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Short sequence-paper

Molecular cloning of a cDNA encoding human ribosomal protein L39 [1]

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Abstract

A cDNA clone encoding a human ribosomal protein L39 (hRPL39) was isolated through a random cDNA sequencing approach to a cDNA library constructed from a human colon carcinoma cell line of COLO 205. Although levels of hRPL39 mRNA were different in several cell lines including carcinoma cell lines from different tissues, they were shown not to be cell cycle-dependent in a human fibroblast cell line of TIG-1.

Keywords: Ribosomal protein L39; Random cDNA sequencing; (Human)

Carcinogenesis is thought to occur as a consequence of multistep alterations in genes [1,2]. In addition to mutations or deletions of certain genes, secondary molecular changes in carcinomas contribute to their malignant phenotypes to a certain extent. These secondary changes provide us with important information concerning the molecular basis of carcinoma and may lead us to find markers for the disease or a new therapeutic target. A random cDNA sequencing approach has recently become an effective method to obtain a group of genes expressed in specific states of cells or tissues by generating expressed sequence tags [3–6]. We applied a random cDNA sequencing approach to a human colon carcinoma cell line of COLO 205 [7], and isolated several cDNAs encoding ribosomal proteins. Among them, one was found to encode a human homologue of rNRPL39.

Cell preparation, cDNA construction, sequencing and analysis of cDNAs, RNA extraction, and Northern blot analysis were performed as previously described [7]. TIG-1 cells (a normal human fibroblast cell line) cultured to subconfluence were synchronized in the quiescent G_0 state by culturing them in DMEM supplemented with 0.5% FCS. The medium was changed on days 2 and 4. On day 6, the medium was changed to DMEM with 10% FCS to stimulate their growth [8]. Total RNA of TIG-1 was extracted by the acid guanidium thiocyanate-phenol-chloroform method [9] at 0, 6, 12, 18, and 24 h after growth stimulation and used for Northern blot analysis. Quantification of Northern blot analysis was performed by Fujix BAS 2000 phosphorimaging plates and a phosphorimager (Fuji, Tokyo, Japan) with the quantities standardized for the GAPDH message as a control.

We randomly picked up 1056 cDNA clones from the cDNA library constructed from COLO 205 and sequenced them by a single path sequencing [7]. The obtained sequences were compared with each other at first to assess the frequency of their appearance in our library, and then with the DNA data base in GenBank using a FASTA program [10,11]. Out of 526 independent cDNA species, we obtained 45 species encoding partial sequences of ribosomal proteins with frequencies of appearance in the library ranging from one to nine times. Of these 45 independent cDNA species, 22 were found only once, and the clone we report here was one of them.

While analyzing the sequences of the cDNA clones, we came across a cDNA which was not identified in humans before and is highly homologous to rNRPL39. Only the partial sequences were found in the DNA databases with the accession numbers L05096 and D28397 [12,13]. The nucleotide sequence determined by sequencing both strands of the cDNA three times and the deduced amino acid sequence are shown in Fig. 1. The cDNA included 37

Abbreviations: DMEM, Dulbecco's modified Eagle's medium; FCS, fetal calf serum; hRP, human ribosomal protein; rNRP, rattus norvegicus ribosomal protein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

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[1] The sequence data reported in this paper have been submitted to the EMBL/GenBank Data Libraries under the accession number D79205.

