Analysis of gene expression profile induced by EMP-1 in esophageal cancer cells using cDNA Microarray

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INTRODUCTION
EMP-1 is a member of the PMP22 family with the similarity in structure. Since EMP-1 was first found by Taylor, it has been isolated independently from human, mouse and rabbit and received many different designations, such as TMP (tumor membrane Protein), PAP (Progression Associated Protein), CL-20 and B4B[1]. All tissues expressing EMP-1 mRNA contain 2.76-kb EMP-1 transcripts. In some regions of the gastrointestinal tract, including the fundus, ileum, cecum, and colon, however, additional transcripts of approximately 1.7 kb hybridize with the EMP-1 cDNA[2]. The 2.76-kb EMP-1 cDNA contains five exons about 0.2kb, 0.12kb, 0.1kb, 0.14kb, and 2.2 kb and four introns about 15kb, 1.9kb, 0.1kb, and 0.7 kb in length respectively, EMP-1 has been mapped to chromosome 12p12 by fluorescence in situ hybridization[3]. EMP-1 is encoded by a single-copy gene with the positions of introns exactly conserved between EMP-1 and PMP22, corroborating the hypothesis that EMP-1 belongs to the PMP22 family[4]. EMP-1 transcript is expressed at high levels in heart, placenta, lung, skeletal muscle, kidney, spleen, colon prostate, ovary, testicle, small intestine and thymus in human[5].

EMP-1 was selected from a series of differential expressed genes obtained from cDNA microarray analysis of expression profiles of esophageal cancer in our previous work. EMP-1 expression was 6 fold down-regulated in esophageal cancer lower than in normal tissue. EMP-1 is highly up-regulated during squamous cell differentiation and in certain tumors, and a role in tumorigenesis has been proposed[6]. Moreover, the overexpression of PMP22 leads to an apoptotic-like phenotype in NIH3T3 growing cells[7] and delays serum-forkosin-stimulated entry of resting Schwann cells from G1 into the S+G2/M phase in Schwann cell[8]. Transient expression of EMP-1 specifically inhibited cellular proliferation by more than 50 %[9]. Preliminary data suggested that EMP-1 was involved in growth control in esophageal cancer cell line EC9706. However, whether there is a similar effect of EMP-1 expression on the cell cycle of epithelial cells remains to be determined and little is known about the function of EMP-1 in growth control in esophageal cancer cell line EC9706.

To elucidate the effect of EMP-1 on EC9706 cell, the open reading frame (ORF) of human EMP-1 was cloned into pcDNA3.1/myc-his, a eukaryotic expression vector. EC9706 was transfected with the integrated plasmid containing EMP-1 to enforce expression of the exogenous EMP-1. Western blotting and RT-PCR were used to analyze positive clones. The cell growth curve was observed and the cell cycle was checked by FACS method. However, the mechanism by which EMP-1 may exert its activity remains unclear. Because the differentiation of mammalian cells is associated with changes in gene expression that is primarily controlled at the level of transcription, we tested the expression alteration with cDNA microarray technology to address the question of which genes are influenced by EMP-1 gene overexpression.

MATERIALS AND METHODS

Sample collection
Fifteen pairs of esophageal tumors and matched adjacent...
normal mucosa were obtained at surgery. Samples were frozen in liquid nitrogen until RNA was extracted.

**Cell lines and cell culture**

Esophageal carcinoma cell line EC9706 was established in our laboratory. The cell lines were maintained in M199 medium with 15 % FBS and cultured at 37 °C in 5 % CO₂.

**The eukaryotic plasmid vector pcDNA3.1-myc-his (-) C**

An Xhol I and Hind III fragment ORF of EMP-1 was cloned into the pcDNA3.1/myc-his vector. The correct construct sequence was confirmed by DNA sequencing.

**Atlas human cancer cDNA expression array**

Atlas Human Cancer cDNA Expression Array (Clontech) was used to analyze EMP-1-induced gene expression which included over 588 genes on the nylon membrane.

