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Profiling of Differentially Expressed Genes in Human Cervical Carcinoma

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Abstract: Using the DDRT-PCR, a series of differentially expressed genes in human primary cervical cancer was isolated. Among the 250 PCR amplimers, 88 gene fragments were confirmed by reverse Northern hybridization. Homology searches indicated that 26 out of 88 were previously known genes including calmodulin, human BBC1, histone H3.3, a series of ribosomal proteins (RPL19, RPS19, and RPS12), translation initiation factor (eIF-4AI), lactoferrin, integrin α6, cell-surface antigens (CD9 and CD59), transcription factor (mbp-1), and mitochondrial proteins. Several unknown clones showed sequence homology with known genes. Furthermore, six of the unknown genes showed identical sequence with expressed sequence tags (EST) of unknown function. Differential expression patterns of identified genes were further examined and confirmed with multiple pairs of cervical cancer samples using Northern hybridization. Our profiling of differentially expressed genes may provide useful information about the underlying genetic alterations in human cervical carcinoma and diagnostic markers for this disease. The precise roles of these genes in cancer development remain to be elucidated

Key words: differential gene expression, cervical cancer, RT-PCR

INTRODUCTION

The human papilloma viruses (HPV), especially the high-risk HPV16 or 18 (Lorincz et al., 1987), are the principal etiological agents for human cervical carcinoma. The viral proteins E6 and E7 inactivate the tumor suppressor genes encoding the p53 and retinoblastoma (Rb) proteins, contributing to uncontrolled growth of cervical epithelium cells (Sheffner et al., 1990, 1992). Numerous *in vivo* and *in vitro* studies have shown that the inactivation of tumor suppressor genes leads to the deregulation of cell cycle regulatory factors and activation of various proto-oncogenes (Park et al., 1995). Although inactivation of tumor suppressor genes by viral proteins is a key regulatory event in human cervical tumorigenesis, little is known about the mechanism of the activation or suppression process. Furthermore, in some cases HPV alone is insufficient to induce cervical carcinogenesis, indicating the presence of other tumorigenic genetic alterations. For example, rearrangement and over expression of the c-myc proto-oncogene (Ocadiz et al., 1987) and mutations and deletions of the c-Ha-ras were reported in cervical cancer (Riou et al., 1988). In particular, structural and numeric alterations of several different chromosomes were identified in cervical cancer (Atkin et al., 1990; Manolaraki et al., 2002; Steekantaiah et al., 1988). But many molecular events involved in cervical tumorigenesis still remain to be elucidated.

To gain an insight into the molecular alterations that occur during the formation and development of cervical tumors, we previously monitored the expression level of 588 known genes on a cDNA expression array. But the result showed very restricted number of differentially expressed genes in human cervical carcinoma. In this study, we characterized differentially expressed genes from whole cDNA pool of human cervical cancer tissues using a differential display based on reverse transcription-polymerase chain reaction (DDRT-PCR)(Liang et al., 1992). Using the DDRT-PCR, we established profiles of differentially expressed genes in human cervical carcinoma using normal cervical tissue as a control.

MATERIALS AND METHODS

Total RNA Isolation from Cervical Cancer Tissues

Cervical cancer biopsies were obtained from patients in

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Department of Obstetrics and Gynecology, Samsung Medical Center, Seoul, Korea. Stages of disease were assigned according to the classification proposed by a clinical staging criteria of International Federation of Gynecology and Obstetrics (FIGO). The stages of cancer were Ib to IIa. All of the cell types were squamous cell carcinoma. We dissected 20 pairs of normal and tumor tissues from 20 patients for this research. After the surgery, sample tissues were immediately frozen in liquid nitrogen and stored in a deep-freezer at −70°C until use. Total RNAs were obtained by extracting tissues in Trizol (GIBCO BRL) according to the manufacturer's instructions. Briefly, normal and cancer tissues (100 mg) were homogenized in 1 mL Trizol solution using a Polytron homogenizer (Polytron). Homogenates were incubated for 5 min at RT and 0.2 mL of chloroform was added to homogenates. After vigorous vortexing for 5 min, inorganic phase was separated by centrifugation at 12,000×g for 20 min at 4°C. RNAs were then precipitated in the presence of one volume of isopropanol, washed with 70% ice-cold ethanol, and dissolved in RNase-free water. Total RNA concentration was assessed by absorbency at 260 nm using an UV-spectrophotometer (LKB).

