometrioid carcinomas (EECs) are estrogen-related tumors, which are frequently euploid and have a good prognosis. Nonendometrioid carcinomas (NEECs; serous and clear cell forms) are not estrogen related, are frequently aneuploid, and are clinically aggressive. We used cDNA microarrays containing 6386 different genes to analyze gene expression profiles in 24 EECs and 11 NEECs to identify differentially expressed genes that could help us to understand differences in the biology and clinical outcome between histotypes. After supervised analysis of the microarray data, there was at least a 2-fold difference in expression between EEC and NEEC in 66 genes. The 31 genes up-regulated in EECs included genes known to be hormonally regulated during the menstrual cycle and to be important in endometrial homeostasis, such as *MGB2*, *LTF*, *END1*, and *MMP11*, supporting the notion that EEC is a hormone-related neoplasm. Conversely, of the 35 genes overexpressed in NEECs, three genes, *STK15*, *BUB1*, and *CCNB2*, are involved in the regulation of the mitotic spindle checkpoint. Because *STK15* amplification/overexpression is associated with aneuploidy and an aggressive phenotype in other human tumors, we used fluorescence *in situ* hybridization to investigate whether *STK15* amplification occurred in ECs. We found that *STK15* was amplified in 55.5% of NEECs but not in any EECs ($P \leq 0.001$). We confirmed this result in an independent series of ECs included in a tissue microarray in which breast and ovarian cancer samples showed an incidence of *STK15* amplification of 15 and 18%, respectively ($P \leq 0.001$). This study demonstrated the usefulness of cDNA microarray technology for identifying differences in gene expression patterns between histological types of EC and implies that alteration of the mitotic checkpoint is a major mechanism of carcinogenesis in NEECs.

Introduction

EC[3] is the most common gynecological malignant tumor in Europe and the United States (1). Clinicopathological, epidemiological, and molecular studies have demonstrated that EC comprises at least two different types of tumor (2). Type I ECs are frequently well-differentiated endometrioid carcinomas that usually develop in pre- and perimenopausal women. They are associated with estrogen stimulation, coexist with, or are preceded by atypical endometrial hyperplasia and are associated with ER positivity and with *K-RAS*, *PTEN*, and *β-catenin* mutations, and microsatellite instability. Conversely, type II tumors are NEECs (papillary serous and clear cell carcinomas) that occur in older women. They are unrelated to estrogen exposure and develop from atrophic endometrium through the so-called endometrial intraepithelial carcinoma. They are associated with *p53* mutations, are ER negative, and have a high degree of chromosomal instability (2).

Large-scale gene expression analysis using high-density cDNA or oligonucleotide arrays is an effective strategy for determining gene expression profiles that may be used for classifying tissues by pathological status. Several recent studies have reported differential gene expression between normal and neoplastic endometrium and between histological types of EC (3, 4). However, because to date these gene expression analyses have been performed on arrays containing a limited set of genes, more studies are needed to characterize ECs and their different histiotypes more accurately. The aim of this study was to determine gene expression profiles in 24 EECs and 11 NEECs by cDNA array to identify genes differentially expressed between histiotypes that could improve our understanding of their specific biological and clinical characteristics.

Materials and Methods

Tissue Samples. We used 24 endometrioid carcinomas that had been previously pathologically and molecularly characterized (5) and 11 NEECs (4 clear cell and 7 serous tumors). These tumors were diagnosed in the Pathology Department of Sant Creu and Sant Pau (Barcelona, Spain) and in the Hospital Materno Infantil (Las Palmas, Spain). Sections of frozen samples were split for confirmatory histology by H&E staining and RNA isolation.

RNA Extraction, Probe Synthesis, and Hybridization on cDNA Arrays. Total RNA was isolated using TRIZOL reagent (Life Technologies, Inc., Gaithersburg, MD) as indicated by the manufacturer. Purity of isolated RNA was evaluated spectrophotometrically by the A_{260}/A_{280} absorbance ratio. Three μg of total RNA from endometrial samples and Universal Human Reference RNA (Stratagene), used as control, and T7-$(dThd)_{24}$ oligo primer were used to amplify the double strand cDNA synthesis by the Superscript Choice System (Life Technologies, Inc.). *In vitro* transcription was conducted with Megascript T7 (Ambion, Austin, TX). Amplified RNA was obtained and purified using TRIZOL reagent, and the integrity was measured spectrophotometrically by the A_{260}/A_{280} ratio or by gel electrophoresis. Three μg of amplified RNA was used to generate fluorescence antisense RNAs by transcriptional synthesis using SuperScript enzyme protocol (Life Technologies, Inc.). All of the endometrial samples were labeled with Cy5-dUTP fluorochrome (Amersham, Uppsala, Sweden), and the reference pool was labeled with Cy3-dUTP fluorochrome (Amersham) as described previously (6, 7).

