Identification and partial characterization of differentially expressed mRNAs in normal human endometria and endometrial carcinomas by differential display RT–PCR

C.Foca¹,², G.E.Rice¹,², M.A.Quinn³ and E.K.Moses¹,²,⁴

¹Perinatal Research Centre, Department of Perinatal Medicine, The Royal Women's Hospital, Carlton, Victoria, 3053, ²Department of Obstetrics and Gynaecology, University of Melbourne, Parkville, Victoria, 3053, and ³Professorial Unit, The Royal Women's Hospital, Carlton, Victoria, 3053, Australia
⁴To whom correspondence should be addressed at: Perinatal Research Centre, Department of Perinatal Medicine, The Royal Women's Hospital, 132 Grattan Street, Carlton 3053, Victoria, Australia. E-mail: emoses@ariel.its.unimelb.edu.au

Introduction

Carcinoma of the endometrium is the most commonly diagnosed invasive cancer of the female reproductive tract, with the most common clinical presentation of disease being post-menopausal bleeding or irregular bleeding. The standard treatment for endometrial carcinoma is abdominal hysterectomy and bilateral salpingo–oophorectomy (Magrina et al., 1999) and a combination of radiotherapy (Jereczek-Fossa et al., 1999), chemotherapy (Gabriele et al., 1998) and hormone therapy (Lhomme et al., 1999) are often used adjunctively to surgery. At present, there is no effective early detection screening method for endometrial cancers. The use of gynaecological malignancy markers, e.g. CA-125, and preventative screening tests, e.g. Papanicolou smears, are often unsuccessful in detecting early stage endometrial carcinoma as they are specifically aimed at the early detection of ovarian and cervical cancers (Berchuck et al., 1995; Westof et al., 1997). Diagnostic and prognostic markers specific to this disease are few, with the depth of myometrial invasion and degree of metastasis, differentiation type and hormone receptor status commonly used as prognostic markers and guides to treatment.

Despite the prevalence of endometrial cancer, little is known of the molecular factors that contribute to the development and evolution of the neoplastic process in the endometrium. The growth factor-like effects of oestrogen on the endometrium are believed to play an important part in the promotion of this cancer (Potischman et al., 1996; Weiderpass et al., 1999). During the initial stages of the multi-step process of cancer development in the endometrium, tumour growth may be dependent on oestrogen stimulation, however with neoplastic evolution, through the loss of functional oestrogen receptors or the uncoupling of receptor and hormone complexes, the tumours may become hormone independent. Therefore, while oestrogen is known to be a major promoter of tumour growth in the early stages of endometrial cancer, the underlying molecular genetic alterations that are essential for the progression to malignant and metastatic disease are still unknown. Changes at the DNA level, such as nucleotide base pair mutations, gene amplifications, chromosome translocations and deletions are common events that occur in the evolution of other cancers. The development of cancer depends on the occurrence of these events in genes that govern cell growth regulation, proteolytic degradation and angiogenesis, ultimately leading to the expression of a non-functional protein or to an alteration in the level of mRNA and subsequent protein expression of these genes.

In an attempt to resolve the mechanisms involved in the progression from a normal to a neoplastic state, earlier studies have examined the association between endometrial carcinoma and previously described tumour suppressor genes and oncogenes originally isolated in other forms of cancer. The expression of the tumour suppressor gene, p53, was commonly
altered in endometrial carcinomas and has been shown to be a useful prognostic indicator for survival outcome (Kohlbarg et al., 1996; Geisler et al., 1999). The role of genes, Her/neu-2 (Hetzel et al., 1992), epidermal growth factor receptor (EGFR) (Scambia et al., 1993), ras p21 (Scambia et al., 1994) and c-fos, c-jun, c-myc (Yokoyama et al., 1998), and their prognostic and diagnostic relevance to this disease have also been examined. However, the occurrence of gene amplifications or alterations to the expression of these genes have not been as frequent as p53 modifications.

