

## ORIGINAL ARTICLE

# The novel human MOST-1 (C8orf17) gene exhibits tissue specific expression, maps to chromosome 8q24.2, and is overexpressed/amplified in high grade cancers of the breast and prostate

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**Aims:** To elucidate genes that participate in the process of oncogenesis, primers based on the E6 genes of genital human papillomaviruses (HPVs) were used to amplify potential expressed sequence tags (ESTs) from the MOLT-4 T lymphoblastic leukaemia cell line.

**Methods:** Using the polymerase chain reaction (PCR) with human papillomavirus E6 gene primers, an EST from the MOLT-4 T lymphoblastic leukaemia cell line was amplified. Via rapid amplification of cDNA ends (RACE) and cycle sequencing from MOLT-4 and fetal lung cDNA libraries, overlapping cDNAs of 2786 bp and 2054 bp of the corresponding novel human intronless gene designated MOST-1 (for MOLT-4 sequence tag-1) were characterised and assigned the symbol C8orf17 by the HUGO Nomenclature Committee.

**Results:** Both cDNAs contained a potential open reading frame (ORF) of 297 bp incorporating a methionine codon with an ideal Kozak consensus sequence for translation initiation, and encoding a putative hydrophilic polypeptide of 99 amino acids. Although reverse transcription PCR (RT-PCR) demonstrated MOST-1 expression in all 19 cancer and two normal cell lines tested, differential expression was seen in only nine of 16 normal tissues tested (heart, kidney, liver, pancreas, small intestine, ovary, testis, prostate, and thymus). A 388 bp fragment was amplified from the NS-1 mouse myeloma cell line, the sequence of which was identical to that within the MOST-1 ORF. The MOST-1 gene was mapped by fluorescent in situ hybridisation to chromosome 8q24.2, a region amplified in many breast cancers and prostate cancers, which is also the candidate site of potential oncogene(s) other than c-myc located at 8q24.1. Analysis of paired biopsies of invasive ductal breast cancer and adjacent normal tissue by semiquantitative and real time RT-PCR revealed average tumour to normal ratios of MOST-1 expression that were two times greater in grade 3 cancers than in grade 1 and 2 cancers. Quantitative real time PCR of archival prostatic biopsies displayed MOST-1 DNA values that were 9.9, 7.5, 4.2, and 1.4 times higher in high grade carcinomas, intermediate grade carcinomas, low grade carcinomas, and benign hyperplasias, respectively, than in normal samples.

**Conclusions:** These data suggest a role for MOST-1 in cellular differentiation, proliferation, and carcinogenesis.

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Following the publication of a working draft of the human genome sequence, the Human Genome Project faces new challenges. Major tasks ahead include the identification of the estimated 35 000 genes residing within three billion base pairs (bp) of DNA, and the characterisation of their regulatory elements and of an even greater number of transcriptional units and translated products.<sup>1</sup> To isolate the numerous expressed genes among large tracts of non-coding genomic DNA, expressed sequence tags (ESTs) represent an efficient and economical "short cut" route for gene identification. The idea of exploiting ESTs has been established as a practical approach for the discovery of novel human genes. ESTs are instrumental in the evaluation of human gene diversity and expression patterns, in addition to the delineation of exons and alternative splicing events. These cDNA fragments are also useful for the annotation of physical maps, and function as handles for the classification of multigene families.<sup>2,3</sup>

"To isolate the numerous expressed genes among large tracts of non-coding genomic DNA, expressed sequence tags represent an efficient and economical "short cut" route for gene identification"

The search for ESTs and their corresponding genes implicated in the causation of human cancers is intensifying in the quest for better diagnostic markers and therapeutic agents.<sup>4,5</sup> Certain viruses contribute greatly to the development of specific cancers; for example, the association between human papillomaviruses (HPV) and genital carcinomas. The E6 and E7 early genes and oncoproteins of high risk genital HPV types possess transforming abilities and are crucial in genital carcinogenesis.<sup>6–9</sup> In an attempt to elucidate genes that participate in the process of oncogenesis, primers based on the E6 genes of genital HPVs were used to amplify potential ESTs from the MOLT-4 T lymphoblastic leukaemia cell line. Reverse transcription polymerase chain reaction (RT-PCR) of MOLT-4 RNA using primers targeting the E6 genes of HPV types 11 and

**Abbreviations:** C<sub>t</sub>, threshold cycle; EST, expressed sequence tag; FISH, fluorescence in situ hybridisation; G3PDH, glyceraldehyde-3-phosphate dehydrogenase; HPV, human papillomavirus; nt, nucleotides; N, normal; ORF, open reading frame; PBS, phosphate buffered saline; PCR, polymerase chain reaction; RACE, rapid amplification of cDNA ends; RT, reverse transcription; SDS, sodium dodecyl sulfate; T, tumour; UTR, untranslated region

18 generated an EST, the sequence of which revealed no significant homology to a known gene in the GenBank database. Arising from this novel EST, we report here the characterisation of a novel human gene designated MOST-1 isolated via rapid amplification of cDNA ends (RACE) and cycle sequencing, its localisation to chromosome 8q24.2, its overexpression in breast cancers, and its amplification in prostatic neoplasia relative to their respective normal tissues.