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[3] The abbreviations used are: EC, endometrial carcinoma; NEEC, nonendometrioid carcinoma; EEC, endometrioid carcinoma; FISH, fluorescence *in situ* hybridization; BAC, bacterial artificial chromosome; LTF, lactotransferrin; ER, estrogen receptor.

Hybridization was performed in 4× SSC, 1× BSA, 2 μg/ml DNAs, and 0.1% SDS at 42°C for 15 h. Slides were washed, dried, and then scanned in a Scanarray 5000 XL scanner (GSI Lumonics, Kanata, Ontario, Canada) at wavelengths of 635 and 532 nm for Cy5 and Cy3 dyes, respectively, to obtain 10-μm resolution images, which were quantified using the GenePix Pro 4.0 program (Axon Instruments, Inc., Union City, CA).

The cDNA array chip is a new version of the CNIO Oncochip (7) manufactured by the CNIO Genomic Unit (http://bioinfo.cnio.es/data/oncochip). This version contains 9726 clones corresponding to 6386 different genes. The chip includes 2489 clones that have been printed in duplicate to assess reproducibility.

cDNA array hybridization was performed in duplicate in all cases, using reciprocal labeling. In these experiments, the endometrial samples were labeled with dUTP-Cy3, and the reference pool was labeled with dUTP-Cy5. Duplicate comparisons showed correlation coefficients of 0.93–0.97 (data not shown).

Data Analysis. Fluorescence intensity measurements from each array element were compared with the median of local background in each channel, and the elements with values less than this median were excluded. In addition, all spots smaller than 25 μm were manually deleted. After these filters were applied, a total of 7921 spots were evaluated. The expression ratios of the duplicated spots on the array were averaged. For statistical analysis, we selected genes with expressions that differed by a factor of at least 2-fold with respect to the reference pool in 30% of patients. This selects genes with large variation in expression levels across the 35 patients and ensures that the genes considered do show relevant differences with respect to the pool, at least doubling or halting of expression levels, so that the genes considered can be regarded as effectively repressed or overexpressed. By requiring that the repression or overexpression be shown by at least 30% of the patients, we make sure that the patterns found are not spurious results from just a few outlying patients.

To find a set of genes that were differentially expressed in EEC ($n = 24$) and NEEC ($n = 11$) endometria, we used Welch's t test, which does not require equal variances between groups (8). However, because we were testing for differential expression of many genes, we needed to account for multiple testing to avoid an excessive number of false positive results. Thus, we used the step-down maxT method (9, 10).[4] This method controls the family-wise error rate but is more powerful than traditional single-step procedures (such as the Bonferroni) because it takes into account the order of the *P*s, makes successively smaller adjustments, and also considers covariance between genes. Because the sample size was small, the adjusted *P*s were obtained by random permutation using 50,000 random permutations. We considered genes to be differentially expressed in the two groups if their adjusted P was ≤ 0.05. Statistical comparison was performed with the POMELO program (http://www.genoma.wi.mit.edu/MPR/software). The SOTA and TreeView programs (http://bioinfo.cnio.es/cgi-bin/tools/clustering/sotarray) were used for clustering analysis, assuming euclidean distances between genes.

Quantitative Real Time PCR. Quantitative real time PCR (TaqMan) was conducted to validate data of cDNA microarrays of selected genes. Analysis was performed with the ABI PRISM 7700 Sequence Detection System Instrument and software (Applied Biosystems, Foster City, CA), using the manufacturer's recommended conditions. Each reaction was performed in triplicate from two cDNA dilutions. TaqMan reactions for target and internal control genes were performed in separate tubes. The comparative threshold cycle (C_t) method was used to calculate the amplification factor as specified by the manufacturer. The internal standard human glucuronidase (*GUS*; Applied Biosystems) was used to normalize variations in RNA quality in the quantities of input cDNA. The amount of target and endogenous reference was determined from a standard curve for each experimental sample. The standard curve was constructed by 5-fold serial dilutions of cDNA from 0.5 μg. The sequence of oligonucleotides and TaqMan probes used for the analysis of: *DEK*, *BUB1*, *STK15*, *MGB2*, *CTNNB1*, and *MYC* were obtained using the Assays-by-Design (SM) File Builder program (Applied Biosystems).

