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SHORT COMMUNICATION

Differential expression of a subset of ribosomal protein genes in cell lines derived from human nasopharyngeal epithelium

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Extraribosomal functions of human ribosomal proteins (RPs) include the regulation of cellular growth and differentiation, and are inferred from studies that linked congenital disorders and cancer to the deregulated expression of RP genes. We have previously shown the upregulation and downregulation of RP genes in tumors of colorectal and nasopharyngeal carcinomas (NPCs), respectively. Herein, we show that a subset of RP genes for the large ribosomal subunit is differentially expressed among cell lines derived from the human nasopharyngeal epithelium. Three such genes (*RPL27*, *RPL37a* and *RPL41*) were found to be significantly downregulated in all cell lines derived from NPC tissues compared with a nonmalignant nasopharyngeal epithelial cell line. The expression of *RPL37a* and *RPL41* genes in human nasopharyngeal tissues has not been reported previously. Our findings support earlier suspicions on the existence of NPC-associated RP genes, and indicate their importance in human nasopharyngeal organogenesis.
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INTRODUCTION

Products of ribosomal protein (RP) genes are essential for cellular protein biosynthesis. Besides this, studies have also linked them to human congenital disorders and cancers. For instance, *RPS4* has been implicated in Turner's syndrome,[1] and the mutant *RPS19* was found in individuals with Diamond–Blackfan anemia.[2] In colorectal carcinoma, the overexpressions of *RPS3*,[3] *RPS19*[4] and *RPL7a*[5] have been reported. *RPL23*, a tumor metastasis-related gene, was found to induce high invasiveness of a human lung adenocarcinoma cell line.[6]

In our previous study, 33 RP genes were overexpressed in tumors of colorectal carcinoma relative to their normal controls.[7] We have also recently identified two RP genes (namely *RPS27* and *RPS26*) to be downregulated in nasopharyngeal carcinoma (NPC) tumors compared with a normal control.[8]

Despite the increasing number of cancer-associated RP genes identified thus far, the full repertoire of RP genes linked to human cancers remains unclear. In this study, 18 RP genes encoding proteins for large ribosomal subunits were tested on cell lines derived from NPC tissues and the normal nasopharyngeal epithelium. This effort was aimed at identifying nasopharyngeal-associated RP genes.

MATERIALS AND METHODS

Cell lines and RT-PCR

Total RNAs were from the nonmalignant nasopharyngeal epithelial (NPE) cell line, NP69;[9] and from the NPC cell lines of TW01, HONE1 and SUNE1. Complementary DNAs (cDNAs) were constructed from the RNA, and RT-PCR was carried out using specific 20-mer primers designed for RP genes *MRPL3*, *RPL3*, *RPL7a/surf3*, *RPL9*, *RPL10*, *RPL12*, *RPL18*, *RPL21*, *RPL23a*, *RPL26*, *RPL27*, *RPL27a*, *RPL29*, *RPL30*, *RPL35a*, *RPL37a*, *RPL38* and *RPL41*. The RT-PCR products ranged in size from 105–433 bp.

Differential expression analysis

Expressions of all 18 RP genes and the internal control, glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), were assessed through band intensity measurement using the AlphaEaseFC (Alpha Innotech, San Leandro, CA, USA) software. Intensity values of genes for each cell line are normalized to the *GAPDH* value of the respective cell line. For calibration of band intensity measurement, bands from a DNA size reference marker (100-bp ladder) was evaluated for delimits in the range of measurable intensities. A replicate test was conducted for all genes in all cell lines. The mean normalized value of band intensities was then plotted in a bar (with s.d.) and line charts for descriptive statistical analysis. Analysis of the *MRPL3* gene was omitted because of undetectable expression. For quantitative statistical analysis, expression data

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for each gene (except *MRPL3*) in NPC-derived cell lines was compared with the NPE cell line using unpaired Student's *t*-test. Significance was accepted at 95% confidence limit ($P<0.05$).

RESULTS

RT-PCR expression analysis

The RT-PCR assay showed expression of all genes (Figures 1a–i), except for the mitochondrial RP gene, *MRPL3*. No DNA band was observed for cell lines tested on expression analysis of the *MRPL3* gene (Figure 1c). Assessment of the overall expression profile showed *RPL9* (Figure 1d) and *RPL21* (Figure 1e) to be high expressors, whereas low expressors were *RPL12* (Figure 1e), *RPL23a* (Figure 1f), *RPL35a* (Figure 1h), *RPL37a* (Figure 1h), *RPL38* (Figure 1i) and *RPL41* (Figure 1i).