**Isolation of RNA and semi-quantitative RT-PCR**

Paired esophageal cancer tissues and adjacent normal mucosa tissues (-100 mg) were homogenized mechanically in 1 ml of TRIzol reagent (Life Technologies, Inc.), and cell pellets were harvested to isolate the total RNA. According to the manufacturer’s protocol (Life Technologies, Inc.). First-strand cDNA was synthesized from 5 μg of total RNA using Superscript II reverse transcriptase (Life Technologies, Inc.) and OligotIT12-18 primers following the company’s protocol. The same amount of cDNAs was subsequently used for PCR amplification. PCR reaction was performed in 25 μl buffer containing 1 μl cDNA, dATP, dCTP, dGTP, dTTP each 0.2 mmol/L, 1×PCR buffer, 1.5 mmol/L MgCl₂ and 1.5U Taq DNA polymerase (GIBCO). The primers for GAPDH are 5’-ACC ACA GTC CAT GCC ATC AC-3’ and 5’-TTT GTA GTC CTG TTT CCA AAG-3’; Human type XI collagen (COL11A1), 5’-TCC TGT TTG TTT TCT TGG CT-3’ and 5’-TTA TGA TTT TCA AAG CTT TTG T-3’; Human tissue-type plasminogen activator (PA), 5’-AGT GCA TTC TCC CGG CAT ATA CT-3’ and 5’-TTG GTG GTC TCT TCA CCA AAG-3’; Human interleukin-6, 5’-AGG CAC TGG CAG AAA ACA AC-3’ and 5’-TCA GAG AAA TGA TGT GCT GC-3’; Human alpha-1 type XI collagen (COL11A1), 5’-GCC TTC GTC GGT GTC TCA CCA AAG AC-3’ and 5’-TCC GAG AAA TGA TGT GCT GC-3’. The amplification was performed with one denaturing cycle of 5 min at 95 °C, then 94 °C for 40s, 55 °C for 40s, 72 °C for 40 s for 25-27 cycles, and one final extension of 7 min at 72°C. RT-PCR products were analyzed on 1.2 % agarose gel. The number of cycles and melting temperature was adjusted depending on the genes amplified.

**In vitro transfection and cell culture**

According to the manufacturer’s instructions (Invitrogen), the day before transfection, seed 3×10⁶ EC9706 cells in 3 ml of the 15 % serum complete growth medium. Combine diluted DNA with diluted Lipofectamine™ Reagent. For each transfection, add 0.8 ml of medium without serum to the tube containing the complexes. Incubate the cells with the complexes for 5 hours at 37 °C in a CO₂ incubator. Following incubation, add 3 ml of growth medium containing 30 % serum without removing the transfection mixture. Add G418 after 3 days and select monoclonal cell to a fresh bottle and keep on culturing with G418 (1 mg/ml). The positive clones together with the negative parental vector without EMP-1 and EC9706 Cells (3×10⁵ in 2 ml) were seeded and incubated at 37 °C in 5 % CO₂ with G418 (1 mg/ml), Cells were harvested and then quantitated every 24 hours.

**Flow cytometry assay**

Flow cytometry was performed to assess the cell cycle alteration. Trypsinized adherent and floating cells were collected, washed twice with cold PBS, and resuspended in PBS containing 0.1 % Triton X-100 and 0.1 % RNase for 5 min at room temperature. The samples were then stained with propidium iodide (0.1 mg/ml), filtered through a 300-μm-pore-size nylon mesh, and analyzed in a cell sorter (Coulter, epics® elite ESP).[10]

**Western blotting analysis**

Cells were rinsed twice with PBS, and protein preparation was performed according to the manufacturer’s instruction (Santa Cruz). Protein concentration in homogenates was determined using a BSA-Protein assay (Hyclone) employing bovine serum albumin as the standard. The extracted proteins (20 μg) were subjected to 15 % polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate under reducing conditions, and then transferred onto poly (vinylidene difluoride) (PVDF) membranes (Hybond-P). Mouse monoclonal anti-human hexad-HIS antibodies (Santa Cruz; final dilution 1:1 000) were used as the primary antibody, and horseradish peroxidase labeled anti-mouse immunoglobulin (Santa Cruz; final dilution 1:1 500) was used as the secondary reagent. Detection was performed using an ECL system (Amersham-Pharmacia).

**cDNA microarray analysis**

Total RNA was extracted by TRIzol from positive clone 2 cells and the parental vector without cDNA insert pcDNA3.1-myc-his (-) C cells as negative control. Poly (A) RNA was purified using an Oligotex-dT30 mRNA purification kit (TAKARA). One μg of highly purified poly (A) RNA from the negative control and and cell clone 2 was performed for cDNA microarray analysis. The mRNA was reverse transcribed with 2P-dATP (Amersham). The paired reactions were purified with a TE-30 column (Clontech, Palo Alto, CA). The radioactively labeled probe was then applied to the array for hybridization at 68 °C for 12 hours. After hybridization, the membrane was washed with buffer of decreasing ionic strength. The X-ray film was scanned at a resolution of 16-bit. AtlasImage software (version 1.01a, Clontech) was used for image analysis. The area surrounding each element image was used to calculate a local background, which was then subtracted from the total element signal. Background subtracted element signals are used to calculate intensity ratios. The average of the resulting total intensity signal gives a ratio that is used to balance or normalize the signals. Then semi-quantitative RT-PCR analysis of 4 randomly selected genes confirms the microarray results.