The genes commonly involved in neoplastic transformation in the endometrium have yet to be identified. The characterization of these genes and their protein products should give a clearer understanding of the transformation process in the endometrium and result in specific diagnostic and therapeutic approaches for endometrial carcinoma. The many physiological differences between normal and cancer cells are the consequence of differential gene expression arising from changes occurring at the DNA level. In this study, we describe the use of differential display reverse transcription–polymerase chain reaction (RT–PCR) methodology to identify the genes altered in endometrial tumorigenesis through the direct comparison of mRNA expressed in normal endometrial cells and endometrial carcinoma cells.

Materials and methods

Patients and samples

This project was approved by the Royal Women’s Hospital Research and Ethics Committees and written informed consent was obtained from participating patients. Clinical specimens were obtained (prior to any treatment) at the time of surgery from pre-menopausal and post-menopausal women undergoing abdominal hysterectomy and bilateral salpingo-oophorectomy for gynaecological malignancies. Normal samples were obtained either by dilatation and curettage or biopsies were excised from women undergoing hysterectomy for other gynaecological malignancies. These samples were deemed normal by both gross and microscopic pathology. Samples obtained from patients prescribed hormone modulation medication (contraceptives or hormone replacement therapy) or tamoxifen, were excluded at the time of surgery from pre-menopausal and post-menopausal women undergoing abdominal hysterectomy and containing one tenth of a reverse transcription reaction, 0.2 µmol/l of oligo dT primer-T 11M (Table I). Following a 5 min incubation at 65°C, 1 µl of Maloney murine leukaemia virus (MMLV) reverse transcriptase was added to the reaction. Reverse transcription was performed at 37°C for 1 h, after which the enzyme was inactivated by heating the reactions to 75°C for 5 min. Subsequent cDNA amplification by PCR was performed in a 20 µl reaction containing one tenth of a reverse transcription reaction, 0.2 µmol/l of each of an arbitrary sequence primer (Table I) and the anchored oligo dT primer used in the RT reaction, 2 µl PCR buffer (100 mmol/l Tris–Cl pH 8.4, 500 mmol/l KCl, 15 mmol/l MgCl 2, 25 mmol/l dithiothreitol (DTT)), 20 µmol/l of each dNTP, 0.2 µmol/l of oligo dT primer-T 11M (Table I). Following a 5 min incubation at 65°C, 1 µl of Maloney murine leukaemia virus (MMLV) reverse transcriptase was added to the reaction. Reverse transcription was performed at 37°C for 1 h, after which the enzyme was inactivated by heating the reactions to 75°C for 5 min. Subsequent cDNA amplification by PCR was performed in a 20 µl reaction containing one tenth of a reverse transcription reaction, 0.2 µmol/l of each of an arbitrary sequence primer (Table I) and the anchored oligo dT primer used in the RT reaction, 2 µl PCR buffer (100 mmol/l Tris–Cl pH 8.4, 500 mmol/l KCl, 15 mmol/l MgCl 2, 0.01% gelatin), 2 µmol/l dNTP, 0.3 µl [α-35S]-dATP (1200 Ci/ml, Amersham, UK), 1 IU AmpliTag DNA polymerase (Perkin Elmer). Cycle conditions were as follows; 95°C for 1 s, 40°C for 30 s and 72°C for 1 min for 40 cycles, followed by 72°C for 5 min. Thermocycling was performed with an Omni-E Thermocycler (Hybaid, UK). The PCR products were size fractionated by electrophoresis on a 6% denaturing polyacrylamide gel in 1× Tris/borate/EDTA (TBE) buffer. Of each PCR 15% were loaded onto the gel.

