Trends in Undergraduate Research (2020) 3(1): a13-17
https://doi.org/10.33736/tur.1994.2020

Comparative Analysis of Ribosomal Protein Gene, *eL14* Expression between Two Types of Colorectal Carcinoma Cell Lines

MELINDA MEI-LIN LAU & EDMUND UI-HANG SIM*

Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia
*Corresponding author: uhsim@unimas.my

ABSTRACT

Association between the expression of ribosomal protein (RP) genes and cancer is widely known. More specifically, the extra-ribosomal functions of RPs have been linked to carcinogenesis. The ribosomal protein gene, *eL14* has been reported to be associated with malignancy of the colorectum, albeit of mechanism yet unclear. Its expression in cells derived from different tissue origin of colorectal carcinoma (CRC) has never been explored. Therefore, this study aims to comparatively analyse the expression pattern of *eL14* between two different CRC cell lines (DLD-1 and HCT116). It involved a conventional gene expression analysis, the Reverse-Transcriptase PCR (RT-PCR) assays. Products of RT-PCR assay were resolved via an agarose gel electrophoresis method, and band intensities of amplicons were documented and quantified using TotalLab Quant software. We observed differential expression patterns of *eL14* between DLD-1 and HCT116 cells, but statistical analysis revealed insignificant differences. Therefore, the relevance of *eL14* as a biomarker to distinguish between different colorectal cancer cells is suggestive but not conclusive.

Keywords: Colorectal carcinoma, *eL14*, gene expression, ribosomal protein genes, RT-PCR

Copyright: This is an open access article distributed under the terms of the CC-BY-NC-SA (Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License) which permits unrestricted use, distribution, and reproduction in any medium, for non-commercial purposes, provided the original work of the author(s) is properly cited.

INTRODUCTION

Ribosomal protein (RP) genes are necessary and ubiquitous components of ribosome structures essential in the process of protein biosynthesis. Increasing evidences have also pointed to the linked between RPs many diseases, including cancer (Chen *et al.,* 2016). In fact, aberrancies in ribosome biogenesis may be partly responsible for cancer and/or susceptibility to cancer (Ruggero & Pandolfi, 2003). Beyond ribosome-related context, the extra-ribosomal roles of RPs have also been established, of which perturbation(s) of such roles has been linked to carcinogenesis also (Wang *et al.,* 2014).

Ribosomal protein *eL14* is a member of the RPs for the large ribosomal subunit (60S) group. It is located at Chromosome 3, in the position of 3p22.1, and consists of 6 exons and 5 introns (Huang *et al*., 2006). eL14 contains a variable-length polyalanine tract that is encoded by a highly polymorphic trinucleotide repeat array (Shriver *et al*., 1998). Studies have demonstrated the down-regulated pattern of *eL14* in lung and oral cancers (Shriver *et al*., 1998), and oesophageal cancer (Huang *et al.*, 2006). The *eL14* gene lies with a genomic region (3p21) that has a high frequency of alteration (including translocation, deletion and allelic loss) that may contribute to the transcriptional loss. These are possible scenarios responsible for malignancy in lung and oral tissues (Shriver *et al*., 1998). Indeed, loss of heterozygosity (LOH) in *eL14* is linked to lung and oral carcinomas (Shriver *et al*., 1998). In the case of oesophageal cancer, genetic alteration and LOH in *eL14* have been reported (Huang *et al*., 2006). Besides these cancer types, *eL14* has also been shown to be associated with hepatocellular carcinoma or liver cancer (Liu *et al*., 2007).

To date, involvement or connection of eL14 to cancer of the colon and rectum region (colorectal carcinoma) has not established. Prior to this study, expression analysis of *eL14* in any cancer model of colorectal carcinoma (CRC) has not been reported in the literature. Therefore, this study is among the first exploration to investigate expression behaviour of *eL14* in cases of CRC. In our case, the CRC cell lines were the cancer model used.