**RESULTS**

**Differential expression of EMP-1 judged by RT-PCR**

RT-PCR was used to detect the EMP-1 gene expression in the pair of esophageal cancer (C) and normal mucosa (N). We checked 15 pairs of esophageal cancer and its adjacent normal tissue and found the expression of EMP-1 was higher in normal tissue than that in cancer tissue in 14 pairs, and lower in only 1 pair (Figure 1).

**Selection of positive transfectant clone by RT-PCR**

Since the expression of EMP-1 should be up-regulated after transfection, RT-PCR was used to analyze the positive clones and the parental vector as negative control. The clones 1, 2, 3,
4, 5, 6, 8 and 10 showed a comparable increased expression of EMP-1 (Figure 2).

Expression of EMP-1 in positive clones detected by western blotting

Since the vector carrying 6xHis peptide as a selective marker, it can be detected by anti-His monoclonal antibody with Western blot. Hexad-His peptide has been expressed in host cell clone 1, clone 2, clone 3, clone 5, clone 6 and clone 8, however, EMP-1 protein was not identified in clone 4 which was highly expressed in transcriptional level for unknown reasons (Figure 3).

**Figure 1** Different expression of EMP-1 detected in matched pairs of esophageal cancer tissue. The upper band represents EMP-1 (702 bp); the lower band represents GAPDH (452 bp). The expression of EMP-1 is higher in normal (N) tissue than its cancer(C) tissue. RT-PCR for GAPDH was used as equal loading control.

**Figure 2** Different expression of EMP-1 cell clones transfected with pcDNA3.1-EMP-1-myc-his (-) C vector and the parental vector without cDNA insert pcDNA3.1myc-his (-) C cells as negative control. Clones 1, 2, 3, 4, 5, 6, 8 and 10 showed the increased expression of EMP-1. RT-PCR for GAPDH was used as equal loading control. B represents the parental vector as negative control.

**Figure 3** Western blotting check the expression of EMP-1 after transfected into the EC9706 cell with anti-HIS monoclonal antibody. His peptide has been expressed successfully in host cell clones (C1, C2, C3, C5, C6 and C8). Control represents the negative control.

**The growth curve of positive clone and the parental vector without the cDNA insert as negative control**

The cell proliferation rate had been observed and the cells displayed a decreased proliferation rate in clone 1, clone 2, clone 3, clone 6, clone 8 and clone 10, especially in clone 2. The proliferation rates of clone 4, clone 7 and clone 9 are close to that of negative control. The expression of EMP-1 in clone 1, clone 2, clone 3, clone 6 and clone 8 was up-regulated in both RNA and protein levels by RT-PCR and Western blot, respectively, and a reduced ability to proliferate was as shown in these clones (Figure 4).

**Figure 4** The growth curve of EC9706 cell line after transfected into EMP-1. The clones 1, 2, 3, 5, 6, 8 and 10 slow down obviously, and the clones 4, 7 and 9 are close to the vector control. Vector represents the negative control.

**Flow cytometry analysis**

Five of ten clones showed similar results after flow cytometry analysis. The percentages of cells in G1 and S phases of clone 1, clone 2, clone 3, clone 6 and clone 8 were 66.6 %, 17.9 %; 64.0 %, 30.2 %; 63.8 %, 29.1 %; 60.9 %, 34.6 %; and 69.5 %, 24.2 %; respectively. The percentage of cells in G1 and S phases of negative control were 52.2 % and 41.7 %, respectively. Comparing the cells transfected with cDNA insert and the negative control, we found that S phase was arrested and G1 phase was prolonged. Then cell clone 2 was used as a representative because its proliferation has been inhibited obviously (Figure 5).