## METHODS

### Preparation of cDNA from cell lines

Cell lines were cultured in appropriate growth media recommended by the American Type Culture Collection. Cells were harvested, washed with phosphate buffered saline (PBS), pelleted, and resuspended in 10mM Tris/HCl (pH 7.5), 0.15M NaCl, 1.5mM MgCl<sub>2</sub>, and 0.65% Nonidet-P40. After removal of nuclei by centrifugation, the cytoplasmic lysate (supernatant) was transferred to an equal volume of buffer containing 7M urea, 1% sodium dodecyl sulfate (SDS), 0.35M NaCl, 10mM Tris/HCl (pH 7.5). The mixture was extracted with phenol/chloroform/isoamylalcohol, and RNA was precipitated with cold absolute ethanol. Total cellular RNA was reverse transcribed in a reaction mix comprising first strand buffer, dithiothreitol, RNase inhibitor, random primers, four dNTPs, and SuperScript II reverse transcriptase (Gibco BRL, Rockville, Maryland, USA), incubated at room temperature for 10 minutes, 42°C for one hour, and 99°C for five minutes.

### Extraction of MOLT-4 genomic DNA

Cells were centrifuged, washed with PBS, suspended in 0.3M sodium acetate, 0.5% SDS, 5mM EDTA (pH 7.0), extracted with phenol/chloroform/isoamylalcohol, and DNA precipitated with 4M LiCl and cold absolute ethanol.

### Isolation of the original MOST-1 EST

The initial EST fragment representing MOST-1 was amplified from MOLT-4 cDNA using HPV primers 11q (5'-CTTCCATGCATGTTGTCCAG-3') and 18c (5'-GGT TTCTGGCACCAGCA-3').<sup>10</sup> The PCR was subjected to 95°C for one minute, followed by 35 cycles each of 95°C for 30 seconds, 50°C for 30 seconds, and 72°C for one minute, and the PCR products were analysed by agarose gel electrophoresis. Three selected amplified bands were excised, the DNA eluted, and reamplified for an additional 25 cycles. PCR products were purified using the QIAquick gel extraction kit (Qiagen, Hilden, Germany) or GENE CLEAN II kit (Qbiogene, Carlsbad, California, USA), and cycle sequenced using the ABI PRISM BigDye terminator cycle sequencing ready reaction kit, and an ABI PRISM 377 DNA sequencer (PE Applied Biosystems, Foster City, California, USA). Database searches were performed with the BLAST algorithm at the National Center for Biotechnology Information website (<http://www.ncbi.nlm.nih.gov/blast>) to authenticate that the MOST-1 EST had not been reported before.

### Identification of MOST-1 cDNA by RACE

Marathon-Ready MOLT-4 and human fetal lung cDNA libraries ligated with adaptor oligonucleotides (Clontech, Palo Alto, California, USA) were used as templates for RACE reactions. Based on the original MOST-1 EST, gene specific primers TF1 (nucleotides (nt) 1108–1127) and TR4.2 (nt 1299–1277) were designed. Before RACE, PCR was performed with these primers to confirm that the cDNA libraries contained the cDNA of interest. Primers TR4.2 and TF1 were individually paired with the adaptor primer AP1 in initial RACE experiments via long distance PCR using Advantage 2 polymerase (Clontech). Nested primers TR1 (nt 1125–1102) and TF2 (nt 1136–1158) were then paired with the adaptor primer AP2 in subsequent RACE to obtain the 5' and 3' RACE fragments, respectively. Thermal cycling conditions were 94°C for 30 seconds, followed

by 25 cycles each of 94°C for five seconds and 68°C for four minutes. The RACE products were cycle sequenced as described above. Amino acid sequence analysis and motif searches were performed using bioinformatics software (<http://www.expasy.ch>).

### Organisation of the MOST-1 gene

To determine the presence of exon(s) or intron(s), primer pairs flanking 200–400 bp target fragments were designed based on the full length MOST-1 cDNA sequence derived by RACE. With these MOST-1 primer pairs, PCR products amplified from MOLT-4 genomic DNA and cDNA were sequenced, compared, and subjected to Grail computational analysis (<http://compbio.ornl.gov/Grail-1.3>).

### Chromosomal localisation of MOST-1

The chromosomal position of MOST-1 was mapped by fluorescence in situ hybridisation (FISH), using a genomic probe of ~2.6 kb generated from MOLT-4 genomic DNA and primers TF10 (nt 34–57) and TR8 (nt 2670–2648). Probe labelling, hybridisation procedures, and visualisation methods were carried out as described previously.<sup>3, 11–14</sup> The MOST-1 sequence was also subjected to computational analysis with the UniGene program (<http://www.ncbi.nlm.nih.gov/UniGene>).