RT-PCR Amplification and Polyacrylamide Gel Electrophoresis

For cDNA synthesis, total RNA (4 μg) was denatured at 65°C for 5 min in the presence of 100 pmol of each $GT_{11}VN$ (V=A, G and C; N=A, G, C, or T; GeneHunter) oligonucleotide in a final volume of 20 μL. After brief centrifugation at 4°C, 20 μL of master mix [400 U of MMLV reverse transcriptase (Boehringer Mannheim), 8 μL dNTP mix (0.25 μM each), 0.5 μL RNasin (26 U/μL; GIBCO BRL), 8 μL of 5× RT buffer (50 mM Tris-HCl, pH 8.3, 75 mM KCl, 3 mM $MgCl_2$, 10 mM dithiothreitol)] was added to the denatured RNA/oligonucleotide mix, and the reaction mixture was incubated further at 35°C for 1 hr. The temperature was then raised to 65°C for 5 min in order to terminate RT reaction. PCR amplification was carried out with 1 μL of RT reaction mixture in 10 μL of a PCR reaction solution contained 1 μL of 10× PCR buffer (supplied by Boehringer Mannheim), 0.5 μL of dNTP mix (100 μM), 0.1 μL of $\alpha[^{35}S]$dATP (1,000 Ci/mmol, Amersham) and 0.5 U of Taq polymerase (Boehringer Mannheim). In each reaction, 5 pmols of primers were added. As 5'-primers, we used 39 arbitrary primers (10-mers) that were commercially available from GeneHunter and Genosis. The PCR reaction mixture was then overlaid with 15 μL of buffer-saturated mineral oil (Sigma) and subject to amplification on PCR cycler (DNA thermal cycler 480, Perkin Elmer). The PCR consisted of 40 cycles with the following parameters; 95°C for 30 sec, 42°C for 1 min, and 72°C for 1 min. The final cycle was extended to 10 min at 72°C. The 6 μL of PCR products was then applied to a 5% non-denaturing polyacrylamide gel electrophoresis. Gels were dried and exposed to X-ray film (Fuji) for 12 hr.

Re-amplification of DDRT-PCR Amplicons, TA cloning, and Sequence Analysis

RT-PCR products were recovered from polyacrylamide gel slices by shaking in 10 μL elution buffer (0.5 M ammonium acetate, 10 mM magnesium acetate, 0.1% SDS, 1 mM EDTA, pH 8.0) at 37°C overnight. Re-amplification of the eluted fragments was performed in 40 μL PCR reaction mixture containing 1 mM dNTP mix, 10 pmol primers, and 1 μL of the eluted cDNA solution, under the same conditions as the DDRT-PCR step, except radio-labeled nucleotides were not included. After PCR amplification, 6 μL of PCR products were applied on 2% agarose gel electrophoresis. PCR amplicons were directly cloned into TA cloning vector (pCR2.1) according to the supplier's instructions (Invitrogen). Nucleotide sequences of cloned cDNAs were determined by Sanger's dideoxy termination method using a T7 Sequenase version 2.0 (USB) according to the supplier's protocol.

Labeled cDNA Synthesis and Dot-blot Analysis of PCR Amplicons

To verify the results of DDRT-PCR, reverse Northern-blot analysis was performed as previously described (Chunming et al., 1996). PCR amplicons (10 ng) obtained by re-amplification of DDRT-PCR products on polyacrylamide gel slices, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA (10 ng) internal control were blotted onto nylon membrane (Nytran; Schleicher & Schuell). cDNAs were then permanently attached by UV-illumination. Two sets of labeled cDNAs, which were used as probes, were synthesized from both normal and tumoric total RNAs (20 mg each) by reverse transcription in the presence of $\alpha[^{32}P]$-dCTP (3,000 Ci/mmol, Amersham) with ReversePrime cDNA synthesis kit (GeneHunter). After gel purification of labeled cDNAs as described by Sambrook et al. (Sambrook et al., 1989), equivalent amounts of radioactively labeled probes were applied to prehybridization solution. Hybridization was performed at 65°C in a rolling bottle overnight. After first wash with 2× SSC and 0.1% SDS, second stringent wash with 0.1× SSC and 0.1% SDS at 68°C was performed twice, and membranes were then exposed to X-ray film (Fuji) for 1 day at −70°C. To control possible loading error, doublet of the same cDNA was blotted onto membranes.