**Figure 5** Flow cytometric analysis of cell cycle of negative control (left) and cell clone 2 (right). The percentages of negative cells and the clone 2 cells in G1, G2 and S phase are 52.2 %, 62.2 %, 41.7 %, 64 %, 58 % and 30.2 %, respectively.

cDNA microarray results of the parental vector pcDNA3.1/myc-his and EMP-1 transfected cell

By cDNA microarray analysis, 35 genes showed an over 2.0-fold change in expression level after transfection, with 28 genes
being consistently up-regulated and 7 genes being down-regulated in clone 2 cells (Figure 6).

**Figure 6** cDNA microarray analysis of parental negative control expression (left) and the EMP-1 transfected cell clone 2 (Right). 35 genes show an over 2.0-fold change in expression level after transfection, with 28 genes being consistently up-regulated and 7 genes being down-regulated.

**RT-PCR validation of the microarray results**
To confirm the gene expression profile, semi-quantitative RT-PCR was carried out for 4 randomly selected differentially expressed genes. As a result, the expression of these 4 genes was similar with the microarray analysis (Figure 7).

**Figure 7** Verification of up-regulated and down-regulated gene expression in EC9706 induced by overexpression of EMP-1. Interleukin-6, plasminogen and P19INK4D increased their expression and COL11A1 decreased its expression. Pre represents the sample not transfected with the vector carried with the ORF of EMP-1, and post represents the sample transfected with EMP-1. RT-PCR for GAPDH was used as equal loading control.

**Gene expression profile induced by overexpression of EMP-1**
In further analysis of the data from this study provided an insight into the alteration in the EMP-1 induced genes. Because these 588 genes are cancer associated genes and include almost all aspects involved in carcinogenesis and development of tumor and can be the representative of tumor, these data provide an independent and relatively unbiased estimate of the EMP-1 induced expression profile. In general, genes that expression were was more than 2.0-fold were informative and valuable (Table 1).

**Table 2 Summary of genes regulated by EMP-1**

| Major category        | Sub category        | Up-regulated | Down-regulated |
|-----------------------|---------------------|--------------|----------------|
| Cell division         | General             | 1            |                |
|                       | DNA synthesis/replication | 1           |                |
|                       | Apoptosis           | 2            |                |
|                       | Cell cycle          | 1            | 4              |
|                       | Chromosome structure| 1            |                |
|                       | Total               | 6            | 4              |
| Cell signaling        | Cell adhesion       | 1            |                |
| or cell communication | Channels/transport protein | 1     |                |
| and adhesion          | Effectors/modulators|              |                |
|                       | Extracellular       | 1            |                |
|                       | Hormones/growth factors | 4           | 1              |
|                       | Intracellular       | 2            |                |
|                       | Transducers         | 3            |                |
|                       | Protein modification| 2            |                |
|                       | Receptors           | 3            | 2              |
|                       | Total               | 13           | 3              |
| Cell structure/       | General             | 3            |                |
| motility              | Cytoskeletal        |              |                |
|                       | Extracellular matrix Proteins | 2 |               |
|                       | Motor proteins      |              |                |
|                       | Total               | 5            |                |
| Cell/organism defense | General             | 1            |                |
|                       | Homeostasis         |              |                |
|                       | Immunology          | 1            |                |
|                       | Total               | 1            |                |
| Gene/protein expression | RNA synthesis      | 1            |                |
|                       | Protein synthesis   |              |                |
|                       | Total               | 1            |                |
| Metabolism            | General             | 1            |                |
|                       | Amino acid          | 2            |                |
|                       | Cofactors           |              |                |
|                       | Energy/TCA cycle    | 2            |                |
|                       | Lipid               |              |                |
|                       | Nucleotide          | 1            |                |
|                       | Protein modification|              |                |
|                       | Sugar/glycolysis    |              |                |
|                       | Transport            | 2            |                |
|                       | Total               | 2            |                |
|                       | Unclassified        | 1            |                |
|                       | Total               | 1            |                |
cDNA microarray analysis offers the opportunity to monitor changes in gene expression across the entire set of expressed genes in cells. These 588 known genes are classified on the basis of the biological function of the encoded protein, using a modified version of a previously established classification scheme\[11,12\]. The classification scheme was composed of six major functional categories and several minor functional categories within the major categories. As shown in Table 2, 34 genes were classified as known function genes, and one gene was categorized as unclassified.