MATERIALS & METHODS

Cell Culture

Colorectal carcinoma cell lines used in the study were DLD-1 and HCT116. These commercially-available cell lines were procured from ATCC. DLD-1 was derived from a Stage C cancer tissue at the sigmoid colon of a 50-year old male, and is a non-metastatic cell with moderate cellular differentiation (Dexter *et al*., 1981; Schneider *et al*., 2012). HCT116 is a human epithelial colorectal carcinoma cell line derived from a Stage D tumour at the ascending colon, and is poorly differentiated and metastatic (Brattain, Fine, Khaled, Thompson & Brattain, 1981; Wahab *et al*., 2017). All cell lines were grown at 37°C, 5% CO_2, and 95% humidity until the confluent stage of 70-90% before harvested for total RNA.

RNA Extraction

The TRIzol method (Chomczynski & Sacchi, 1987) was used for RNA extraction. Basically, monolayer cells in culture flask were rinsed with ice-cold PBS prior to the addition of 1ml TRIzol reagent. Then, the cells were gently scraped, and mechanically lysed by repeated pipetting before incubation for 5 min at room temperature. Subsequently, the lysate was transferred to a 1.5 ml sterile microcentrifuge tube, followed by the addition of 0.2 ml chloroform. This mixture was thoroughly mixed by vortexing for 15 sec, and then incubated at room temperature for 3 minutes. Then, the homogenate was centrifuged at 12,000 rpm for 15 min at 4°C. The aqueous phase was transferred into a new 1.5 ml sterile microcentrifuge tube, followed by the addition of 0.5 ml isopropanol. This was incubated at room temperature for 10 min, and then centrifuged at 10,000 rpm at 4°C for 10 min. The supernatant was removed, and the resulting RNA pellet was washed once with 1ml of 75% ethanol. It was air dried for 10 min, prior to dissolution 30 µl nuclease-free. The RNA solution was stored at -80°C until use. Quantity and quality check were carried out using spectrophotometric analysis.

Reverse-transcription and Polymerase Chain Reaction

Complementary DNA (cDNA) was synthesised using Moloney Murine Leukemia Virus Transcriptase (M MLV-RT). Two microlitre of random primers was added to 2 µg of RNA sample in a sterile 1.5ml tube. Nuclease-free water was then added to make the total volume of 15 µl. The mixture was incubated for 5 min at 70°C, and cooled on ice for 1 min. Then 1.25 µl of 10mM dNTPs, 5 µl M-MLV 5X reaction buffer, 0.6 µl of Recombinant RNAsin® Ribonuclease Inhibitor and 1 µl of M-MLV RT were added. The tube was flicked gently to mix the solution. Then, nuclease-free water was added to make the total volume to 25 µl. This was incubated at 37°C for 60 min, and then at 70°C for 15 min. Polymerase Chain Reaction assay was carried out according to the protocol for GoTaq® Polymerase (Promega, USA). The primer pair for *eL14* consists of the forward (5'- TTCTTCCTTCTCGCCTAA CG-3') and reverse (5'-CCTCCTAACTCCAGCCTCAA-3') primers, and the pair for *GAPDH* comprises 5'-AGATCATCAGCAATGCCTC-3' and 5'-TACCAGGACATGAGCTTGAC-3' as the forward and reverse primers, respectively. Expected amplicon size for *eL14* and *GAPDH* is 877bp and 507bp, respectively. The *GAPDH* gene functioned as an internal control for consideration of the equalizing amount of loaded sample, and allowed normalisation of data among the test results. For each PCR test, the components included 0.5 ug of template DNA, 1X Green Go Taq® Flexi Buffer, 1.0 mM $MgCl_2$, 0.2 mM dNTP mix, 0.4 µM each of the forward and reverse primers, and 1.25 U of Go Taq® Polymerase. Nuclease-free water was added to each mixture to a total volume of 25 µl. The thermal cycling conditions were initial denaturation at 95°C for 2 min, followed by 35 cycles of denaturation at 95°C for 30 sec, primers annealing (53°C for *eL14*, and 58°C for *GAPDH*) for 30 sec, and extension at 72°C for 1 min. A final extension of 5 min at 72°C was also included. PCR was done using a SensoQuest (Germany) PCR machine. The PCR products were resolved using agarose gel electrophoresis (AGE) method, and visualised using EtBr-stained gel on a UV transilluminator. Biological replicates were applied for each test.