Northern-blot Hybridization

For Northern-blot hybridization, total RNA solution (10 μg) was denatured in the presence of 50% formamide, 2.2 M formaldehyde, 20 mM MOPS [3-(N-morpholino) propanesulfonic acid], 4 mM sodium acetate, 0.5 mM

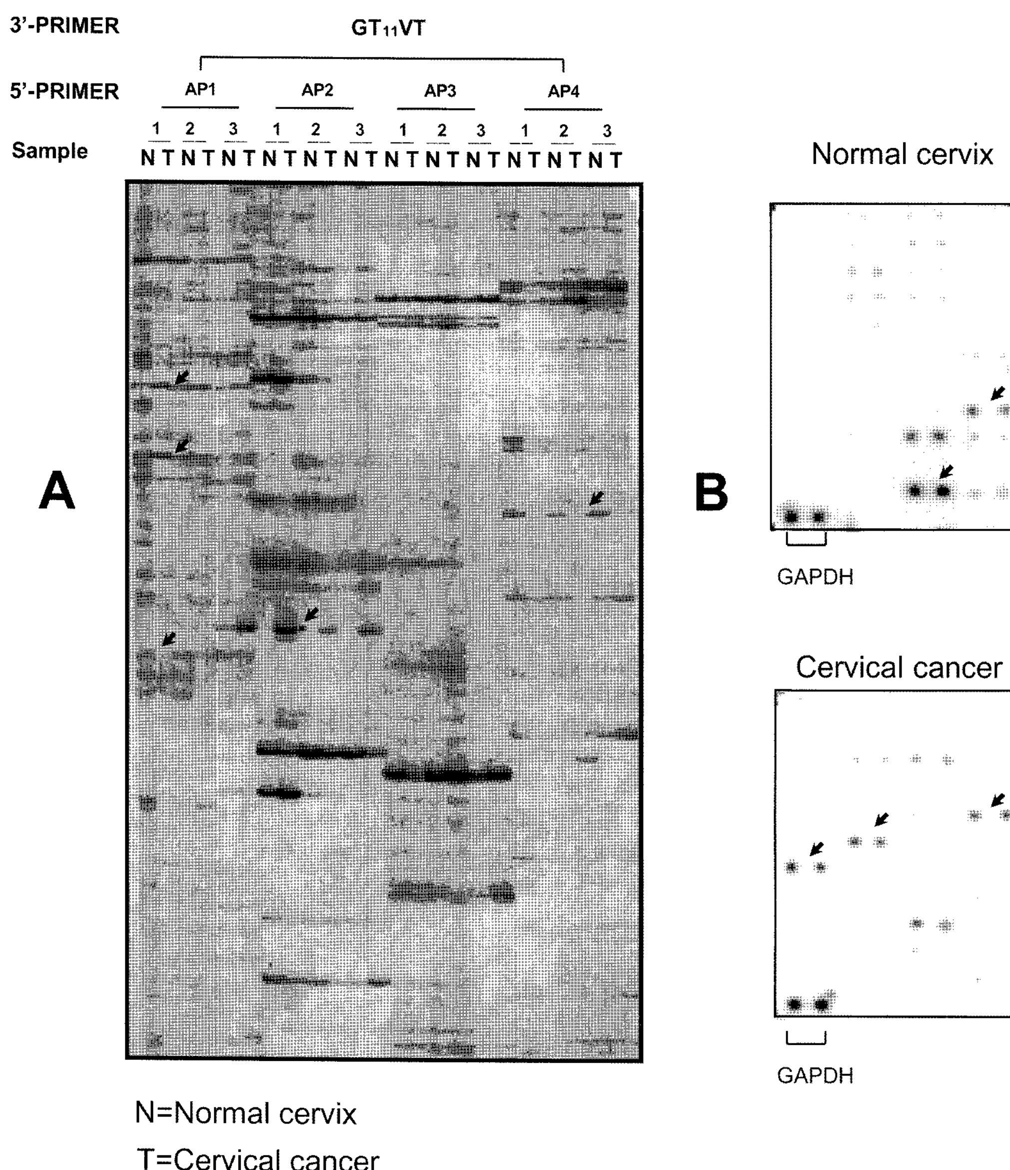

Fig. 1. (A) Representative results of DDRT-PCR analysis. Total RNAs were isolated from three pairs of normal cervix (N) and cervical carcinoma (T) and subjected to RT-PCR in the presence of $\alpha[^{35}S]dATP$. As 3'-primers, 4 anchored polyT ($GT_{11}VN$) primers were used for separate RT reactions and PCR amplifications. Thirty-nine arbitrary primers (AP1 to AP39; 10-mers) were used as 5'-primers. RT-PCR was performed as described in *Materials and Methods* and resulting amplimers were analyzed on a non-denaturing polyacrylamide (5%) gel. Bands Significantly altered in intensity in cancer samples vs. the control normal tissues were excised from gels. Re-amplification of PCR amplimers and cloning into TA vectors followed. (B) Reverse Northern-blot analysis of DDRT-PCR amplimers. RT-PCR products were blotted directly onto nylon membrane by diffusion. Duplicate membranes were hybridized separately with ^{32}P-labeled cDNAs synthesized from total RNAs (20 μg) of normal vs. tumeric cervix samples using a Reverse-Prime kit according to the manufacturer's instructions. To control for differences in hybridization efficiency of the two separate reactions, human GAPDH was used as an internal control.

EDTA at 65°C for 10 min. After Gel electrophoresis in 1.2 % agarose gel containing 2.2 M formaldehyde, the RNA was transferred onto nylon membrane (Nytran; Schleicher & Schuell) by capillary action under 10× SSPE (1x SSPE: 0.18 M NaCl, 10 mM Na_2HPO_4, pH 7.7, 1 mM EDTA). RNA transfer and loading efficiency were estimated by staining either the agarose gel with ethidium bromide or extra membrane with 0.1% methylene blue. For Northern hybridization, the membranes were washed in 6× SSPE for 5 min, air dried and RNA was permanently attached to the membrane by UV illumination for 1 min using an UV cross-linker (LKB). To prepare hybridization probes, EcoRI fragments of cloned cDNAs were labeled with $\alpha[^{32}P]$-dCTP (3,000 