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        10         20         30         40         50         60
CAGCCATCGTGGTGTGTTCTTGACTCCGCTGCTCGCCATGTCTTCTCACAAGACTTTCAG
                                        M  S  S  H  K  T  F  R
        70         80         90        100        110        120
GATTAAGCGATTCCTGGCCAAGAAACAAAAGCAAAATCGTCCCATTCCCCAGTGGATTCG
  I  K  R  F  L  A  K  K  Q  K  Q  N  R  P  I  P  Q  W  I  R
       130        140        150        160        170        180
GATGAAAACTGGAAATAAAATCAGGTACAACTCCAAAAGGAGACATTGGAGAAGAACCAA
  M  K  T  G  N  K  I  R  Y  N  S  K  R  R  H  W  R  R  T  K
       190        200        210        220        230        240
GCTGGGTCTATAAGGAATTGCACATGAGATGGCACACATATTTATGCTGTCTGAAGGTCA
  L  G  L  *
       250        260        270        280        290        300
CGATCATGTTACCATATCAAGCTGAAAATGTCACCACTATCTGGAGATTTCGACGTGTTT
       310        320        330        340        350        360
TCCTCTCTGAATCTGTTATGAACACGTTGGTTGGCTGGATTCAGTAATAAATATGTAAGG
       370
CCTTTCTTTTT
```

Fig. 1. The full length nucleotide sequence of hRPL39 and its deduced amino acid sequence. The numbered positions of nucleotides are indicated above the sequence and the polyadenylation signal is underlined.

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(A)
ATGTCTTCTCACAAGACTTTCAGGATTAAGCGATTCCTGGCCAAGAAACAAAAGCAAAAT
********************** ** ************** ******************
ATGTCTTCTCACAAGACTTTCAGAATCAAGCGATTCCTGGCAAAGAAACAAAAGCAAAAT

CGTCCCATTCCCCAGTGGATTCGGATGAAAACTGGAAATAAAATCAGGTACAACTCCAAA
***** ***** ** ********************** ** ***************** **
CGTCCTATTCCTCAATGGATTCGGATGAAAACTGGTAACAAAATCAGGTACAACTCTAAG

AGGAGACATTGGAGAAGAACCAAGCTGGGTCTA
** ***** ***** ***** ************
AGAAGACACTGGAGGAGAACGAAGCTGGGTCTA

(B)
MSSHKTFRIKRFLAKKQKQNRPIPQWIRMKTGNKIRYNSKRRHWRRTKLGL
**************************************************
MSSHKTFRIKRFLAKKQKQNRPIPQWIRMKTGNKIRYNSKRRHWRRTKLGL
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Fig. 2. Comparison of the nucleotides and deduced amino acids of hRPL39 with those of rNRPL39 within the open reading frame. (A) hRPL39 showed 90.8% similarity at the nucleotide sequence and (B) 100% similarity at the amino acid sequence with the rat counterpart. (upper panel, hRPL39; lower panel, rNRPL39).

5′-non-coding nucleotides, an open reading frame of 153 nucleotides, and 181 nucleotides in the 3′-untranslated region, followed by a poly(A) tail. The polyadenylation signal, AATAAA, was located at nucleotides 346 to 351. The open reading frame specified a protein of 51 amino acids with the theoretical molecular weight of 7307. The nucleotide sequence of the cDNA shared 90.8% similarity (14 different nucleotides out of 181 nucleotides) within the coding region (Fig. 2A), and its deduced amino acid sequence shared 100% similarity with that of rNRPL39 (Fig. 2B). These results identified this cDNA as that for hRPL39.

We examined the levels of hRPL39 mRNA among different organs by Northern blot analysis (Fig. 3A). The signals were observed by cross-hybridization of an hRPL39 cDNA probe with mRNAs in organs of mice due to difficulty to obtain human tissues. RPL39 was ubiquitously expressed in all organs we examined. Lung and spleen showed about two-fold increased amounts of RPL39 mRNA when compared with those in other organs.