Interestingly, the largest categories (13 out of 28 genes) of EMP-1-induced genes are those involved in cell signaling, cell adhesion and cell-cell communication, which included integrin beta 7 (ITGB7), integrin beta 8 (ITGB8) and cadherin 5 (CDH5). These results indicated that EMP-1 might be related with cell adhesion and cell-cell communication. Cadherin-5 and the alpha E beta 7 integrin mediated heterotypic adhesive interactions between epithelial cells and intraepithelial lymphocytes in vitro\[13\]. Integran beta 7 (ITGB7) and integrin beta 8 (ITGB8) are heterodimeric (alpha/beta) transmembrane receptors for extracellular matrix (ECM) ligands. The beta subunit of integrins are considered important for regulation of stimulated cell adhesion and adhesion-dependent signal transduction. Through interactions with molecular partners at cell junctions, they provide a connection between the ECM and the cytoskeleton and regulate many aspects of cell behaviors. Integrins play an important role in lymphocyte adhesion to cellular and extracellular components of their microenvironment\[14\]. Cadherin 5 was also induced by EMP-1 in our microarray analysis. It is generally accepted that cadherins are a group of cell adhesion molecules located at intercellular junctions, and they play an important role in

| Coordinate | Gene Name                                      | Ratio | Category       | Sub Category                        |
|------------|-----------------------------------------------|-------|----------------|-------------------------------------|
| A3i        | Cyclin-dependent kinase 4 inhibitor 2D        | 2.04  | Cell division  | Cell-cycle regulators               |
| A7d        | Cytokeratin 1                                 | 2.26  | Cell structure | Intermediate Filament Proteins      |
| B3i        | Caspase 9                                     | 4.11  | Cell division  | Cell-apoptosis                      |
| B7b        | Chromatin assembly factor 1 p48 subunit       | 2.93  | Cell division  | Chromosome structure                |
| B7j        | Adhesion-related gene                         | 4.49  | Tumor Suppressors | Oncogenes & Suppressors          |
| C2e        | DNA damage-inducible transcript 3             | 2.80  | Cell division  | Apoptosis-Associated Proteins      |
| C2j        | Ras associated with diabetes protein 1        | 2.47  | Cell signaling | Intracellular transducers          |
| C3e        | UV excision repair protein RAD23              | 5.15  | Cell division  | DNA synthesis/replication           |
| C4b        | Wingless-related MMTV integration 5a         | 3.09  | Cell Adhesion  | Extracellular Communication Proteins|
| C4k        | Dishevelled homolog 1-like protein            | 4.13  | Cell signaling | Intracellular Transducers          |
| C7m        | Retinoic acid receptor beta                   | 5.47  | Cell division  | Death Receptors                    |
| D3e        | Vitronectin (VTN);                           | 2.36  | Cell structure | Extracellular Matrix Proteins       |
| D4j        | Integrin beta 7 (ITGB7)                      | 2.27  | Cell Adhesion  | Matrix Adhesion Receptors           |
| D4k        | Integrin beta 8 (ITGB8)                      | 2.45  | Cell Adhesion  | Cell-Cell Adhesion Receptors       |
| D5g        | Ezrin; cytofilin 2; villin 2 (VIL2)           | 3.06  | Tumor Suppressors | Oncogenes & Tumor Suppressors     |
| D6i        | Caveolin 1                                    | 3.29  | Metabolism     | GTP/ GDP Exchangers                 |
| E1h        | Matrix metalloproteinase 11 (MMP11)          | 4.64  | Cell structure | Extracellular matrix Proteins       |
| E1m        | Matrix metalloproteinase 16 (MMP16)          | 3.06  | Protein modification | Metalloproteinases      |
| E2b        | Tissue inhibitor of metalloproteinase 1       | 3.82  | Protein modification | Protease Inhibitors                |
| E2h        | Tissue-type plasminogen activator            | 4.39  | Protein modification | Serine Proteases                  |
| E5b        | RHO GDP-dissociation inhibitor 1              | 2.42  | Metabolism     | GTP/ GDP Exchangers                 |
| E5h        | Cadherin 5 (CDH5)                             | 2.64  | Cell Adhesion  | Cell-Cell Adhesion Receptors       |
| F1h        | Bone morphogenetic protein 2A (BMP2A)        | 12.45 | Cell communication | Cytokines                       |
| F3j        | Early growth response protein 1               | 16.06 | RNA synthesis  | Transcription Activators & Repressors|
| F4l        | Interleukin 6 (IL6)                           | 4.28  | Cell communication | Interleukins & Interferons      |
| F5e        | Interleukin 13 (IL13)                         | 2.62  | Cell communication | Interleukins & Interferons      |
| F5k        | Interferon gamma (IFN-gamma; IFNG)            | 6.04  | Cell communication | Interleukins & Interferons      |
| F5l        | Leukocyte interferon-inducible peptide       | 2.73  | Unclassified   | Functionally Unclassified Proteins |
| A1j        | Cell division cycle 25 homolog A             | 0.35  | Cell division  | Cell-cycle regulators               |
| 2m         | Cyclin D2 (CCND2)                             | 0.34  | Cell division  | Cell-cycle regulators               |
| A2n        | G1/ S-specific cyclin D3 (CCND3)             | 0.30  | Cell division  | Cell-cycle regulators               |
| A3k        | Polo-like kinase (PLK)                       | 0.47  | Cell division  | Cell Cycle-Regulating Kinas         |
| D1m        | Collagen V1 alpha 2 subunit (COL6A2)         | 0.32  | Cell communication | Extracellular Matrix Proteins |
| D2b        | Collagen XI alpha 1 subunit (COL11A1)        | 0.17  | Cell communication | Extracellular Matrix Proteins |
| D7e        | Vascular endothelial growth factor C         | 0.11  | Cell communication | Growth Factors,                  |