Differentially expressed mRNAs in neoplastic endometria

Table I. RNAimage™ primer sequences used in differential display RT–PCR

<table>
<thead>
<tr>
<th>Primer no.</th>
<th>Primer sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>HT11G (oligo dT)</td>
<td>AAAAGTTTTTTTTTTTG</td>
</tr>
<tr>
<td>HT11C (oligo dT)</td>
<td>AAAAGTTTTTTTTTTTC</td>
</tr>
<tr>
<td>HT11A (oligo dT)</td>
<td>AAAAGTTTTTTTTTTTA</td>
</tr>
<tr>
<td>H-AP1</td>
<td>AAGCTTGTATTGCC</td>
</tr>
<tr>
<td>H-AP2</td>
<td>AAGCTTGGAGCTG</td>
</tr>
<tr>
<td>H-AP3</td>
<td>AAGCTTGGTGTCAG</td>
</tr>
<tr>
<td>H-AP4</td>
<td>AAGCTTCTCAACG</td>
</tr>
<tr>
<td>H-AP5</td>
<td>AAGCTTAGTAGGC</td>
</tr>
<tr>
<td>H-AP6</td>
<td>AAGCTTGGACCAT</td>
</tr>
<tr>
<td>H-AP7</td>
<td>AAGCTTAAGGAG</td>
</tr>
<tr>
<td>H-AP8</td>
<td>AAGCTTTTACCAG</td>
</tr>
</tbody>
</table>

Proteinase K (10 IU/µg RNA, 37°C for 30 min) and subsequently DNase 1 (10 IU/µg RNA, 37°C for 15 min) to remove any residual protein and genomic DNA. The quantity and quality of RNA after extraction and DNase treatment was determined from optical density measurements taken at 260 and 280 nm using a spectrophotometer (UV-2101/3101 PC; Shimadzu Corporation, Kyoto, Japan).

Differential display RT–PCR

Reverse transcription was performed on total RNA (250 ng), using the RNAimage™ mRNA Differential Display System (GenHunter Corporation, Nashville, TN, USA). The 20 µl reactions contained 5 µl 5× RT buffer [125 mmol/l Tris–Cl pH 8.3, 188 mmol/l KCl, 7.5 mmol/l MgCl 2, 25 mmol/l dithiothreitol (DTT)], 20 µmol/l of each dNTP, 0.2 µmol/l of oligo dT primer-T 11M (Table I). Following a 5 min incubation at 65°C, 1 µl of Maloney murine leukaemia virus (MMLV) reverse transcriptase was added to the reaction. Reverse transcription was performed at 37°C for 1 h, after which the enzyme was inactivated by heating the reactions to 75°C for 5 min. Subsequent cDNA amplification by PCR was performed in a 20 µl reaction containing one tenth of a reverse transcription reaction, 0.2 µmol/l of each of an arbitrary sequence primer (Table I) and the anchored oligo dT primer used in the RT reaction, 2 µl PCR buffer (100 mmol/l Tris–Cl, pH 8.4, 500 mmol/l KCl, 15 mmol/l MgCl 2 and 0.01% gelatin), 2 µmol/l dNTP, 0.3 µl [α-35S]-dATP (1200 Ci/ml, Amersham, UK), 1 IU AmpliTag DNA polymerase (Perkin Elmer). Cycle conditions were as follows; 95°C for 1 s, 40°C for 30 s and 72°C for 1 min for 40 cycles, followed by 72°C for 5 min. Thermocycling was performed with an Omni-E Thermocycler (Hybaid, UK). The PCR products were size fractionated by electrophoresis on a 6% denaturing polyacrylamide gel in 1× Tris/borate/EDTA (TBE) buffer. Of each PCR 15% were loaded onto the gel. Following electrophoresis the gels were blotted on a piece of 3MM paper, dried under vacuum and overlaid with X-ray film overnight.

Single stranded conformation polymorphism analysis (SSCP)

Gel slices containing cDNA amplicons that were expressed in all three RNA samples in either the normal or carcinoma groups were excised from the polyacrylamide gel. The corresponding region of the gel, which did not contain the band, was also excised. The DNA was extracted from the gel debris by soaking in 100 µl of H2O for 10 min followed by boiling for 15 min. Glycogen (50 µg) was then added to facilitate precipitation with ethanol.

The precipitated DNA (2 µl) was used in a PCR containing, 20 µmol/l dNTP, PCR buffer (100 mmol/l Tris–Cl, 500 mmol/l KCl and 15 mmol/l MgCl 2), 0.1 µl [α-32P]-dCTP (3000 Ci/mmol), 2 IU AmpliTag DNA polymerase, and the appropriate primer pair (0.2

RNA isolation and preparation

Total RNA was extracted from frozen tissue biopsies as described previously (Chirgwin et al., 1979). The RNA was incubated with...
Table II. Primer sequences used for semi-quantitative RT–PCR analysis of T19 and N22 mRNA. The expected amplicon sizes for each primer pair are listed.