Tissue Microarrays. Representative areas from 200 endometrial, ovary and breast cancers of different histological types (see Results and Discussion) were carefully selected on H&E-stained sections and marked on individual paraffin blocks. Two 1-mm-diameter tissue cores were obtained from each specimen. The tissue cores were precisely arrayed in a new paraffin block using a tissue microarray workstation (Beecher Instruments, Silver Spring, MD) as previously described (11). An H&E-stained section was reviewed to confirm the presence of morphologically representative areas of the original lesions.

FISH of *STK15* Gene. We used 4-μm sections of all tissue microarrays for FISH analysis, which was performed using two different probes simultaneously. For the detection of *STK15* amplification we used the BAC RP5-1167H4, from the Human BAC Clone Library RPC5 (Children's Hospital Oakland Research Institute, Oakland, CA), which spans the entire *STK15* genomic region. We used a commercial probe for chromosome 20 (CEP 20; Vysis, Downer's Grove, IL) as a control for the ploidy level of chromosome 20. The slides were deparaffinized, boiled in a pressure cooker with 1 mM EDTA (pH 8.0) for 10 min, and incubated with pepsin at 37°C for 30 min. The slides were then dehydrated. The probes were denatured at 75°C for 2 min after overnight hybridization at 37°C in a humid chamber. Slides were washed with 0.4× SSC and 0.3% NP40. The FISH analysis was performed by two investigators (S. R. and J. C. C.) who had no prior knowledge of the genetic, clinical, or immunohistochemical analysis results. Fluorescence signals were scored in each sample by counting the number of single-copy gene and centromeric signals in an average of 130 (60–210) well-defined nuclei. Amplification was defined as the presence (in >5% of tumor cells) of either >10 gene signals or >3 times as many gene signals as centromere signals of chromosome 20. Cutoff values for the copy number changes were obtained from the analysis of normal adjacent epithelia in each experiment.

Results and Discussion

We analyzed 35 endometrial tumors (24 EECs and 11 NEECs) and hybridized them against a Universal Reference RNA pool in cDNA arrays containing 6386 genes represented by 9726 clones. Our main aim was to identify differences in the gene expression profile between histiotypes, so we did not use any normal endometrial samples, because the expression profile of the endometrium varies depending on the hormonal status of women (proliferative, secretory, and atrophic). After array processing, 549 genes were identified as at least 2-fold up- or down-regulated with respect to the reference sample (data not show). Using unsupervised analysis, we identified differences in the gene expression pattern between EECs and NEECs. The hierarchical cluster had two major branches, one containing most of the EECs and the other most of the NEECs. Other subbranches grouped the rest of the EECs, suggesting that different genes are involved in development in EECs and NEECs (Fig. 1*A*). We observed that the serous and clear cell carcinomas were grouped in the same main branch but produced two separate subgroups. Although, this may indicate differences in gene expression profile between these two types of EC, as previously reported (4), supervised analysis did not reveal any gene that differed significantly between these tumor types. Therefore, they were lumped for subsequent analysis. To compare pairs of conditions based on individual histology, we examined only the set of genes that had already been identified as differing significantly between conditions (adjusted $P < 0.05$) (12, 13). Sixty-six genes showed a statistically significant difference in expression between the 2 tumor histological types (Table 1), including 31 genes up-regulated in EECs and 35 genes up-regulated in NEECs (Fig. 1*B*). To validate the quality of our array data, a subset of four genes (*BUB1*, *DEK*, *STK15*, and *MGB2*) differentially expressed between histiotypes and 2 genes the expression of which did not differ (*MYC*, and *CTNNB1*) were examined in all samples using TaqMan PCR. This confirmed the array results (Fig. 1*C*).