Data Analysis

Band intensities of PCR products from AGE were measured using TotalLab Quant Software (TotalLab, USA) for the analysis of differential expression levels of both RPeL14 and GAPDH. The normalised value of each gene in each cell line was calculated based on the formula by Sim, Chee, Vasudevan, Ng and Chan (2018).

$$\text{Normalised value of test A} = \frac{\text{Average value of } \textit{GAPDH} \text{ band intensity}}{\text{Intensity value of } \textit{GAPDH} \text{ for test A}} \times \text{Intensity value of test A}$$

Validation of relative quantification of gene expression patterns can be done using Paired Student's *t*-test with a confidence interval of 95%. A significant difference was taken at $p < 0.05$.

RESULTS

Observable transcript expression, as far as RT-PCR products, was detected for *eL14* and *GAPDH* (internal control) in both DLD-1 and HCT116 cells (Figure 1). Eye-balling assessment (based on band intensities) revealed a general difference in the expression level of *eL14* between DLD-1 and HCT116 cells. The expression is apparently higher in DLD-1 compared to HCT116 cell lines.

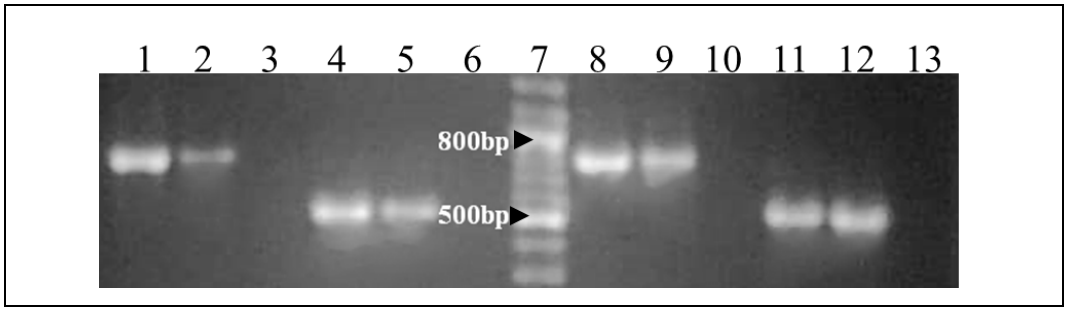

Figure 1. AGE analysis representation of RT-PCR assays of *eL14* and *GAPDH* expression pattern in DLD-1 and HCT116 cell lines. Lanes 1 and 8 are *eL14* in DLD-1 cells, Lanes 4 and 11 are *GAPDH* in DLD-1 cells, Lanes 2 and 9 are *eL14* in HCT116 cells, Lanes 5 and 12 are *GAPDH* in HCT116 cells, Lanes 3 and 10 are negative controls for *eL14*, Lanes 6 and 13 are negative controls for *GAPDH*, and Lane 7 is the size marker, 1kb DNA ladder. Lanes 1 to 6, and Lanes 8 to 13 represent Replicate 1 and 2, respectively.

The band intensities of both *eL14* and *GAPDH* in DLD-1 and HCT116 were documented and analysed using TotalLab Quant Software (TotalLab, USA). The result of both raw and processed data (normalised band intensities) are presented in Table 1. The normalised data of *eL14* expression in both DLD-1 and HCT116 is illustrated in a bar chart (Figure 2). Based on the bar chart, the band intensity of *RPeL14* in DLD-1 is observably higher than in HCT116. Statistical analysis via Student's *t*-test was applied to the normalised band intensity data. The results indicated that the difference in expression of *eL14* between DLD-1 and HCT116 cell lines is not significant (p = 0.069).