Ci/mmol, Amersham) using a RediPrime random priming kit (Amersham). Hybridization was performed for 2 hr in hybridization buffer (RapidHyb; Amersham) at 65°C. After hybridization, membranes were washed twice with 2x SSC and 0.1% SDS at 65°C for 10 min with shaking. The second wash was performed under high stringent conditions (0.1× SSC and 0.1% SDS at 70°C for

30 min). The membranes were then exposed to X-ray film (Fuji) overnight at −70°C. As an internal control, human GAPDH cDNA was re-hybridized with same blot after the initial probing.

RESULTS

Three pairs of normal and cervical tumor samples were subjected to DDRT-PCR. We isolated 250 clones differentially expressed in human cervical cancer compared with the normal cervix.A representative example is shown in Fig. 1A. To verify the results of DDRT-PCR, we performed reverse Northern analysis by dot-blot hybridization of PCR amplimers. ^{32}P-labeled cDNA probes were synthesized by reverse transcription from normal or tumor RNAs and used as templates (a representative example is shown in Fig. 1B). As a positive control to equalize loading efficiency, human GAPDH cDNA was applied onto all of the blots, and the result of autoradiogram signals were normalized with the GAPDH signals. Plasmid vector GEM4Z was used as a negative control, as it does not hybridize with human cDNA. By this selection process, we identified 88 re-amplified PCR clones which showed either decreased or increased expression in cervical cancer compared with the control cervix (40% cut off point). These cDNAs were then cloned into plasmid vectors by TA cloning strategy and subjected to sequence analysis. Nucleotide sequences were then matched with the GeneBank databases. Sequence analysis showed that 26 of the differentially expressed clones were known genes including calmodulin, human BBC1, histone H3.3B, ribosomal protein L19(RPL 19), S12(RPS12), lactoferrin, and integrin α6, among others (Table 1). Several clones showed extensive sequence homology with known genes, and a large number of clones were identified as novel genes (data not shown). Among the novel sequences, some showed a complete sequence identity with expressed sequence tags (EST) of unknown function (Table 1).