### Screening for MOST-1 expression in normal human tissues and cancer cell lines

Multiple tissue cDNA panels generated from poly(A)<sup>+</sup> RNA of 16 human adult tissues (0.8 ng each; Clontech), and cDNAs from 19 human cancer and two normal cell lines (200 ng each) were screened for MOST-1 expression. PCR with primers TF5 (nt 860–882) and TR4.2 flanking a 440 bp fragment was performed using a profile of 95°C for one minute, followed by 25 cycles each of 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds. A subsequent amplification was repeated using a 50× dilution of the first PCR product as template. To serve as controls for the human adult tissues and cell lines, housekeeping genes were also subjected to PCR using glyceraldehyde-3-phosphate dehydrogenase (G3PDH) specific primers,<sup>15</sup> and/or HUEL gene specific primers: 5R2 (5'-AAGTATGTAATGGAAAGTCGTG-3') and R3 (5'-AAGCATAAAGTCTCTAGTTCTTCAGG-3').<sup>3</sup> All reactions were performed in duplicate.

### Semiquantitative and real time RT-PCR of frozen breast tissue biopsies

Total RNAs were previously isolated from 27 pairs of snap frozen invasive ductal breast carcinoma and adjacent normal tissue samples.<sup>16–18</sup> The breast cancers were histologically graded according to modified criteria of the Bloom and Richardson system.<sup>19, 20</sup> After reverse transcription, each cDNA sample (equivalent to about 80 ng of total RNA) was subjected to PCR amplification using primers TF5 and TR4.2 at 95°C for 30 seconds, followed by 35 cycles each of 95°C for 30 seconds, 65°C for 30 seconds, and 72°C for one minute. Semiquantitative analysis of MOST-1 mRNA was facilitated by concurrent RT-PCR of the G3PDH housekeeping gene.<sup>15</sup> A prior PCR cycle optimisation was conducted to ensure that all reactions remained in the linear region (by removal and analysis of samples at 15, 20, 25, 30, 35, and 38 cycles). The resultant RT-PCR products were electrophoresed along with DNA markers in agarose gels stained with ethidium bromide, visualised under ultraviolet light, and analysed by densitometric scanning using a GS-700 Imaging Densitometer and Quantity One software (Bio-Rad, Hercules, California, USA). MOST-1 expression was quantified by calculating the ratio of the intensity of each RT-PCR product of MOST-1 to that of G3PDH.<sup>16–18</sup> Relative MOST-1 expression was further presented as the differential expression between corresponding tumour (T) and normal (N) tissues as follows: overexpression in