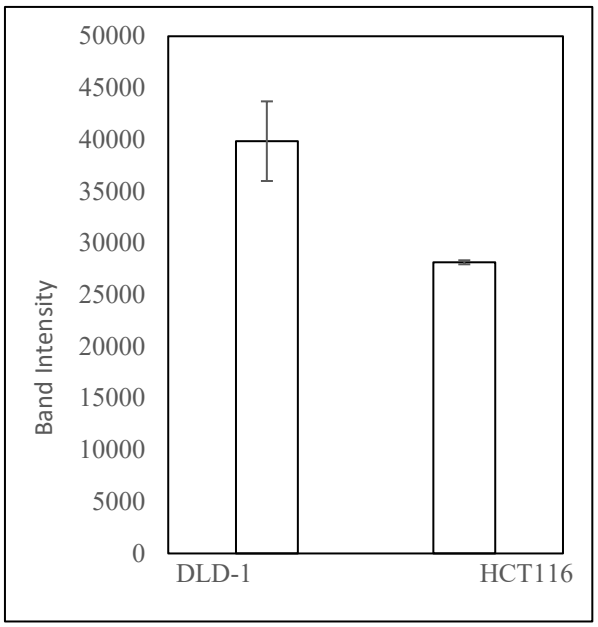

Figure 2. Bar chart of the normalised band intensities of *eL14* amplicons in DLD-1 and HCT116 cell lines.

Table 1. Band intensities of *eL14* and *GAPDH* (and the normalized value of *eL14*) for the CRC cell lines used.

Cell lines	Replicate	Band Intensity					Standard Deviation
		eL14	*GAPDH*	Average GAPDH	Normalised	Normalised Average	
DLD-1	1	38228	34697	38646	42579	39857	3850
	2	39937	41561		37135		
HCT116	1	22569	30834		28286	28148	195
	2	34422	47492		28010		

DISCUSSION

Our results indicate observable differential expression of *eL14* between DLD1-1 and HCT116, but the difference is not statistically significant. Nevertheless, expression of *eL14* in both CRC cell lines has been proven and reported for the first time here. Prior to this, its expression has been found in lung, oral (Shriver *et al*., 1998) and oesophageal carcinoma (Huang *et al*., 2006), of which was reportedly down-regulated in the diseased situation. In our case, the lack of availability of a normal cell lines for comparative limited our analysis to cancer cell lines. We were unable to infer whether *eL14* is up- or down-regulated in CRC models/cases. Between CRC cell lines of different origin, cell types and stages, the expression of *eL14* was initially observed to be different in level. However, this difference was not substantiated after a statistical analysis. Further studies, involving more different CRC cell lines will have to be carried out before any conclusion regarding the association of *eL14* to CRC scenario can be inferred. More tellingly, a normal control is required to be comparatively analysed with a variety of CRC cell lines for the hypothesis of a connection between *eL14* to CRC tumourigenesis to be accepted. A better model to be studied is, perhaps, actual tumours from CRC patients.

Besides our study, another research involving *eL14* expression in CRC cell lines (HCT116 and SW480) has been done by other members of our research team (Sim, Mutsamy & Teh, 2020). Our data here concurs with this other study, in that expression of *eL14* is detected in CRC cell lines. Our data here indicated higher expression (albeit insignificant) of *eL14* in cells (DLD-1) derived from an earlier stage (Stage C) of CRC compared to that (HCT116) from a more advanced stage (Stage D). In contrast, the findings by Sim *et al.* (2020) demonstrated a different scenario, whereby expressed transcripts of *eL14* is significantly higher in a cell line (HCT116) derived from an advanced stage of CRC compared to that (SW480) that is from an early stage (Stage B) of the cancer. The inconsistency of expression trends in *eL14* between all these studies suggests a comprehensive and simultaneous expression assay on many different CRC cell lines, together with relevant normal controls is warranted.

In addition to more cell lines, future expression study should utilise the quantitative PCR strategy for a more accurate and sensitive assessment of gene expression activity. The use of conventional RT-PCR method entails inaccuracy due to the fact that it is limited to the evaluation of end-point PCR product quantity. Quantitative PCR, however, allows for real-time measurement of the amplification process. Naturally, immunohistochemical techniques on CRC tissues to detect and quantify *eL14* expression would be an even better strategy for future study of its relevance in cancer of the colorectal region.

CONCLUSION

Expression of *eL14* has been detected in two cell lines (HCT116 and DLD-1) derived from CRC tissues. The level of transcripts is observably higher in DLD-1 (from Stage C cancer) than HCT116 (from Stage D). However, this difference in expression level is not statistically significant.