Table 1. Known genes that are differentially expressed in cervical cancer cells

GeneBank Locus	Gene identification	Expression
HUMCMYCQ	Human DNA binding protein (mbp-1)	+
HSRPL19	Ribosomal protein L19	-
HSRPS12	Ribosomal protein S12	+
HUM4AI	eIF-4AI	+
HUMCD59A	CD59/MEM43	+
HSBBC1	BBC1	+
HUMRCC1	Human RCC1	+
HUM927A	Interferon-induced 17kDa protein	+
HUMMTA	mCytochrome C oxidase I	+
HUMANTCD9	MRP-1/CD9	+
HSINTA6	Integrin a	+
HUMLFERR	Human lactoferrin	+
HUMHISH3B	Histone 3.3B	+
HUML101	Human L1 repetitive sequence	-
HSU85268	Mitochondrial COI gene	+
HUMMTM1	Mitochondria ORF1	-
HSAC000119	Human BAC clone RG104104	+
HUMALDN	Calmodulin	+
HSU85195	Human BAC 129	+
D87953	Human RTP	+
E02379	Autonomously replicating sequence	-
Hs214K23	Human DNA sequence PAC 214K23	+
HUMIGLAC	IgG kappa chain	-
HUMS19RP	Ribosomal protein S19	+
HUMALNE32	Alu transcripts	-
HAPRPS2	Phosphoribosyl pyrophosphate synthase II	+
N45290	Homosapiens cDNA clone 283219 3'	+
H79977	Homosapiens cDNA clone	-
T05990	Homosapiens cDNA clone	+
AA6106671	Homosapiens cDNA clone	+
L17796	Human STS UT1314	-
AA844605	Homosapiens cDNA clone IMAGE:1391034 3'	+
AA600127	Homosapiens cDNA clone 950291 3'	-
AA923553	Homosapiens cDNA clone IMAGE:1536220 3'	-

+: increase, -: decreased

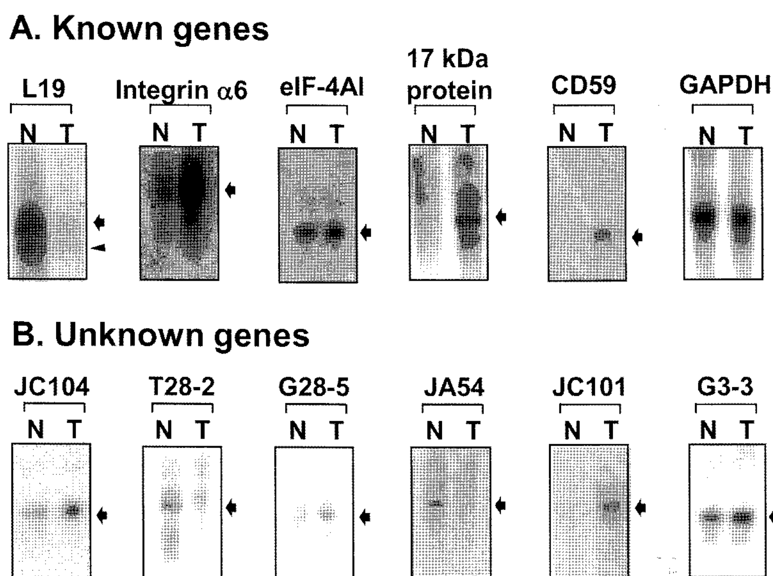

Fig. 2. Northern hybridization analysis for identified individual clones by DDRT-PCR. Representative results were for known (A) and unknown genes (B); known genes include ribosomal protein L19, integrin α6 chain, eukaryotic translation initiation factor (eIF-4AI), interferon-induced 17 kDa membrane protein, CD59. Same membrane was used for all hybridization reactions after repeated re-probing. Ten mg of total RNA was blotted onto nylon membrane after size-fractionation by denaturing agarose gel electrophoresis. Loading efficiency and RNA intactness were confirmed by ethidium bromide (EtBr) staining of agarose gel. Hybridization was performed with ^{32}P-labeled cDNA probes synthesized by random priming of EcoRI fragments of individual clones.