**A**

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CTCAGTTTTC TAATCTGAAAAATGAGAATATTAACCATAGACAACCTGCCACTCACTGAACACCAGCTGCAGTAGAGCCGAGCATACACGTAATCCTCACC 100
GCAACCATAGGAGCTGGATCCTGACATCCCATCTCACAGAGGAGGAGACTGAGGTAAATGACGTGGCAAATATCCACAAATGACAGTTTCTGCAAGTCTT 200
TCCTAGTGCAATCACCCACAACACAGCCCTAGAAAGAAGGATGAACACTCTTCCACAGAGGCTGGCACGCACCTTAAGGCAGGTGCCACTGGAAACCTT 300
GTCTGTGGTGTCAAGGATTTTACAGTTGACCTTGAAGACCACCTAGTCCAAAGCTCTCATTGTACAAAAGGCCAAGCCAGAGGTCACATAAAGCTTTGG 400
ACCTGAGCCTCTGGGCCCGGTCTGGAAGTGTCTCTTCTGATCAGCTGGGGCTCAACTGAGGTGGTCCAGTCTGCAGATCTACATCCTCTGG 500
CCCGCTGAGCAGAGCCGGTGTGGGACCACTGCCGATCTCCACGATTTGTCAGCCCATCTGATAAGTCTGAGGCTCTGCCCATCATATCTCATCACAGC 600
ATCATTAGGATACAGTGCATGCGGTAGAAAGGAAAGGAAACACGAAACAAAAGGATGAGAAATTAAGCCCATTCACCCGAAAAGATGACTCTGAT 700
TTATTGCTCTATAGCAGCTGCAGATGGCTGTGAGAAATTTCTGTGTGAAAATAAAGAATAAAAAATCACCCAGAGTTGTTTTGCCACACATCCAAAT 800
AGAAAATGCTACTCTCCGGATGATTTATGCTTCAATTTTTTCCCTTATTCACAGAGAATTTGTCCAGCATTACAAGAGCACCTAAGAGCAATAATACAA 900
TCAAAGGAGATAGAAAGAGCCGCAGAGGTGTGATGTGCGGCTGAGGGCGGGGTGTGCCCCAAAGGCAGGGCTTTGGTGTCTGAGAAGCATTGACCTTA 1000
CAGGGAGGAACAAGGAGAAACCAGCCAGGCAGGGCCCGGCAGGCAGCCACAGAGCCACTTCGCCGTGCAGCCGAGGGAGTCCCGCACCCAGGGGCTCGC 1100
TCAGGGTAGCCACDATTGGAGAATGTCCACCTCGTGGATGTGAGGCTTGGCCACAGGTCTTGGCCACGGGACCTGAGCAGAGTGACATATGCCACAC 1200
TGGATCAGAAGCACGCTGGACCACCAGTGGTATGGCTCCCTTTCTTCTCCGTCATAAGTACAAGATGTTGGCTGACCTGACTCTCGGGTAGAAATG 1300
TGCTGTAGACACTGGGCGCGGTCTCACGCTTCAATCCAGCCTTTGGAGGGCGGAGCCAGGTGGATTGCCCTGAGCTCAGGAGTTCGAGACCAGCCT 1400
GGACAACATGCTGAAACCCCGTCTCTACTAATTTACAAAAAATAGCCAGGTGTGTGGCAGATGCCCTGTAGTCCAGCTACGTGGGAGGCTGAGGCAC 1500
GAGAATTCCTGAACCCAGAGGTGGAGTTGCAGTGAGCCGAGATGGTACCCTGCACTGCAGCCTGGGCAACAGAATGAGACTGTGTCTCAAAAAA 1600
AAAAAGCACTGTCTGACAGGGCAGGGTCCCAAGGCCCCAGCCCACTCTCGTTGACATGTAATGTAAGAAGATGTCAACTCATGCTTATAGTCACTGACA 1700
TTTAGGAATAGTCTGTTACCACGGCATAAGCTGGTAAAAAATAATCATACAGCTCTTCTTCCCGGACGCCCTGCACCTCTGCCTCCGGTGCACAAAT 1800
GCTTCTCTTCAAGGCATGGCGCAAAAAGCCACTACTTCCAGAAGCATCTCTCATTTCCCGCCGGGAGCAACATCTCTTCTTCGAGACCCAGCCCT 1900
TCCGCTGTCTACCCATGGACTTCTCTAGTGTGTTTTTCCGATGATTTCTTGTGACCAAGCTGTTTTCTCAGCTGCCCTGAGGACATGCCCTAGGGAA 2000
AGTGCTCTGAATCGTACC TGGATTCTGTGCTACCTGCTCCATCTAAAGCAGAAAATCCACAATAAGATCTCTTCTAACATTTAAGGAATAGAGCCTA 2100
ATTAGCCATGAATACGTTGAACTGTGAATGAATCAATGAGTATGGAACACTACATTATACGGCAGTGTCTGATAGAACCAAGTTGCCAAGGGAGATATCA 2200
TCTCTCCTGCAGGAAGGAAGTCTGCTCCCACTGATCCACTCAGGCACAGCCTTGAACAAAACAGAGCAAGAGTTATAACCTTCAGATGTGGGGGAG 2300
AATAGGCCATTTGGGAGACACAGGCCAGGAAACACAGGGCCAGCGTCTTGGCCCTCGATCAGACGCCCTCCCGCCACCGCGGCCGCTTCTCCAC 2400
GCTTTTGGCCAGATCTCTGCACCGCTGGCCCGGTCTTGGCTCCCTGAGGACACACTGGCTGGCAGCAACCAACTCGACTTCTACTTACACTTGGCTCAG 2500
CATTTTTGTACATCCCTGCCCTTGGCCAAATCCCTGTCTTGGTTCTGCTCTTCCACACAAGTTTGGGAATCAATTCATACATATTTGAGATTAAACAA 2600
AGTCCATGATCAGTTCTGGACCCTATGATTCCAGATCCACCTTACGTAGCCGTGTGAGCTCCGGTAAATACATAACCCATCAAAATTTCTTTATCTAT 2700
AAAGCAGCAACAATAATGTACGAAGATTAAGGTAAGATGAGAAATAGTATAGCAAAATGATCTTCCAAACCTGTTATCTTTT 2786
    
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**B**

MGECPHLVDVRLGHRSLATGPEQSDICTHGTSEARWTTWYGLSFSRHKYKMLADLTGVMESCRHWARWLTPIVLPALWKAEGGLPELRSSRPAAWTTW

**Figure 1** (A) cDNA sequence of the human MOST-1 gene (GenBank accession number AF220264). The 2786 nucleotide (nt) sequence was derived from the MOLT-4 cDNA library, whereas an overlapping and identical 2054 nt sequence (nt 664–2705) was generated from a human fetal lung cDNA library. The italicised nucleotides in bold face correspond to the 3' segments of primers targeting human papillomavirus E6 genes. MOST-1 cDNA contains a potential open reading frame of 297 nt (nt 1115–1411), encoding 99 amino acids, with the presumed translation start and termination codons within boxes. Three potential mRNA destabilising signals (ATTTA) are shown as underlined nucleotides. (B) Deduced amino acid sequence of a putative MOST-1 polypeptide comprising 99 residues.

tumour tissue compared with matched normal tissue ( $T > N$ ); decreased expression in tumour tissue compared with matched normal tissue ( $T < N$ ); and no difference ( $T = N$ ).<sup>21</sup> All reactions were performed in triplicate or duplicate, and mean values derived. Real time PCR analysis with the LightCycler-DNA Master SYBR Green I (Roche Diagnostics, Mannheim, Germany) was carried out for 12 representative sample pairs using the iCycler iQ real time PCR detection system (Bio-Rad), as described below.