<table>
<thead>
<tr>
<th>mRNA</th>
<th>Primer sequence</th>
<th>Amplicon size</th>
</tr>
</thead>
<tbody>
<tr>
<td>N22</td>
<td>5'-TCG CAA GCA GAA GCA ATC-3' S</td>
<td>229 bp</td>
</tr>
<tr>
<td></td>
<td>5'-TGG ACC GAC CAA ATG TAT AAA C-3' AS</td>
<td></td>
</tr>
<tr>
<td>T19</td>
<td>5'-GGG TGG TAA GCA CAA TGA A-3' S</td>
<td>140 bp</td>
</tr>
<tr>
<td></td>
<td>5'-TGC TAA TGT AGG AGA CTG A-3' AS</td>
<td></td>
</tr>
<tr>
<td>GAPDH</td>
<td>5'-GAA ATC CCA TCA CCA TCT TCC-3' S</td>
<td>805 bp</td>
</tr>
<tr>
<td></td>
<td>5'-CCA GGG GTC TTA CTC CTG-3' AS</td>
<td></td>
</tr>
</tbody>
</table>

µmol/l) to a final volume of 20 µl. The cycle conditions were as follows; 94 °C for 30 s, 40 °C for 30 s and 72 °C for 1 min, for 22 cycles, followed by 5 min at 72 °C. The PCR products were electrophoretically separated on a 6% acrylamide gel containing 10% glycerol. The products were mixed with 18 µl of loading dye (90% formamide, 0.05% Bromophenol Blue, 0.05% xylene cyanol, 5 mmol/l EDTA), heated to 95 °C for 3 min and loaded onto the gel. The gel was run in 1× TBE buffer at 8 W for 18 h. The gel was then blotted on 3MM paper, dried under vacuum and overlaid with Biomax film (Kodak) for 24 h. Differentially expressed bands verified by SSCP analysis were then excised from the gel and were each separately cloned into PCRscript™ plasmid (Strategene, La Jolla, USA).

**Sequence analysis**

Recombinant plasmids containing amplicon inserts were purified (High pure plasmid isolation kit, Boehringer Mannheim) and subsequently sequenced using a PE Applied Biosystems 377 DNA sequencer and a Dye terminator reaction kit (Perkin Elmer, Promega) immobilized by UV cross-linking. Membranes were incubated for 20 cycles, followed by 5 min at 72 °C. The PCR products were sequenced using a PE Applied Biosystems 377 DNA sequencer and a Dye terminator reaction kit (Perkin Elmer, Promega) to (0.84 M NaCl, 0.048 M NaH₂PO₄, 20 µmol/l NaOH/1.5 mol/l NaCl) buffer alongside pGEM DNA markers (Promega) to

**cDNA probe isolation and preparation**

To confirm the sequence composition of RT–PCR products, specific probes were isolated from the three recombinant plasmids containing N22, T19 and GAPDH, by restriction enzyme digestion with EcoRI and SacI (Boehringer Mannheim) and purification from agarose gel after electrophoresis. The cDNA was excised and purified from the agarose gel using a DNA purification resin column (Promega, Wisconsin, USA). The cDNA was labelled with 5 µl of [α-32P]-dCTP, 3000 Ci/mmol (Amersham, UK) in a 50 µl reaction containing 250 mmol/l Tris–HCl, pH 8.0; 25 mmol/l MgCl₂, 10 mmol/l DTT, 1 mol/l HEPES, pH 6.6 and 1 IU of DNA polymerase 1, Large (Klenow) fragment (Promega). The cDNA was then used as a probe to confirm the specificity of the RT–PCR by Southern blot analysis.