Genes up-regulated in EECs included some involved in cell secretion (*MGB2*, *LTF*, *END1*, *END3*), adhesion (*CTNNA1*), extracellular matrix remodeling (*HSPG2*, *MMP11*), transcription (*NFYC*, *HOXB5*, *CHD3*, and *REST*), and other basic cellular functions (*PPAP2C*). Interestingly, the most up-regulated genes in EEC were secretory

[4] S. Dudoit, J. P. Shaffer, and J. C. Boldrick. Multiple hypothesis testing in microarray experiments, submitted for publication.

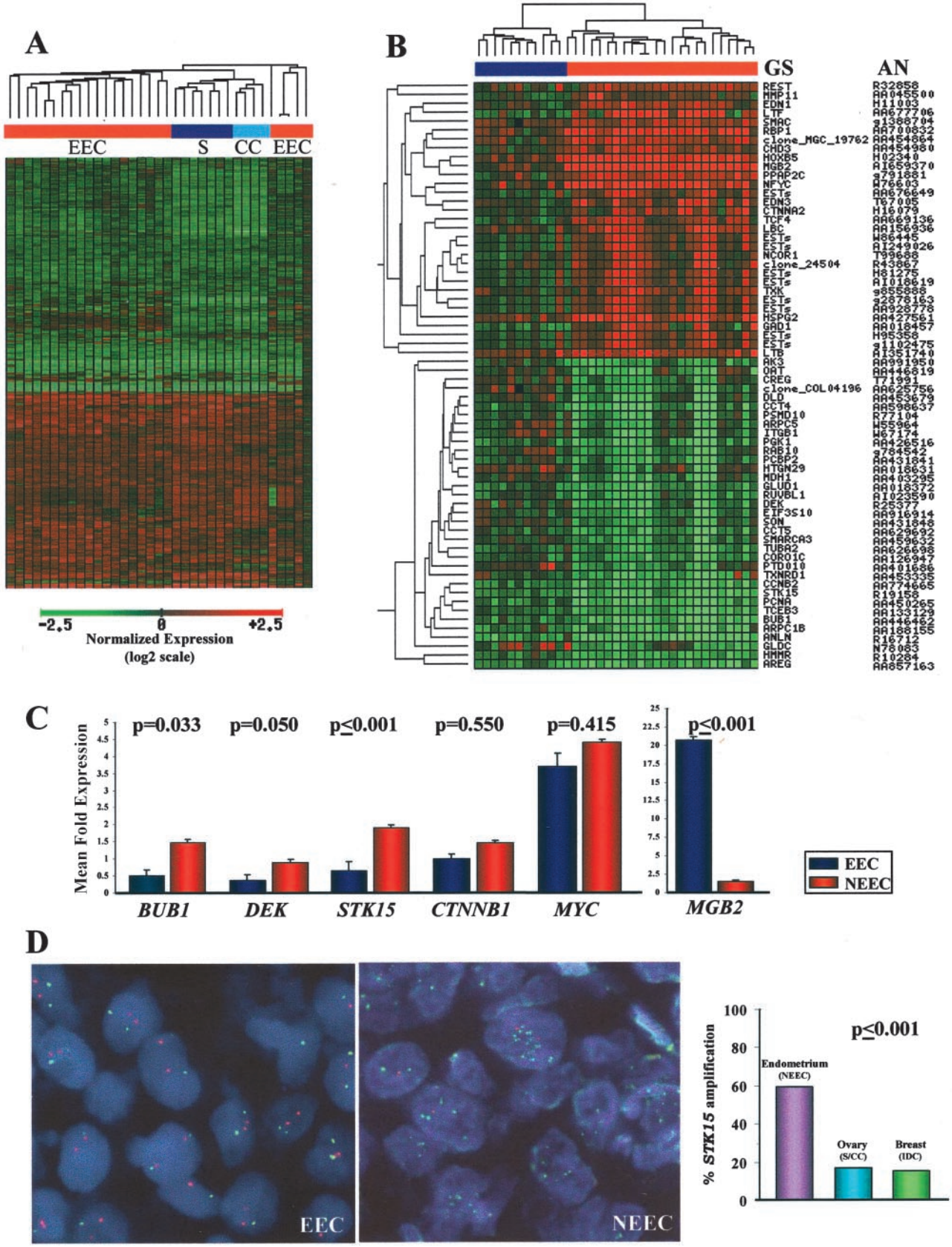

Fig. 1. Gene expression profile in ECs. *A*, unsupervised analysis of 35 tumor samples: 24 EECs and 11 NEECs. *S*, serous; *CC*, clear cell). With this analysis, we found 549 gene modified at least 2-fold with respect to reference pool. *B*, hierarchical clustering of 66 genes with differential expression between EECs and NEECs ($P \leq 0.05$) using a 2-fold threshold. The symbol for each gene (*GS*) and the Gene Bank accession number (*AN*) of the clones spotted into the cDNA array are indicated on the right. *C*, validation data of selected genes using quantitative real time RT-PCR. Statistical significance between tumor classes for each of the analyzed genes is indicated at the top. *D*, FISH analysis of *STK15* in neither of 24 EECs and in 9 of 15 NEECs. The graph shows the percentage of STK15 amplification in 9 of 15 (60%) NEECs, 7 of 49 (15.2%) ovarian carcinomas, and 7 of 53 (13.2%) breast carcinomas. *IDC*, infiltrating ductal carcinomas.

Table 1 *Genes differentially expressed in endometrial carcinomas ($P \leq 0.05$)*

Gene name	Fold	Description (accession no.)
Genes up-regulated in EECs		
Secretory proteins		
MGB2	10.4	Mammaglobin 2
LTF	6.5	Lactotransferrin
END3	2.4	Endothelin 3
END1	2.1	Endothelin 1
Cell adhesion		
CTNNA2	2.3	Catenin (cadherin-associated protein), α2
Cell death		
SMAC	2.1	Second mitochondrion-derived activator of caspase
Extracellular matrix		
HSPG2	2.5	Heparan sulfate proteoglycan 2 (perlecan)
MMP11	2.1	Matrix metalloproteinase 11 (stromelysin 3)
Immune response/cytokines		
LTB	2.1	Lymphotoxin β (TNF superfamily, member 3)
Metabolism		
GAD1	3.2	Glutamate decarboxylase 1 (brain, 67 kDa)
Oncogene		
LBC	2.2	Lymphoid blast crisis oncogene
Transport		
RBP1	3.1	Retinol-binding protein 1, cellular
Transcription- related		
NFYC	3.9	Nuclear transcription factor Y, γ
HOXB5	3.8	Homeobox B5
TCF4	2.6	Transcription factor 4
NCOR1	2.2	Nuclear receptor corepressor 1
CHD3	2.2	Chromodomain helicase DNA-binding protein 3
REST	2.1	RE1-silencing transcription factor
Basic cellular function		
PPAP2C	3.1	Phosphatidic acid phosphatase type 2C
TXK	2.1	TXK tyrosine kinase
Miscellaneous		
ESTs	2.9	ESTs (AA676649)
ESTs	2.6	ESTs (AI018619)