Next, we performed Northern blot hybridization analysis on total RNA isolated from cervical cancer and matched normal tissues with the cloned PCR fragments as the hybridization probes. Fig. 2 shows representative results of Northern hybridization of several clones that were identified by DDRT-PCR as differentially expressed genes in cervical cancer. We included several known genes in the Northern analysis, such as ribosomal protein L19, human integrin α6 chain, eukaryotic initiation factor 4AI (eIF-4AI), interferon-induced 17 KDa membrane protein, and CD59 (Fig. 2A). Nothern hybridization of the clones that contained sequences from unknown genes (Fig. 2B) showed that these clones hybridized to discrete mRNAs and were expressed in a tumor-specific manner. Densitometric scanning of the autoradiograms demonstrated that the level of integrin α6 mRNA was increased about 4-fold in cervical cancer vs. normal tissue (data not shown). The expression levels of eIF-4AI, interferon-induced 17 kDa membrane protein and CD59 were increased significantly in cervical cancer than in the normal cervix. The RPL19 cDNA probe detected one additional cross-hybridizing bands smaller than the major 0.9 Kb transcript that were increased significantly in cervical cancer tissue.

We also performed Northern hybridization using the unknown clones as probes and used the same membrane in Fig. 2B. Two mRNA species that hybridized to the T28-2 and JA54 clones were absent from in cancer samples, although they were normally detected in control cervix. In contrast, the G28-5 and JC101 clones hybridized to a discrete mRNA band that were detectable only in cancer tissues. The levels of mRNA species that hybridized to the JC104 and G3-3 clones were slightly altered in cervical cancer vs. the matched normal tissue. Densitometric autoradiograms demonstrated that the level of JC104 and G3-3 mRNA were increased about three- and two-fold each in cervical cancer vs. normal tissue (data not shown).

To examine whether the differential expression of specific and previously identified genes is a general event in human cervical cancer, we performed additional northern blot analysis with multiple pairs of cervical cancer and matching normal tissues. Representative autoradiograms of these northern hybridization experiments are shown in Fig. 3. Among the known genes, histone H3.3, lactoferrin, BBC1, interferon-induced 17 kDa membrane protein, mbp-1, and ribosomal protein S19 were included. A densitometric scanning of the autoradiograms was performed to compare the relative levels of expressed genes in normal cervix and cervical cancer. The expression level was considered to be altered (increased or decreased) when the amount of RNA in the cervical cancer tissue was 50% more or less than that in normal cervix. The overall expression patterns (percent expression) of each gene are summarized in Table 2. The number of tissue pairs represented in Table 2 differ for each gene because of difficulties encountered in tissue collection, especially the normal tissue, due to scarce availability. As shown in Fig. 3, the fold changes of mRNA levels in cervical cancer compared with matched normal cervix varied in different cancer patients. For example, the mRNA

Table 2. Expression patterns of DDRT-PCR amplimers from cervical tumor tissue vs. normal control cervix as determined by Northern hybridization

Genes	Total pairs (N vs. T)[1)]	Increased (%)	Unchanged (%)	Decreased (%)
Lactoferrin	20	11(55)	6(30)	3(15)
Interferon-induced 17-kDa protein	20	10(50)	6(30)	4(20)
BBC1	14	9(64)	2(14)	3(21)
PRDII-BPI/mbp-1	7	4(57)	2(29)	1(14)
Histone H3.3			7 5(71)	2(29)
RPL19	12	4(33)	1(8)	7(58)
RPS19	7	5(71)	2(29)	
RPS12	8	6(75)	2(25)	
eIF-4AI	14	10(71)	3(21)	1(7)
CD9	8	6(75)	2(25)	
CD59	11	8(73)	2(18)	1(9)

[1)]Total number of normal and cervical tumor tissue pairs.

levels of interferon-induced 17kDa membrane protein were higher in 10 of 20 cancer patients, and its levels were increased about 1.5- to 5-fold in cervical cancer vs. normal cervical tissue. Although we could not examine the expression of all differentially expressed genes by northern hybridization(limitation of tissue collection), our results demonstrated that DDRT-PCR analysis combined with reverse Northern hybridization is specific enough to detect multiple differentially expressed genes in primary human tumors.

Cancer tissue-specific expression was also observed with the unknown clones identified by DDRT-PCR. As shown in Fig. 4, unknown clones hybridized to discrete mRNAs in Northern hybridization experiments. The G3-3 clone hybridized to an ~1.5 Kb mRNA whose levels were higher in two of five cancer patients. The KG102 clone detected an ~2 Kb mRNA, which was increased in three patients and two transcripts hybridized by the JG61 clone were decreased in all cervical cancer tissues examined. Taken together with the data in Fig. 2B, these results indicate that the DDRT-PCR clones of unknown sequences were highly tumor-specific. Full sequence analysis of these unknown clones is in progress and should help to determine the role of these genes in cervical tumorigenesis.