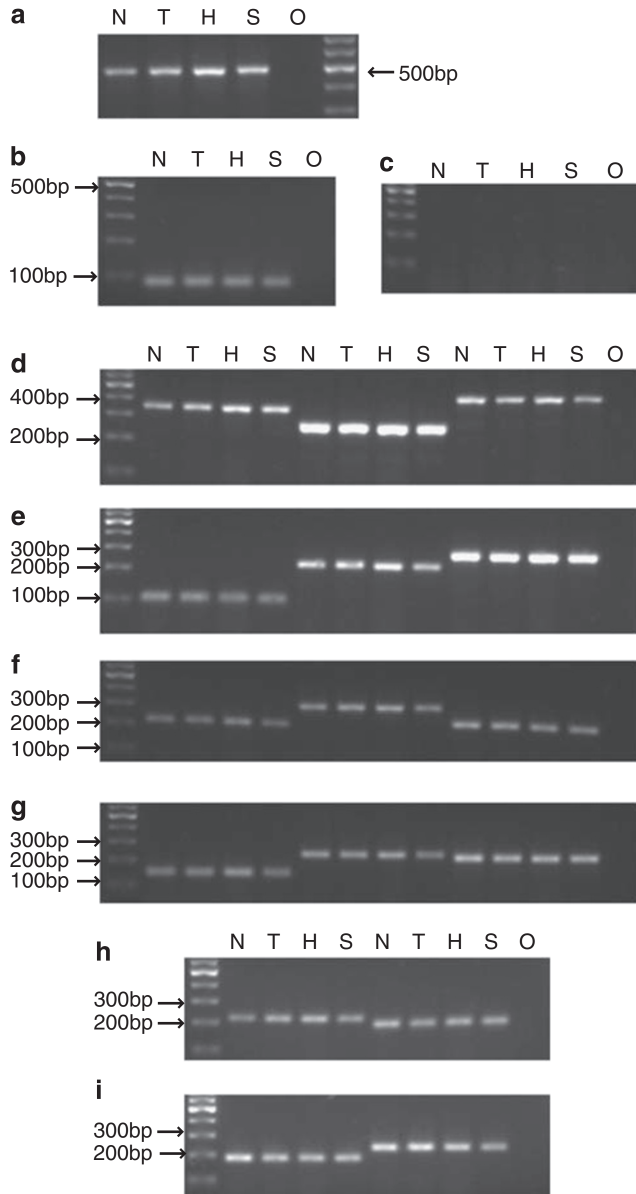

Figure 1 RT-PCR results of RP genes' expression in cell lines (N=NP69, T=TWO1, H=HONE-1 and S=SUNE-1). (**a**) is L3, (**b**) is GAPDH, (**c**) is MRPL3, (**d**) is L7a, L9 and L10 (from left to right), (**e**) is L12, L18 and L21 (left to right), (**f**) is L23a, L26 and L27 (left to right), (**g**) is L27a, L29 and L30 (left to right), (**h**) is L35a and L37a (left to right), and (**i**) is L38 and L41. Negative control=O (deionized water as PCR template). Size reference marker used is the 1-kb DNA ladder. Each set of analysis (for each gene) comprises N, T, H and S cell lines. RT-PCR, reverse transcriptase-PCR; RP, ribosomal protein.

Differential expression of RP genes

The expression of RP genes was compared between NP69 (the nonmalignant NPE cell line) and the three NPC-derived cell lines of TWO1, HONE1 and SUNE1. With the exception of *RPL3*, *RPL7a*, *RPL29*, *RPL30* and *RPL35a*, which were overexpressed in SUNE1, all other RP genes were observably underexpressed in the three NPC cell lines (Figure 2). The overall expression pattern of the 17 RP genes studied for each cell line was similar in profile among all cell lines studied (Figure 3). In general, expression for all RP genes is generally lower in HONE1 compared with NP69, TWO1 and SUNE1 (Figure 3). Approximately 47% (8 of 17) of the genes showed relatively higher expression in NP69 compared with the three NPC cell lines (Figure 3).