ESTs	2.5	ESTs (AI249026)
ESTs	2.4	ESTs (H81275)
Clone 24504	2.4	*Homo sapiens* clone 24504 mRNA sequence (R43867)
ESTs	2.3	ESTs, weakly similar to FYB_human FYN-binding protein *[H. sapiens]* (H95358)
ESTs	2.3	ESTs (AA928778)
Clone MGC:19762	2.2	*H. sapiens*, clone MGC: 19762 IMAGE: 3636045, mRNA, complete cds (AA454864)
ESTs	2.1	ESTs (g1102475)
ESTs	2.1	ESTs (W86445)
ESTs		ESTs, weakly similar to S59501 interferon receptor JFNAR 2-1 *[H. sapiens]* (g2878163)
Genes up-regulated in NEECs		
Cell adhesion		
ITGB1	2.8	Integrin, β_1 (fibronectin receptor, β-polypeptide, antigen CD29 includes MDF2, MSK12)
Oncogene		
DEK	2.6	DEK oncogene (DNA binding)
Cell cycle		
CCNB2	2.6	Cyclin B2
STK15	2.4	Serine/threonine kinase 15
BUB1	2.3	Budding uninhibited by benzimidazoles 1 (yeast homologue)
PCNA	2.3	Proliferating cell nuclear antigen
Cell death		
SON	2.3	SON DNA-binding protein
Cell growth		
AREG	2.6	Insulin-like growth factor 1 (somatomedin C)
RUVBL1	2.1	Amphiregulin (schwannoma-derived growth factor) RuvB (*Escherichia coli* homologue)-like 1
Cell motility		
CORO1C	2.3	Coronin, actin-binding protein, 1C
HMMR	2.1	Hyaluronan-mediated motility receptor (RHAMM)
Cytoskeleton		
ARPC5	2.6	Actin-related protein 2/3 complex, subunit 5 (16 kDa)
ANLN	2.4	Anillin (*Drosophila* scraps homologue), actin-binding protein
ARPC1B	2.2	Actin-related protein 2/3 complex, subunit 1A (41 kDa)
TUBA2	2.1	Tubulin, α_2
Metabolism		
GLDC	3.3	Glycine dehydrogenase (decarboxylating; glycine decarboxylase, glycine cleavage system protein P)
DLD	2.5	Dihydrolipoamide dehydrogenase (E3 component of pyruvate dehydrogenase complex, 2-oxoglutarate complex, branched chain keto acid dehydrogenase complex)
MDH1	2.5	Malate dehydrogenase 1, NAD (soluble)
OAT	2.3	Ornithine aminotransferase (gyrate atrophy)
PGK1	2.1	Phosphoglycerate kinase 1
AK3	2.1	Adenylate kinase 3
GLUD1	2.1	Glutamate dehydrogenase 1
Transport		
RAB10	2.6	RAB10, member RAS oncogene family
Transcription related		
CREG	2.4	Cellular repressor of E1A-stimulated genes
TCEB3	2.2	Transcription elongation factor B (SIII), polypeptide 3 (110 kDa, elongin A)
SMARCA3	2.1	SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily a, member 3
Basic cellular function		
CCT4	2.7	Chaperonin-containing TCP1, subunit 4 (δ)
TXNRD1	2.2	Thioredoxin reductase 1
PSMD10	2.1	Proteasome (prosome, macropain) 26S subunit, non-ATPase, 10 poly(rC)-binding protein 2
PCBP2	2.1	Chaperonin-containing TCP1, subunit 5 (ε)
CCT5	2.1	Eukaryotic translation initiation factor 3, subunit 10 (θ, 150/170 kDa)
EIF3S10	2.1	
Miscellaneous		
Clone COL04196	2.8	*H. sapiens* cDNA: FLJ23249 fis, clone COL04196 (AA625759)
PTD010	2.6	PTD010 protein (AA401686)
HTGN29	2.3	HTGN29 protein (AA018631)