ACKNOWLEDGEMENTS

This is supported by the teaching allocation provided for the Resource Biotechnology by the Faculty of Resource Science and Technology (UNIMAS), and also the research grant from the Ministry of Education, Malaysia [grant code: FRGS/ST03(02)/1299/2015(16)].

REFERENCES

Brattain, M. G., Fine, D. W., Khaled, M., Thompson, J., & Brattain, D. E. (1981). Heterogeneity of malignant cells from a human colonic carcinoma. *Cancer Research, 41*, 1751-1756.

Chen, J. W., Wei, Y., Feng, Q. Y., Ren, L., He, G. D., Chang, W. J., Zhu, D., Yi, T., Lin, Q., Tang, W., & Xu, J. (2016). Ribosomal protein S15A promotes malignant transformation and predicts poor outcome in colorectal cancer through misregulation of p53 signaling pathway. *International Journal of Oncology, 48*(4), 1628-1638.

Chomczynski, P., & Sacchi, N. (1987). Single-step method of RNA extraction by acid guanidinium thiocynate-phenol-chloroform extraction. *Analytical Biochemistry, 162*(1), 156-159.

Dexter, D. L., Spremulli, E. N., Fligiel, Z., Barbosa, J. A., Vogel, R., VanVoorhees, A., & Calabresi, P. (1981). Heterogeneity of cancer cells from a single human colon carcinoma. *The American Journal of Medicine, 71*(6), 949-956.

Huang, X. P., Zhao, C. X., Li, Q. J., Cai, Y., Liu, F. X., Hu, H., Xu, X., Han, Y.L., Wu, M., Zhan, Q.M., & Wang, M. R. (2006). Alteration of RPL14 in squamous cell carcinomas and preneoplastic lesions of the esophagus. *Gene, 366*(1), 161-168.

Liu, Y. F., Zhu, X. J., Zhu, J., Liao, S. B., Liu, K. K., Guan, X. H., Zhang, J., & Feng, Z. Q. (2007). Identification of different expression of genes in hepatocellular carcinoma by suppression subtractive hybridisation combined cDNA microarray. *Oncology Reports, 18*, 943-951.

Ruggero, D., & Pandolfi P. P. (2003). Does the ribosome translate cancer? *Nature Reviews Cancer, 3*, 179-192.

Schneider, M., Huber, J., Hadaschik, B., Siegers, G. M., Fiebig, H. H., & Schüler, J. (2012). Characterization of colon cancer cells: a functional approach characterizing CD133 as a potential stem cell marker. *BMC Cancer*, *12*, 96.

Shriver, S. P., Shriver, M. D., Tirpak, D. L., Bloch, L. M., Hunt, J. D., Ferrell, R. E., & Siegfried, J. M. (1998). Trinucleotide repeat length variation in the human ribosomal protein L14 gene (RPL14): Localisation to 3p21.3 and loss of heterozygosity in lung and oral cancers. *Mutation Research Genomics, 406* (1), 9-23.

Sim, E. U. H., Chee, C. S. M., Vasudevan, L., Ng, K. H., & Chan, S. L. L. (2018). Selective differential expression of the ribosomal protein genes *eL14* and *uS19* in a well-differentiated epithelial cell line of nasopharyngeal carcinoma. *Malaysian Applied Biology Journal*, *47* (1), 247–253.

Sim, E. U. H., Mutsamy, S., & Teh, Z. Y. (2020). Expression patterns of the human ribosomal protein genes *eL14* and *uS19* in colon cancer is dependent on the type and stage of the cancer cell. *Malaysian Applied Biology Journal*, *49*(3), in press.

Wahab, R., Gopalan, V., Islam, F., Smith, R. A., Qiao, B., & Lam, A. K. (2017). Cellular expression, in-vitro ad in-vivo confirmation of *GAEC1* oncogenic properties in colon cancer. *European Journal of Cell Biology, 96*(6), 487-495.

Wang, W., Nag, S., Zhang, X., Wang, M. H., Wang, H., Zhou, J. W., & Zhang R. W. (2014). Ribosomal proteins and human disease: Pathogenesis, molecular mechanisms, and therapeutic implications. *Medical Research Reviews, 35*(2), 225-285.