**Southern blot analysis**

PCR products (10% of final PCR volume) were resolved on 2% agarose gels prepared in 1× TAE (0.04 M Tris-acetate, 0.001 M EDTA, pH 8.0) buffer alongside pGEM DNA markers (Promega) to confirm PCR product sizes. The gels were prepared for Southern transfer by sequential soaking in 0.5 mol/l NaOH/1.5 mol/l NaCl solution for 40 min and 1 mol/l Tris/1.5 mol/l NaCl for 40 min. The amplicons were transferred to Hybond-N membrane by capillary blotting with 20× sodium chloride/sodium citrate (SSC) and were immobilized by UV cross-linking. Membranes were incubated for 1 h at 42 °C in hybridization buffer [50% formamide, 6× SSC/0.1% SDS/0.1% SDS/0.2% skimmed milk powder] then hybridized with the radio-labelled probe overnight at 42 °C. The membranes were washed in 1× SSC/0.1% SDS at 42 °C for 15 min followed by 0.1× SSC/0.1% SDS at 55 °C for 15 min. The membranes were overlaid with Kodak XOMAT-AR film for 2 h. The autoradiograms were scanned and the PCR products were quantified using laser densitometry (Molecular Dynamics, USA). For semi-quantitative analysis, the intensity of hybridization signal for either N22 or T19 amplicons was expressed as a ratio of a positive control on the membrane and was normalized against the GAPDH amplicon signal.

**Statistical analysis**

The data were analysed for homogeneity of variance using Levene’s test. Non-parametric statistical tests (Kruskal–Wallis analysis of variance and Mann–Whitney U-test) were used to analyse non-homogenous data. P < 0.05 was considered to be statistically significant.

**Results**

**Selection of differentially expressed mRNAs by differential display**

The mRNA expression pattern derived from three normal endometrial tissues and three endometrial carcinoma tissues was compared using differential display RT–PCR methodology at which PCR product amount was optimal and within the linear portion of the curve, well before saturation point. PCR negative controls for each sample were prepared by mixing the identical reagents as per the test samples, however the conjugated enzyme for reverse transcription and PCR was added after the RT incubation prior to the RT enzyme inactivation period. PCR products were not detected in these controls.
Differentially expressed mRNAs in neoplastic endometria

![Figure 1. Differential display reverse transcription–polymerase chain reaction (RT–PCR) of normal endometrial mRNA (n = 3) and endometrial carcinoma mRNA (n = 3). The region of the RNA fingerprinting gels showing the differentially amplified (A) T19 and (B) N22 cDNAs (arrowed). The size in bp of fragments derived from a DNA sequence ladder run in parallel are shown.](image)

(Diagram showing RT-PCR results)

![Figure 2. Single stranded conformation polymorphism analysis (SSCP) of the differentially amplified T19 and N22 cDNAs.](image)

(Diagram showing SSCP results)

Figure 1. Differential display reverse transcription–polymerase chain reaction (RT–PCR) of normal endometrial mRNA (n = 3) and endometrial carcinoma mRNA (n = 3). The region of the RNA fingerprinting gels showing the differentially amplified (A) T19 and (B) N22 cDNAs (arrowed). The size in bp of fragments derived from a DNA sequence ladder run in parallel are shown.

Figure 2. Single stranded conformation polymorphism analysis (SSCP) of the differentially amplified T19 and N22 cDNAs.

on the basis of single-stranded conformation polymorphism (SSCP) (Figure 2). SSCP analysis of cDNA bands re-amplified from RNA fingerprinting gels was successful in separating cDNAs of similar size, since each cDNA species had a distinct mobility pattern on a SSCP gel due to the difference in sequence composition (Figure 2a,b). From ~24 cDNA bands excised from fingerprinting gels, only four cDNA bands showed reproducible differences and were selected for further analysis.

**Semi-quantitative RT–PCR analysis**

The four cDNA species selected for further analysis were cloned and sequenced. These cloned cDNA sequences were purified from their respective plasmid vectors, radiolabelled and then hybridized to nylon membranes containing total RNA derived from the original normal endometria and endometrial carcinoma samples. Northern blot analysis results indicated two of the four cDNAs were not differentially expressed and the remaining two cDNA probe hybridizations (N22 and T19) produced ambiguous results. These two putative mRNA species were either not detectable (N22) or produced only very faint hybridization signals (T19) by Northern blot analysis. These results highlighted the lack of sensitivity of Northern blot analysis for detecting low abundance messages and therefore led to the adaptation of semi-quantitative RT–PCR analysis of these two cDNAs isolated by differential display.