proteins, some of which were hormonally regulated, supporting the notion of EEC as a hormonally driven neoplasia, in contrast to NEEC. Mammaglobin 2 (*MGB2*) was the most up-regulated gene found in EECs: ~10-fold with respect to NEECs. *MGB2* is a protein initially characterized in rat uterus and human endometrium (14, 15) that is up-regulated during the endometrial window of implantation (3) and is principally involved in glandular secretions in hormone-responsive tissues (14). *MGB2* was overexpressed in primary breast tumors, and in stomach and colon carcinomas (16). *LTF* was the second most up-regulated gene (6.5-fold) in EECs. LTF is an iron-binding glycoprotein present in most exocrine secretions. Initially, Walmer *et al.* (17) described that *LTF* mRNA and protein were expressed in the uterus. Subsequent studies demonstrated that LTF was frequently overexpressed in EC (16). These studies also provide evidence of a role for LTF in endometrial carcinogenesis, supported by the *in vitro* evidence that LTF promotes cell proliferation of normal and neoplastic endometrial cells (18). Other secretory proteins included two members of the endothelin proteins, END1, END3. Endothelin-1 and its mRNA are present in human endometrium. Human endometrial epithelial cells are the major source of endothelin-1, and its expression varies throughout the menstrual cycle, provoking powerful contractile actions in myometrium and other types of smooth muscle. It is also mitogenic or comitogenic for fibroblasts, vascular smooth muscle and other cells. Human endometrial adenocarcinoma cells express endothelin-1 (19). Endothelin-3 has been reported to be down-regulated in the endometrium during the implantation window (3).

ER-mediated transcriptional activity in hormone-dependent tissues and tumors is influenced by several regulatory factors know as coactivators and corepressors, which, respectively, activate or repress the transcription of ER-responsive genes (20). *NCOR1* is an ERα corepressor gene, with levels of expression that are associated with those of ERα in breast carcinoma (20). In addition, low *NCOR1* expression was associated with significantly shorter relapse-free survival in thin neoplasm (21). In accordance with these observations, differences in the level of *NCOR1* expression in EECs and NEECs might be associated with the high level of ER in EECs.