**Real time PCR analysis of DNAs from archival prostatic biopsies**

DNA was extracted from randomly selected, paraffin wax embedded tissue sections of normal, hyperplastic, and malignant prostatic biopsies using buffer comprising 0.5M Tris, 10mM NaCl, 20mM EDTA, 1% SDS (pH 9.0), and proteinase K. This was accompanied by phenol/chloroform/isoamylalcohol extraction and ethanol precipitation. DNA samples were reconstituted to the same concentration of 100 ng/μl, and 100 ng of each sample was used for amplification. Real time PCR was performed with primers TF1 and TR4.2 using the iCycler iQ SG-1 quantitative PCR reaction buffer kit (Bio-Rad) and LightCycler-DNA Master SYBR Green I (Roche Diagnostics), at 95°C for four minutes, followed by 40 cycles each of 95°C for 30 seconds, 65°C for 30 seconds, and 72°C for one minute. Also included was the amplification of the G3PDH housekeeping gene.<sup>15</sup> All reactions were performed in triplicate or duplicate, and mean values derived. Real time PCR conditions were optimised by varying MgCl<sub>2</sub> concentrations between 1.5mM and 5mM. Different starting amounts of template were also used for validation of amplification efficiency. For each sample, the threshold cycle ( $C_T$ ) values for both MOST-1 and G3PDH were determined, and the difference

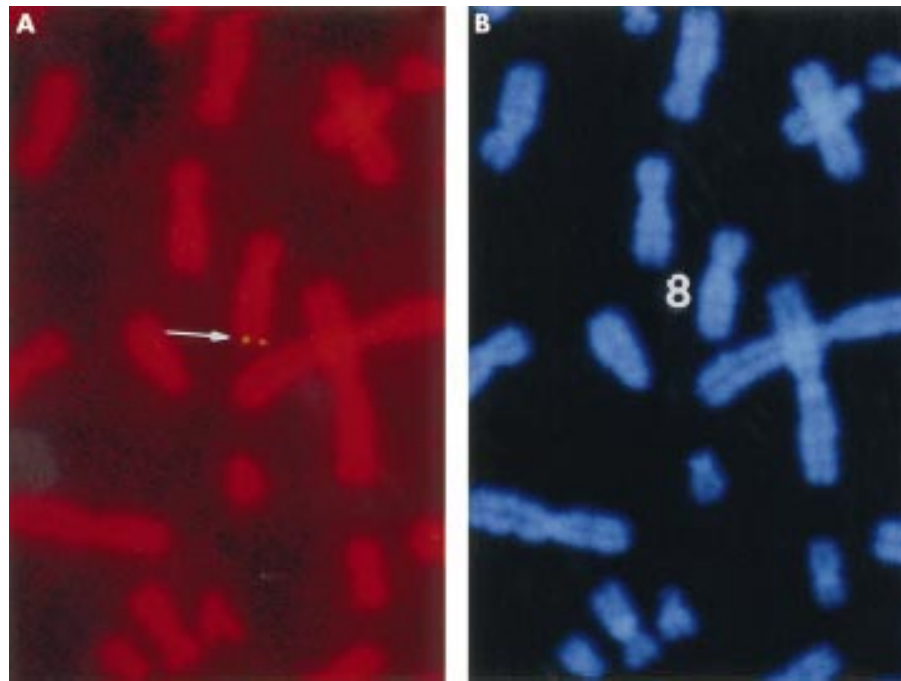
between their  $C_T$  values ( $\Delta C_T$ ) was calculated as recommended by the manufacturer of the real time PCR kit (Bio-Rad). The mean  $\Delta C_T$  values of each category of normal samples, hyperplastic samples, low, intermediate, and high grade carcinoma samples were calculated using SPSS for Windows (release 10.0.5). These mean  $\Delta C_T$  values were then subjected to analysis by multiple comparisons, and the p values between their differences were computed using the Student's paired sample *t* test. Acting as the reference, the mean  $\Delta C_T$  of normal tissue samples was compared against the mean  $\Delta C_T$  values of hyperplastic samples and carcinomas of varying grades to obtain the relative DNA values presented as a numerical fold change for each category of prostatic neoplasia.<sup>21 22</sup>

**RESULTS**

**Structural analysis of MOST-1 cDNA**

Amplification of the initial MOST-1 EST of ~ 350 bp from MOLT-4 cDNA can be explained by the homology between the 3' ends of the HPV 18c and 11q primers with nt 1036–1044 and nt 1399–1410 of the novel cDNA, respectively (fig 1A). BLAST searches of the GenBank database revealed no significant homology between this EST fragment and a known gene.