Because ribosomal proteins were reported to be overexpressed in carcinoma cells and tissues [14–21], the levels of hRPL39 mRNA were examined in cultured human cell lines. Fig. 3B shows a representative Northern blot analysis of hRPL39 in COLO 205, CW2 (a human colon carcinoma cell line), hNCI-H520 (a human lung squamous carcinoma cell line), and TIG-1. In comparison to hRPL39 mRNA in TIG-1, it was expressed almost at the same level in CW2, about two-fold higher in COLO 205 and three-fold higher in hNCI-H520, based on the quantitation using a phosphorimager (Fuji) (data not shown). These different expression levels may reflect the origin of tissues from which they were derived, because hNCI-H520 and lung showed a relatively high hRPL39 mRNA expression level. However, the mRNA level of hRPL39 was two-fold higher in COLO 205 than that in CW2, despite that they were derived from the same colonic origin. These altered mRNA levels may reflect different malignant phenotypes of these cells, because the mRNA level of a certain ribosomal protein was reported to correlate with colorectal carcinoma progression and its biological aggressiveness [14].

The mRNA of another human ribosomal protein S13 which was also overexpressed in carcinoma cells, was reported to correlate with the in vitro proliferative rate [15]. In addition, expression of several growth-associated

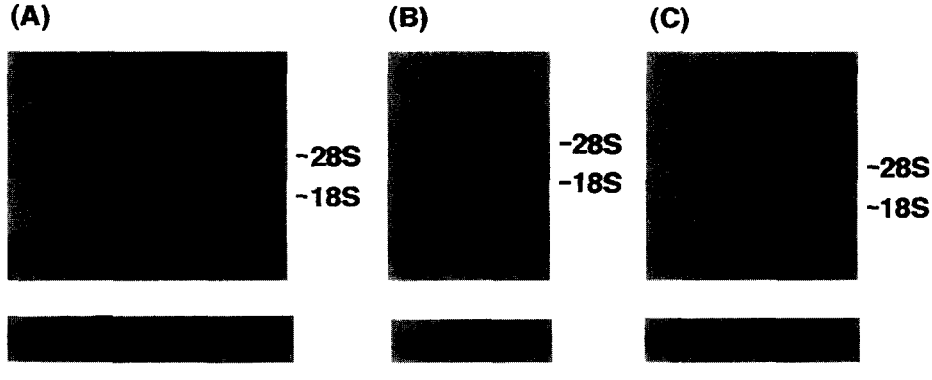

Fig. 3. Northern blot analysis of hRPL39. (A) RNA samples of brain, heart, lung, liver, spleen, kidney, and intestine which were abbreviated as Br, He, Lu, Li, Sp, Ki, and In, respectively, were extracted from C57BL/6 mice. Twelve micrograms each of total RNA were cross-hybridized with an hRPL39 cDNA probe. (B) Twelve micrograms of total RNA samples from COLO205, CW-2, hNCI-H520, and TIG-1, which were abbreviated as C, W, N, and T, respectively, were hybridized with an hRPL39 cDNA probe. (C) Total RNAs of TIG-1 at 0, 6, 12, 18, and 24 h after growth stimulation were hybridized with an hRPL39 cDNA probe. All blots were rehybridized with a murine GAPDH cDNA probe as a control (lower panel). The positions of 18S and 28S rRNA are indicated.

genes such as c-*myc* [22], c-*myb* [23], cyclin, and thymidine kinase [24], was reported to be cell cycle-dependent. However, little is known about the relationship between the cell cycle and the expression level of ribosomal proteins. Thus, we examined the expression of hRPL39 for its cell cycle-dependency in TIG-1. TIG-1 was used because hRPL39 was expressed at a fairly high level, and because they had been well analyzed for their cell cycles. TIG-1 cells were first synchronized to G_0 phase with serum starvation by 0.5% FCS, and their growth was stimulated by increasing the FCS concentration up to 10% as described [8]. As shown in Fig. 3C, the hRPL39 mRNA levels standardized for the GAPDH message were not significantly changed in TIG-1 (about 1.3-fold at most) during 24 h after the cell growth stimulation, suggesting that hRPL39 mRNA is stably expressed throughout its cell cycle phases. These results are consistent with the notion that the altered protein synthetic capacity in different growth states is based on changes in translation of ribosomal protein mRNA, rather than changes in mRNA abundance or stability, except for the condition where decreased mRNA levels were observed associated with differentiation [25].

It is likely that a subset of ribosomal proteins is overexpressed to produce other essential proteins to keep up with the high level proliferation and/or maintaining malignant phenotypes in certain carcinomas, because ribosomal proteins play an important role in protein synthesis via formation of ribosomal subunits [26]. Careful future studies on the relationship between malignant phenotypes of carcinomas and expression patterns of ribosomal proteins should help us to understand the significance of their overexpression in carcinomas, which may afford us therapeutic targets of carcinomas.

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