Table 1 Genes whose expression was altered more than 2.0-fold
embryogenesis and morphogenesis in animals and humans due to their adhesive and cell-signaling functions. Disturbances of the expression or function of cadherins and their associated proteins are crucial for the initiation and development of many pathological states. Cadherin 5 is an epithelium-specific cadherin that is required for the development and maintenance of the normal function of all epithelial cells in tissues. The loss or down-regulation of cadherin 5 is a key event in the process of tumour invasion and metastasis[95] Cadherin 5 has the major role in intercellular adhesion in esophageal mucosa[16]. Appropriate cadherin expression reflects the differentiation of squamous cell carcinoma[17]. These results support the putative function of EMP-1, which was a potential maker of differentiation[18] and was related to cell proliferation, differentiation, regulation and cell death[10].

In addition, the second largest categories of EMP-1-induced genes were those involved in cell division. Such as cyclin-dependent kinase 4 inhibitor 2D, DNA damage-inducible transcript 3, and retinoic acid receptor beta. The up-regulated cyclin-dependent kinase 4 inhibitor 2D and the down-regulated genes in this categories including cell division cycle 25 homolog A[19] and cyclin D2 (CCND2) and cyclin D3 (CCND3) affect the cell cycle collaboratively. These support our finding that the inhibition of cell proliferation after overexpression of EMP-1 associates with the induction of S phase arrested and the prolonged G1 phase with flow cytometric assay.

Retinoic acid receptors beta (RAR-β) is another up-regulated gene induced by EMP-1. It is well established that retinoids can modulate epithelial cell growth, differentiation, and apoptosis in vitro and in vivo by binding to specific nuclear retinoid receptors, which include RAR-β[21]. Retinoids can prevent abnormal squamous cell differentiation in nonkeratinizing tissues physiologically. Retinoids can also reverse squamous cell metaplasia, which develops during vitamin A deficiency[22]. The expression of RAR-beta varied along with the differentiation level of esophageal cancer. RAR-beta mRNA was expressed in 62.7% (42/67), 55.1% (43/78) and 29.2% (7/24) of well, moderately and poorly differentiated SSCs, respectively[23]. Retinoic acid, which inhibits squamous cell differentiation with RAR-beta involved in, repressed EMP-1 expression in normal human bronchial epithelial cells[3]; EMP-1 is one of down-regulation of a cluster of squamous cell differentiation marker genes[18] and RAR-β may be involved in this process.

Some genes associated with the intracellular signaling pathway, such as dishevelled homolog 1-like protein and ras associated with diabetes protein 1, were induced by EMP-1. It is well established that retinoids could be able to interact with other molecules and could have functional significance. Furthermore, the first hydrophobic region of PMP22/EMPs, between the first and second hydrophobic domains, forms an extracellular loop that contains one or more consensus sequences that can be N-linked glycosylated in vitro, a structure which has been implicated in cell-cell recognition and adhesion processes[24].

In conclusion, the study of the gene expression changes support the putative function of EMP-1 in cell proliferation and cell differentiation after enforcing expression of EMP-1 associates with the induction of S phase arrest and the prolonged G1 phase. The expression profile and localization of EMP-1 suggest a role in cell signaling, adhesion and communication. This is the first demonstration of global gene expression analysis of esophageal cancer cell line EC9706 transfectant with EMP-1, and these results provides a new insight in the study of the relationship between EMP-1 and esophageal cancer.

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