Screening for this novel EST in the two cDNA libraries of the MOLT-4 T lymphoblastic leukaemia cell line and of human fetal lung authenticated its presence in both libraries. We then proceeded to isolate the full length cDNA sequence corresponding to this EST from which specific primers were designed for use in RACE experiments with these libraries. From the MOLT-4 library, products of ~ 1.7 kb and ~ 1.2 kb were generated via 3' and 5' RACE, respectively. The fetal lung library yielded bands of ~ 1.6 kb and ~ 0.5 kb from 3' and 5' RACE reactions, respectively. Cycle sequencing of the overlapping 3' and 5' RACE fragments revealed composite cDNA



**Figure 2** Chromosomal localisation of the MOST-1 gene. (A) Double fluorescence in situ hybridisation signals detected by a MOST-1 specific probe at chromosome region 8q24.2, indicated by the arrow. (B) DAPI staining of the same mitotic figure to identify chromosome 8.

sequences of 2786 bp and 2054 bp in MOLT-4 and fetal lung, respectively (fig 1A). This last cDNA sequence in fetal lung was identical to nt 664–2705 of its MOLT-4 counterpart. The resultant cDNA sequence of 2786 bp was found to contain a continuous open reading frame (ORF) of 297 bp, with an in frame nonsense codon 168 bp upstream of the putative start codon. The first methionine codon lies in an extremely favourable context for translation initiation—that is, GGCACCATGG—which conforms exactly to the ideal sequence for a eukaryotic translation start site.<sup>23</sup> This potential ORF was predicted to encode 99 amino acids (fig 1B), forming a basic, hydrophilic protein (grand average of hydrophobicity of  $-0.417$ ) of 11 219 kDa. This putative protein had an isoelectric point of 8.59, and was classified as unstable in nature with an instability index computed to be 76.92. No notable amino acid sequence motifs were evident. However, three ATTTA mRNA destabilising signals<sup>24</sup> were present in the 3' untranslated region (UTR) of the gene. This novel gene was designated MOST-1 (for MOLT-4 sequence tag-1), assigned the symbol C8orf17 by the HUGO Nomenclature Committee, and its cDNA sequence deposited in GenBank under accession number AF220264.

To search for introns, the sequences of PCR products amplified from genomic and cDNA templates using identical primer pairs were compared but showed no differences. Subjecting the MOST-1 cDNA sequence to computational analysis by exon–intron search engines such as Grail also arrived at the same conclusion—that this gene was intronless.

#### The MOST-1 gene is located on chromosome 8q24.2

A detection efficiency of ~50% was attained with the FISH probe; that is, out of 100 mitotic figures checked, 50 displayed hybridisation signals on one pair of homologous chromosomes. Using DAPI (diamidinophenylindole) banding to identify the specific chromosome, the assignment between the signal from the probe and the long arm of chromosome 8 was obtained. Based on a summary of 10 photographs, MOST-1 was localised to a more precise position at chromosome 8q24.2 (fig 2), and no other loci were detected with the probe. The MOST-1 sequence was also mapped by UniGene computational analysis to chromosome 8q24.3.

#### MOST-1 is ubiquitously expressed in cancer cell lines but differentially expressed in normal human tissues

RT-PCR with MOST-1 specific primers TF5 and TR4.2 yielded expected 440 bp target fragments of detectable but varying intensities from cDNAs of 19 human cancer cell lines and two normal cell lines tested (table 1). However, of the 16 normal human adult tissues tested, MOST-1 RT-PCR products were detectable in only nine tissues (heart, kidney, liver, pancreas, small intestine, ovary, testis, prostate, and thymus), but not in others (brain, lung, skeletal muscle, colon, placenta, spleen, and peripheral blood leucocytes). The G3PDH and/or HUEL housekeeping genes served as cDNA quality controls, and were successfully amplified from the tested human cell lines and adult tissues with good correlation (data not shown).

#### MOST-1 is overexpressed in a subset of breast cancers

MOST-1 expression in paired breast tissue biopsies was compared by first normalising densitometric readings of MOST-1 with G3PDH RT-PCR products, and then calculating the ratios between corresponding tumour and adjacent normal tissues (T : N). Of the 27 paired biopsy samples tested, relative T : N expression of MOST-1 was increased in 11 pairs, unchanged in four, and decreased in another 12. Real time RT-PCR performed on 12 randomly selected pairs of tumour and normal tissues (six grade 1 and 2 cancers and six grade 3 cancers) revealed MOST-1 expression data consistent with semiquantitative RT-PCR results (data not shown). The T : N ratio of MOST-1 expression showed a correlation with breast cancer grade; that is, mean ratios for grade 1 and 2 versus grade 3 were 0.84 versus 1.69 by semiquantitative RT-PCR, and 1.41 versus 2.92 by real time RT-PCR. However, the differences between the means for grade 3 versus grade 1 and 2 cancers were not significant (table 2).