Thirty-five genes were overexpressed in NEECs relative to EECs, including genes involved in control of the cell cycle and mitosis (*STK15*, *BUB1*, *CCNB2*, *PCNA*), oncogenesis (*DEK*), metabolism (*MDH1*, *PGK1*, *GLDC*), transcription (*CREG*, *TCEB3*, *SMARCA3*), and/or transport (*RAB10*). A well-recognized characteristic of NEECs is the higher frequency of aneuploidy than in EEC. Defects in the mitotic spindle checkpoint genes have been implicated in aneuploidy in human neoplasias. Thus, three genes involved in the regulation of the mitotic spindle checkpoint (*STK15*, *BUB1*, and *CCNB2*) were overexpressed in NEEC. It is important that the high level of expression of *STK15* is thought to disrupt the signaling cascade that regulates equal segregation of chromosomes, leading to pronounced aneuploidy and an aggressive phenotype in some human tumors, such as breast, gastric, and bladder carcinomas (22). These *in vivo* observations were consistent with *in vitro* studies demonstrating that *STK15* overexpression overrides the mitotic spindle assembly checkpoint, inducing centrosome amplification, aneuploidy, and transformation (23). Interestingly, elevated *STK15* activity works to trigger mitotic abnormalities through the *BUB1* gene (24); thus, it is not surprising that both genes were overexpressed in our sample of NEECs.

Although the mechanisms of *STK15* overexpression are not fully understood, gene amplification has been observed in several human carcinoma cell lines (22) and primary tumors (25). Thus, *STK15* amplification has been reported in ~5, 13, and 15% of primary gastric, breast, and ovary carcinomas, respectively (22, 25, 26). To determine whether the *STK15* up-regulation in this series of NEECs was attributable to gene amplification, we examined the gene status by FISH, using the specific *STK15* BAC RP5-1167H4. We found that 5 of 9 (55.5%) NEECs available for FISH analysis showed gene amplification, whereas none of the 20 EECs did.

To establish whether this high percentage of *STK15* amplification was characteristic of NEECs or was a property of the series itself, we constructed a tissue microarray that included a new set of ECs with previously reported clinicopathological and molecular characteristics. We also arrayed breast and ovary carcinomas as a control, because the frequency of *STK15* amplification has been previously determined in these tumor types.

FISH analysis gave valuable results in 141 of 200 (70%) arrayed cases. Nonvaluable cases were attributable to cores lost during processing, inadequate morphology, or lack of probe hybridization. We found *STK15* amplification in 9 (2 clear cell, 4 serous, and 3 clear cell/serous carcinomas) of 15 NEECs (60%) but in none of the 24 EECs analyzed ($P \leq 0.001$; Fig. 1*D*). *STK5* amplification was found in 7 of 53 (13.2%) valuable breast carcinomas and in 7 of 49 (15.2%) ovarian carcinomas. These figures are similar to those previously reported (23, 24). *STK15* amplification in breast and ovary carcinomas was observed only in some histological types. The 7 amplified breast carcinomas were infiltrating ductal carcinomas but amplification was not observed in lobular ($n = 7$), papillary ($n = 3$), or colloid ($n = 2$) infiltrating carcinomas. *STK15* was amplified in ovarian carcinomas (4 of 15 clear cell carcinomas and 3 of 24 serous carcinomas) but not in the 7 mucinous and endometrioid carcinomas. The percentages of *STK15* amplification in NEECs (60%), infiltrating ductal carcinomas of the breast (15%), and serous/clear cell carcinomas of the ovary (18%) are significant different ($P \leq 0.001$; Fig. 1*D*).

Although we do not know the cause of the high frequency of *STK15* amplification in NEEC, it might be associated with the high frequency of p53 alterations present in this tumor type. It is well known that loss of wild-type p53 predisposes cells to chromosomal instability. Furthermore, certain mutant p53 proteins have oncogenic potential and increase the frequency of gene amplification by interacting with topoisomerase I (27). Likewise, we have found the frequency of *CCNE* and *CCND1* amplification to be significantly higher in NEECs than in EECs (28).

In summary, cDNA array technology is a powerful tool for identifying differences in gene expression patterns between histological types of EEC and for identifying genes involved in specific pathways of carcinogenesis. Alteration of the mitotic checkpoint secondary to *STK15* amplification/overexpression seems to be a major mechanism of carcinogenesis in NEEC.

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