DISCUSSION

Comparing the genes that are expressed in the carcinoma cells versus the normal cells could lead to the identification of cervical carcinoma-specific oncogenes, tumor suppressor genes and other tumor-associated antigens.

Although the biological functions of the known genes identified in our study remain to be analyzed, some of these genes have been implicated previously in tumorigenesis. For example, Northern hybridization indicated that the levels of lactoferrin mRNA were higher in cervical cancer than in normal tissue (Fig. 3 and Table 2). Lactoferrin is an iron-binding protein found predominantly in milk and secretary fluids of mammals and its RNA is expressed in mammary gland and uterus (Teng et al., 1989). Although lactoferrin is a major component of milk, its involvement in cellular proliferation and the development of neoplasia has been suggested (Walmer et al., 1995). In the uterus of mice, lactoferrin expression is directly correlated with the onset of epithelial proliferation (McMaster et al., 1992). In endometrial adenocarcinoma, an overexpression pattern for lactoferrin was observed both in the mRNA and protein levels, and possible modulation by progesterone receptor expression was suggested (Walmer et al., 1995). And lactoferrin acted early in the HPV uptake process (Drobni et al., 2004). In contrast, normal endometrium expresses lactoferrin in a cyclic manner in a restricted number of grandular epithelial cells (Newbold et al., 1992). Previous observation *in vitro* indicated that lactoferrin inhibits the growth of most tumor cells, suggesting a role for lactoferrin in the regulation of tumor growth (Bezault et al., 1994; Hurley et al., 1994). These results are consistent with our observation that lactoferrin mRNA level was higher in cervical cancer than in normal tissue, suggesting that the altered expression of lactoferrin is closely associated with the development of cervical cancer.

Motility-related protein-1 (MRP-1/CD9) is a trans-membrane glycoprotein belonging to the tetraspanin (TM4) superfamily (Maecker et al., 1997). It is closely associated with suppression of cell motility and reduction of metastatic potential of some tumor cells. The general functions of tetraspanin are to regulate cell adhesion, motility, differentiation, and proliferation by association with other

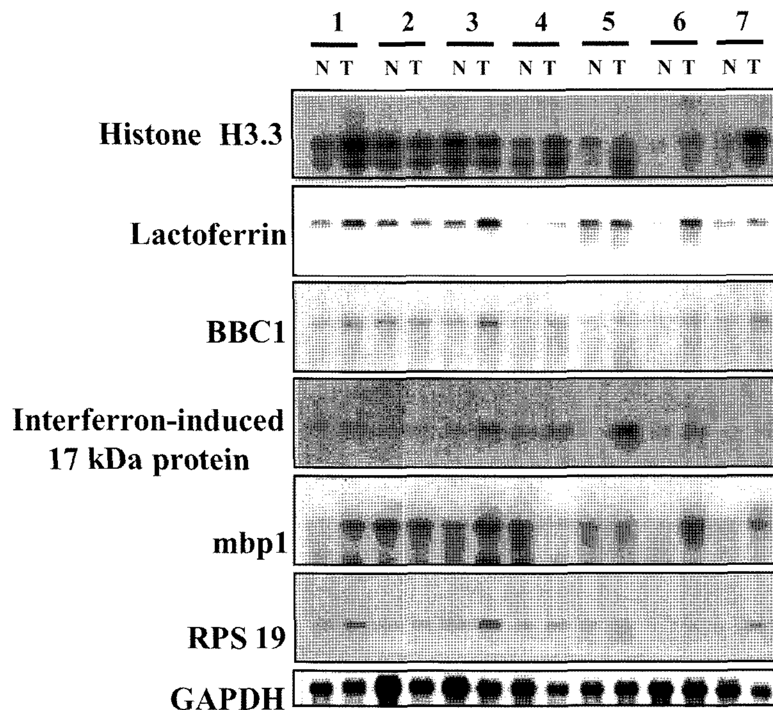

Fig. 3. Northern analysis of several known genes using multiple cancer tissue samples. Several known genes that showed tumor-specific expression in human cervical tissue were analyzed by Northern hybridization using additional cervical cancer samples. Total RNA (10 μg) was extracted from normal cervix (N) and cervical cancer (T) samples. The stages of all cancer samples used in this study were Ib to IIa. All of the cell types were squamous cell carcinoma.