#### MOST-1 DNA copy numbers are increased in prostatic neoplasia

Because of the relatively low incidence of prostate cancers in Singapore, sufficient numbers of paired biopsies of prostatic cancer and adjacent normal tissues for statistical analysis were not readily available. Hence, we resorted to analysing DNA from a total of 61 histologically assessed, archival

**Table 1** Comparative MOST-1 expression in human tissues, normal cell lines, and cancer cell lines

Normal tissues	MOST-1: G3PDH ratio	Cell lines	MOST-1: G3PDH ratio
Pancreas	0.61	MRC-5 fetal lung cells	1.35
Prostate	0.57	Breast myoepithelial cells	1.15
Ovary	0.55	MDA-MB-231 breast adenocarcinoma	2.11
Testis	0.33	Hs578T breast ductal carcinoma	1.14
Liver	0.33	MCF7 breast adenocarcinoma	1.13
Heart	0.31	ZR-75-1 breast ductal carcinoma	0.44
Kidney	0.31	PC-3 prostate adenocarcinoma	1.20
Thymus	0.28	DU145 prostate carcinoma	1.13
Small intestine	0.26	CaSki cervical carcinoma	1.18
Lung*	0.17	HeLa cervical carcinoma	1.06
Peripheral leucocytes*	0.17	SiHa cervical carcinoma	0.90
Skeletal muscle*	0.14	T24 bladder carcinoma	0.86
Placenta*	0.08	HepG2 hepatocellular carcinoma	1.08
Colon*	0.06	Hep3B hepatocellular carcinoma	0.95
Brain*	0.02	PLC/PRF/5 hepatocellular carcinoma	0.69
Spleen*	0.00	Mahlavu hepatocellular carcinoma	0.64
		KATO III gastric carcinoma	0.76
		U-937 histiocytic lymphoma	1.59
		Raji Burkitt's lymphoma	0.96
		HL-60 promyelocytic leukaemia	0.95
		MOLT-4 T-lymphoblastic leukaemia	0.94

All cDNA samples were subjected to PCR with MOST-1 and G3PDH primers, and the individual ratios of the densitometric readings of their amplified products were calculated. \*Samples for which no MOST-1 amplified products were visualised by agarose gel electrophoresis. G3PDH, glyceraldehyde-3-phosphate dehydrogenase; PCR, polymerase chain reaction.

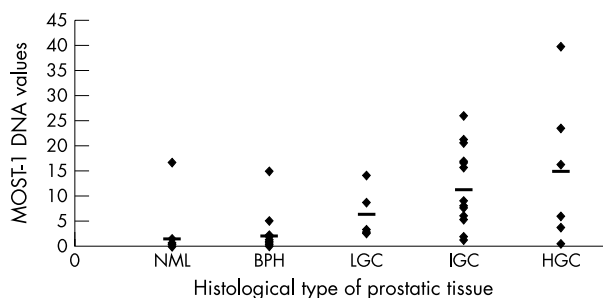
prostate biopsy sections; that is, 15 normal prostates, 21 benign prostatic hyperplasias, and 25 prostatic carcinomas. All DNA samples were subjected to real time PCR experiments to derive each individual  $\Delta C_t$  value—the difference between the threshold cycles for MOST-1 and G3PDH gene detection. The

formula  $2^{-\Delta C_t}$  was used to measure the amount of MOST-1 DNA (fig 3). By comparing the mean  $\Delta C_t$  value of normal samples (as the benchmark reference) with those of prostatic neoplasia, relative MOST-1 DNA values were increased 1.4 times in hyperplasias, and 9.9, 7.5, and 4.2 times in high, intermediate, and low grade carcinomas with the corresponding Gleason scores of 8–9, 6–7, and 3–5, respectively. Multiple comparisons between the mean for normal tissues against the individual means for neoplastic tissues indicated that the differences for hyperplasias or low grade cancers were significant ( $p = 0.014$  and  $p = 0.002$ , respectively), and for intermediate or high grade cancers were highly significant ( $p = 0.000$  for both grades). Interestingly, only one sample each of normal and hyperplastic prostatic tissue appeared to have significant amplification of MOST-1.

**Table 2** Ratio of MOST-1 expression in grade 1–3 breast cancers to expression seen in the corresponding adjacent normal tissues

Breast cancer grade	Tumour:normal ratio of MOST-1 mRNA (mean (SD))	
	Semiquantitative RT-PCR	Real time RT-PCR
1 and 2	0.8370 (0.3781) (n=14)	1.4148 (1.2565) (n=6)
3	1.6874 (1.5545) (n=13)	2.9154 (5.2143) (n=6)

The mean ratios and SD values were calculated from semiquantitative and real time reverse transcription polymerase chain reaction (RT-PCR) experiments.



**Figure 3** Correlation between histological types of prostatic tissues with MOST-1 DNA values. NML and BPH refer to normal prostate and benign prostatic hyperplasia, respectively. LGC, IGC, and HGC denote increasing grades of prostatic cancer (low, intermediate, and high), corresponding to Gleason scores of 3–5, 6–7, and 8–9, respectively. MOST-1 DNA values were calculated according to the formula  $2^{-\Delta C_t}$ , where  $\Delta C_t$  equals the difference between the threshold cycles for MOST-1 and G3PDH (glyceraldehyde-3-phosphate dehydrogenase) gene detection by real time polymerase chain reaction. The horizontal bars indicate mean values.