Expression of T19 (represented by a 190 bp fragment generated by semi-quantitative RT–PCR analysis), while present in all of the normal (n = 3) and carcinoma samples (n = 3), was significantly higher in the tumour group (Figure 3a). A 229 bp cDNA band generated by semi-quantitative RT–PCR, Figure 3b, and representing N22 was visible in the normal RNA samples examined (n = 3) and absent from the tumour RNA samples (n = 3). The cDNA, T19, was selected for further analysis in a larger clinical sample set. T19 mRNA expression was observed in all of the RNA samples examined (normal endometria and endometrial carcinomas). Again,
Figure 5. Relative abundance of T19 mRNA in normal endometrial and endometrial carcinoma tissues. Expression of T19 in each histological tumour grade was significantly higher than that observed in the normal group (Mann–Whitney U-test, *P < 0.05). No significant difference in T19 mRNA expression was observed between each tumour grade (grade 1, n = 4; grade 2, n = 6; grade 3, n = 9). Data are expressed as median values with the 25 and 75% quartiles.

Table III. DNA sequence of T19 cDNA

<table>
<thead>
<tr>
<th>T19 cDNA nucleotide sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>GGG GGA GCC AAT GGT GCC GTA TAG TGA CAA TAT TCG CGA GTG GCC GTA AGG GTA AAG GCA ATT TCG GCT TAT ATG CAT TAG AAA AAA AAA AAA A</td>
</tr>
</tbody>
</table>

of T19 with an EST Genbank database showed a 100% sequence identity with a human mRNA partial sequence (accession no. gbAA148573), isolated from a pregnant uterus (over 270 nucleotides). The N22 (425 bp) sequence analysis and comparison with the EMBL (European Molecular Biology Laboratory) and GenBank sequence databases showed an extremely high degree of similarity at the nucleotide level (~95% over 356 nucleotides) to a human partial cDNA sequence of unknown function (accession no. gbH60694).
cells of the rat upon exposure to growth agonists, platelet-derived growth factor and angiotensin II. Without full cDNA sequence characterization it is difficult to speculate on the potential role of the putative protein product of *T19*, particularly since the sequence similarity with *SM-20* is over a modest section located in the 3′ untranslated region of the mRNA transcript. Comparison of *T19* with an EST database showed a full sequence identity match with a partial mRNA isolated from pregnant human uterus. Interestingly, during trophoblast implantation and throughout gestation, the vasculature at the myometrial/decidual interface is constantly modified and remodelled to accommodate the nutritional requirements of the fetus. Considering the function of *SM-20* and the full sequence identity of *T19* with an EST derived from a pregnant uterus, this protein product may be involved in angiogenesis or vascular remodelling. Complete sequence characterization of *T19* may prove that the putative protein encoded by this transcript contributes to the pathogenesis of endometrial carcinoma development.

The expression of *N22* mRNA, as determined by semi-quantitative RT–PCR, was clearly restricted to the normal endometrial RNA samples, which was consistent with its expression on the RNA fingerprinting gel. This alteration in the level of *N22* expression in tumour tissues may be the result of molecular genetic changes associated with the development of endometrial cancer. Considering the small sample size examined, it is also difficult to conclude whether the loss of *N22* expression in the three tumours examined is a common event in endometrial tumourigenesis or is restricted to this small sample set. The partial sequence characterization of *N22* has given no further suggestions as to the potential function of the putative protein encoded by the mRNA transcript. Comparison of *N22* with the sequence databases EMBL and Genbank revealed a 95% sequence identity with a human cDNA clone of unknown function. It is, therefore, impossible to speculate on the putative role of *N22* in endometrial tumourgenesis, although further sequence isolation and characterization of this transcript may reveal a gene encoding a protein with potential tumour suppressing function.