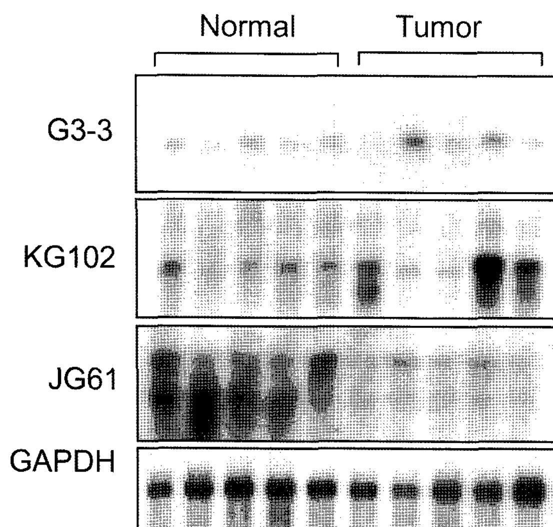

Fig. 4. Northern analysis of unknown genes differentially expressed in cervical cancer samples. RNA extraction and Northern blotting were performed as described in Fig. 3. EcoRI fragments of cloned genes in the pCR2.1 vector were used as hybridization probes.

cell-surface molecules. However, our Northern analysis demonstrates that CD9 mRNA was highly expressed in six of eight cervical cancer patients (Table 2), indicating that the increased levels of CD9 mRNA may be associated with the invasive phenotype of cervical cancer. The biological meaning of increased levels of CD9 mRNA in cervical cancer remains to be elucidated. Exposure of CD9 specific antibodies inhibit suspension-induced terminal differentiation of keratinocytes, showing that CD9 plays a regulatory role in keratinocyte motility and differentiation (Jones et al., 1996). In addition, altered expression of CD9 surface antigen in primary tumor cells and metastatic carcinoma cells were reported in colon carcinoma cell lines (Cajot et al., 1997), lung adenoma carcinoma (Higashiyama et al., 1997), oral squamous cell carcinoma (Kusukawa et al., 2001), ovarian carcinoma (Furuya et al., 2005), breast cancer (Miyake et al., 1995), and non-small cell lung cancer (Higashiyama et al., 1995). It is well known that the various cell surface adhesion molecules and intracellular linker proteins that are associated with the adhesion molecules are not only involved in the maintenance of cellular architecture, but also in the regulation of cellular proliferation and differentiation. Therefore, it is not unusual that cell surface molecules such as CD9, CD59, and integrins are over-expressed in cervical cancer (Tables 1 and 2).

We also detected three differentially expressed ribosomal protein subunits (RPL19, RPS19, and RPS12) by DDRT-PCR. RPL19 and RPS12 mRNA levels were increased in cervical cancer samples when compared with normal tissues (Table 2). In contrast, the levels of RPS19 expression were decreased in seven of the 12 cervical cancer samples examined. Increasing evidence suggested that translational components may act as regulators of

cellular growth and neoplastic transformation (Kondoh et al., 1992; Wong et al., 1993; Henry et al., 1993). The over-expression of RPS19 has been correlated previously with the development of cancer of the colon (Kondoh et al., 1992), and co-amplification of the RPL19 gene with the tumor-specific erbB-2 gene was observed in human breast cancer cells (Davis et al., 1995). Functional enhancement of ribosomal proteins, not only by over-expression at the transcriptional level, but also by post-translational modification as shown for RPS2 (Susa et al., 1989; Wang et al, 2009), is involved in cellular growth and differentiation of a variety of cells. These observations strongly suggested a close correlation between altered translational machinery and cancer development. Furthermore, translation factor, such as protein synthesis initiation factor eIF-4E, level was elevated bin esophageal cancer (Salehi et al., 2006) and also have been shown to function as mitotic regulators in HeLa cells, a human cervical cancer cell line (De Benedetti et al., 1990). Deregulation of the eIF-4AI mRNA levels were higher in cervical cancer than in matched normal tissues in ten of 14 patients examined (Table 2). Although we can not discuss each of the cDNA clones identified in this study, our results clearly demonstrate the significance of these genes in human cervical cancer development. Characterization differentially expressed genes may provide valuable information about the genetic alterations in cervical malignancies.

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