## DISCUSSION

The MOST-1 gene was found to be intronless, and structural analysis of its cDNA predicted an unstable transcript, with a strong consensus sequence at the putative methionine start codon<sup>23</sup> of a potential ORF encoding a relatively small protein. Present within the 3' UTR of MOST-1, the AUUUA motif is a highly conserved sequence repeated three or more times in RNAs encoding many short lived cytokines and protooncogenes.<sup>24</sup> The generally low abundance of MOST-1 mRNA in normal adult tissues was reflected by the necessity for reamplification during screening for tissue expression of MOST-1, and by the inability to detect MOST-1 transcripts by northern blot analysis (data not shown). These features are characteristic of many transiently expressed genes encoding small polypeptides involved in signalling or metabolic cascades that are being discovered at an accelerating pace. For example, human G protein coupled receptors that mediate ligand induced signalling are encoded by predominantly intronless genes.<sup>25</sup> Interestingly, the novel PSGR prostate specific gene with homology to a G protein coupled receptor is overexpressed in prostate cancers.<sup>21</sup>

It is noteworthy that MOST-1 was expressed in all the human cancer cell lines tested, but only in specific normal human adult tissues. Another important finding was the detection of MOST-1 mRNA in human fetal lung but not in

adult lung tissue, suggesting that MOST-1 expression is dependent on tissue differentiation. RT-PCR of the NS-1 mouse myeloma cell line using MOST-1 primers TF7 (nt 1000–1022) and TR4.1 (nt 1387–1368) amplified an EST, the sequence of which was completely identical to nucleotides 1000–1387 within the MOST-1 ORF (data not shown). This indicates the existence of a murine homolog of the MOST-1 gene, and implies its conservation throughout mammalian evolution.

The localisation of MOST-1 to chromosome 8q24.2 is noteworthy because this region (and its vicinity) has been shown by many comparative genomic hybridisation studies to be amplified mainly in breast and prostate cancers, and also in ovarian, testicular, renal, bladder, and colorectal tumours.<sup>26–33</sup> A recent study using FISH in pathological organ confined prostate cancers demonstrated that an additional increase or over-representation of 8q24 together with loss of 8p22 are associated with poor prognosis.<sup>34</sup> Although the localisation of the c-myc protooncogene at 8q24.1 has been cited to account for many poorly differentiated prostate cancers, the entire long arm of chromosome 8 is usually present at an increased copy number, suggesting that other genes may be involved.<sup>26–35</sup> For example, a gene that is differentially expressed during prostate cancer progression has been identified on chromosome 8q11.<sup>36</sup> Amplification of c-myc may not always correlate with 8q amplification in breast and prostate cancers.<sup>29–30</sup> Other genes located at the 8q24.2 region are the prostate stem cell antigen (PSCA) gene, a prostate specific gene that apparently plays a role in prostate cancer progression,<sup>37</sup> and the brain adenylyl cyclase gene HBAC1.<sup>38</sup> This locus has also been implicated in various diseases such as partial trisomy 8q, where patients suffer from psychomotor retardation,<sup>39</sup> and hereditary spastic paraplegia, in which the dominant HSP gene has been localised to 8q23–q24.<sup>40</sup> Diseases associated with 8q for which the exact disease locus has yet to be discovered include autosomal recessive achromatopsia with defective photoreceptors,<sup>41</sup> retinitis pigmentosa,<sup>42</sup> and tibial hemimelia in Langer–Giedion syndrome.<sup>43</sup>

“It is noteworthy that MOST-1 was expressed in all the human cancer cell lines tested, but only in specific normal human adult tissues”

The amplification of the chromosomal locus coinciding with that of MOST-1 in breast and prostate malignancies prompted us to perform quantitative PCR experiments to determine MOST-1 RNA and DNA values in frozen breast cancers and archival prostate tumours compared with normal tissues. Two fifths of breast cancers exhibited MOST-1 overexpression, concurring with previous comparative genome hybridisation studies that revealed amplification of 8q24.2 in these tumours. Compared with lower grade breast cancers, those of grade 3 showed higher T : N ratios of MOST-1 transcript values of up to 13.5. Relative to normal prostate specimens, benign prostatic hyperplasia samples displayed an average 1.4-fold increase in MOST-1 DNA, whereas up to approximately a 10-fold amplification was seen in prostatic cancers, especially those of higher grades and Gleason scores.

Aberrations of MOST-1 expression and copy number appear to be associated with substantial subsets of breast and prostate carcinomas, especially those of higher grade. Taken together, these findings suggest that MOST-1 may serve as a potential marker of breast and prostate tumour progression, and also imply a possible role for MOST-1 in cellular differentiation, proliferation, and tumorigenesis.

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#### Take home messages

- Starting with an expressed sequence tag from the MOLT-4 T lymphoblastic leukaemia cell line, we characterised a novel human, intronless gene, designated MOST-1
- The MOST-1 gene was mapped by fluorescent in situ hybridisation to chromosome 8q24.2, a region amplified in many breast cancers and prostate cancers, which is also the candidate site of potential oncogene(s)
- The MOST-1 gene was overexpressed in certain subsets of breast carcinoma and copy numbers were increased in prostatic neoplasia relative to the respective normal tissues; these effects appeared to correlate with grade
- The MOST-1 gene identified here may play a role in cellular differentiation, proliferation, and carcinogenesis

generous gift of some cell lines. This study was supported by a grant from the Biomedical Research Council, Singapore. JMM Tan is a recipient of a research scholarship from the National University of Singapore.

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