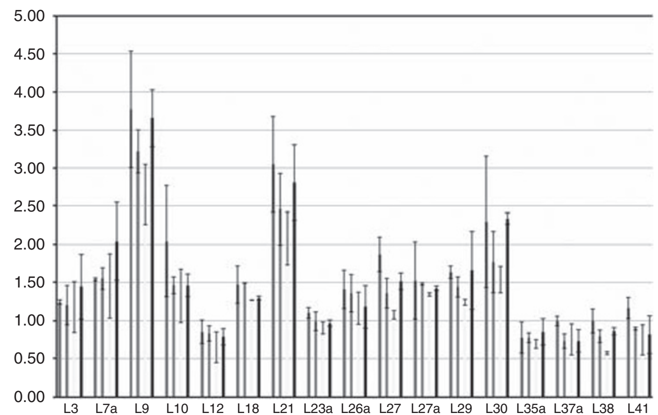

Figure 2 Bar chart illustrating the expression profiles of 17 RP genes in NP69 (blue bar), TWO1 (red bar), HONE1 (yellow bar) and SUNE1 (black bar) cell lines. The *X* axis represents RP genes, and *Y* axis is the normalized mean values processed from PCR product band intensities. Error bars included constitute calculated s.d. of the expression levels for each gene in each types of cell line. RP, ribosomal protein. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

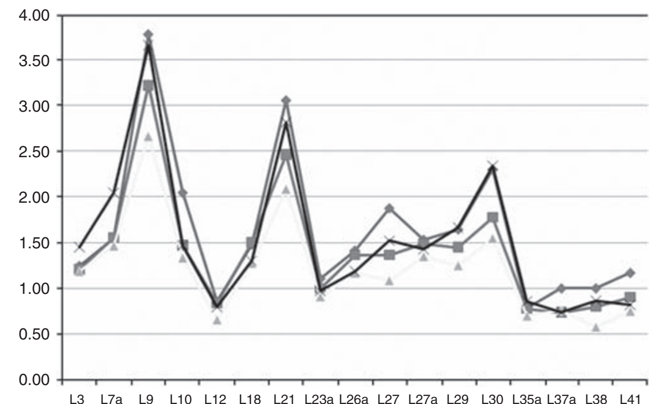

Figure 3 Line chart indicating expression profile of 17 RP genes in NP69 (blue line), TWO1 (red line), HONE1 (yellow line) and SUNE1 (black line) cell lines. RP, ribosomal protein. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

Two genes, *RPL3* and *RPL7a* showed higher expression in SUNE1 relatively to the other cell lines. In all cell lines studied, *RPL12*, *RPL23a*, *RPL27a* and *RPL35* genes showed comparable expression levels (Figures 2 and 3). However, *RPL27*, *RPL37a* and *RPL41* genes exhibited similar expression levels among all NPC cell lines, but not with NP69 (Figure 3). *t*-test analysis showed that they were significantly downregulated in all NPC-derived cell lines relative to NP69 (*RPL27* (P=0.023), *RPL37a* (P=0.032) and *RPL41* (P=0.033)).

DISCUSSION

In this study, RP genes for the large ribosomal subunits appeared largely underexpressed in NPC-derived cell lines compared with the nonmalignant NPE cell line. In all, 3 of the 17 genes tested (*RPL27*, *RPL37a* and *RPL41*) showed significant downregulation. Their expression pattern in NPC cell lines was consistent with our recent reports of the underexpression of RP genes in NPC cases.[8]

Among the significantly downregulated genes, only *RPL27* has been previously reported to be expressed in an NPC-derived cell line. Its expression was found to be inhibited in drug (cisplatin)-resistant NPC-derived cell lines (CNE2/DDP) in comparison with the normal cisplatin-sensitive CNE2 cell line.[10] This finding is somewhat consistent with our observation and indicates its role in maintaining tumorigenic potency of cancer cells.

The *RPL37a* gene was found to be upregulated in astrocytoma,[11] which contradicts our observation of its behavior in NPC cells. Such variation has to be clarified through more research. Nevertheless, the presence of a putative zinc finger in RPL37a,[12] suggests its DNA-binding capabilities, and thus its possible role(s) in the regulation of tumor suppressors, oncogenes or cell-cycle genes.

The observation made by Waxman and Wurmbach[13] on stable expression of *RPL41* in hepatocellular carcinoma varies from the findings by others,[14,15] and those of ours, which suggest its deregulation in cancer cells. In collaboration with p53 and $p27^{kip1}$, it was found to exert tumor-suppressive effects.[15] Therefore, its downregulation in tumors may suggest loss of control on p53 and $p27^{kip1}$, and inhibition of apoptosis. This phenomenon and the instability of *RPL41* in cancer cells of NPC remain to be explored.

On a final note, our findings substantiated the involvement of *RPL27*, *RPL37a* and *RPL41* in NPC tumorigenesis, thus bringing their extraribosomal role(s) into the context of human nasopharyngeal development for the first time.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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