In conclusion, the complex multi-step process of tumour development involves many molecular genetic changes that result in the aberrant expression or loss of expression of genes involved in the maintenance of normal cell function. Few of the genes involved in progression of endometrial tumourogenesis have been identified. The elucidation of these genes and their putative protein products may not only aid in the early diagnosis and treatment of the disease but they may also be useful targets in future treatment therapies specific to carcinoma of the endometrium. While it is tempting to speculate that *N22* may have potential tumour suppressor functions and *T19* may have oncogenic properties, it remains to be determined whether the changes in expression observed for these genes are a fundamental part of the many molecular genetic alterations which presumably contribute to the progression of endometrial carcinoma, or whether they arise as a consequence of these changes. Future mRNA and protein sequence characterization may help to clarify the function of *N22* and *T19* to confirm their true role in endometrial tumour development.

### Table IV. DNA sequence of *N22* cDNA

<table>
<thead>
<tr>
<th>N22 cDNA nucleotide sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAA AAA AAA ACA TGT GTT GGT TAA GAA GCA AAC TTC ACC TAC GAA GCG TTC GCT GAA TGG TAG GGA GTA AGG AGT TTC AAC ACC CGA CAC AAT TGA ATT CAC CCG TAA GTT CAT ATT GTG TCT TTT TGT AGA GAA GAC CTT CTC CTT GTT TCA TGT GAA GAG GTT GTG CCC ACG ACT ATA TAT TAG AAC ATT GTT GTT TAA TNG AGG GAT CTC GAC GCG TAA AGG TCG ATC GTA GGA CGG AAG GGA CAA ATA TTG AAA CCA CGG AGC TTA ATA AAA AAT TAA AAG ACA GGA AAT TGT TTT TGG TCT TGG AAA GAA AAT TAC AAA ACA GTG TGT ACA AGT AAA ACT ATG TCA AGA AGG ATA GGA GCA ATT TGA KCC ATC TTG CCG CGG TGG CCG CCG TGA GGT GAA ACA AGG AAT</td>
</tr>
</tbody>
</table>

Comparison of *N22* to EMBL EST databases using the FASTA program (available at WEBANGIS) showed a 91% similarity (over 260 nucleotides) to a human cDNA of unknown function (accession no. HS694210).

### Discussion

From an estimated 100 000 genes in the human genome, ~15 000 are expressed in any given cell type at any one time (Liang and Pardee, 1992). In this study, almost one third of this number of mRNAs derived from either normal endometrial tissues or endometrial carcinomas were displayed as cDNA bands on fingerprinting gels. Commonly, when employing differential display RT–PCR as a means to identify differentially expressed mRNAs, a large proportion of the differentially amplified cDNA species are subsequently found not to be differentially expressed (Liang et al., 1993). This is thought to be caused in part by slight variations in RNA quantity and quality or from separate reactions causing preferential PCR amplification of cDNAs, which are not actually differentially expressed. In this context, it is not surprising that only two of the 24 differentially amplified cDNAs identified in this study (designated *N22* and *T19*), were subsequently shown to be derived from transcripts differentially expressed in normal endometrium and endometrial carcinoma respectively.

The detection of *T19* mRNA expression in all normal and neoplastic endometria examined, with ~ half of the neoplastic samples expressing higher levels of *T19* mRNA when compared with the normal endometria, suggests that the over-expression of *T19* mRNA may be a common event in endometrial tumourgenesis. It is not known, however, whether the observed increase in *T19* mRNA relative abundance is the result of increased transcription or increased mRNA stability in the endometrial carcinomas. It is difficult to establish whether the change in *T19* mRNA expression is a fundamental part of the many molecular genetic alterations which presumably contribute to the progression of endometrial carcinoma, or whether it arises as a consequence of these changes. The partial sequence characterization of *T19* has revealed a partial sequence identity (at the nucleotide level) with a growth factor responsive gene expressed in vascular smooth muscle cells (*SM-20*) isolated from *Rattus norvegicus* (Wax et al., 1994, 1996). Transcription of the *SM-20* gene is an immediate/early response gene that may be induced in smooth muscle aorta
C.Foca et al.

Acknowledgements

The authors gratefully acknowledge the assistance of Mrs Julene Harro for tissue sample collection. This study was funded by a grant from the 3AW Community Services Trust Fund of the Royal Women’s Hospital, Melbourne, Australia (E.K.Moses, G.E.Rice).

References


Chirgwin, J.M., Przybyla, A.E., MacDonald, R.J. et al. (1979) Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry, 18, 5294–5299.


