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Assessment of *uS17* Expression in Colorectal Cancer Cell Lines HT-29 and HCT116 Reveals Minimal Variation

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Abstract

Colorectal cancer (CRC) is a malignant neoplasm that develops in the large intestine, specifically in the colon and rectum. The ribosomal protein gene uS17 (RPS11) has been found to be overexpressed in CRC tissues, while its presence in normal colorectal mucosa is minimal. However, its expression pattern across different types of CRC remains poorly understood and its prognostic potentials is underexplored. In this study, we investigated the expression levels of uS17 in two CRC cell lines, HT29 and HCT116, to assess potential differences in expression between these cell types. HT29 was derived from a late-stage primary colorectal adenocarcinoma, whereas HCT116 originated from an advanced-stage metastatic colorectal carcinoma. A semi-quantitative Reverse Transcription Polymerase Chain Reaction (RT-PCR) assay, along with a two-tailed unpaired t-test for inferential statistical analysis, were used in our evaluation. Although a higher uS17 expression level was observed in the HT29 cell line compared to HCT116, the difference was not statistically significant, suggesting minimal variation in uS17 expression between two different CRC cell lines of different origins, cellular differentiation, and stages of malignancy. While further studies incorporating additional cell lines and in situ tissue analyses are warranted, our findings offer novel insights into the expression profile of uS17 in specific CRC cell models.

Keywords: *Cell lines, Colorectal carcinoma, Gene expression, Ribosomal protein, uS17.*

1. INTRODUCTION

Colorectal cancer (CRC) develops from a combination of genetic, epigenetic, and environmental factors. Approximately 85% of CRC cases arise through the chromosomal instability (CIN) pathway, characterized by chromosomal aberrations that cause aneuploidy (Markowitz & Bertagnolli, 2009). Key events in this pathway include *APC* (adenomatous polyposis coli gene) mutations, occurring in a majority of cases (Olkinuora et al., 2021). The microsatellite instability (MSI) pathway, responsible for some CRCs, is driven by mutations in DNA mismatch repair (MMR) genes (*MLH1*, *MSH2, MSH6, PMS2*) (Olkinuora et al., 2021). These MSI-related cancers tend to have better prognoses and respond more effectively to immune checkpoint inhibitors (Yamamoto et al., 2020). Another pathway, the CpG island methylator phenotype (CIMP), involves promoter hypermethylation that silences tumour suppressor genes, frequently overlapping with MSI (Advani et al., 2021). CIMP-positive tumours, however, are often linked to poorer survival outcomes (Wang et al., 2022) Additionally, mutations in *TP53* (found in over 40% of CRC cases) (Liebl & Hofmann, 2021) and *BRAF* V600E (present in

about 10%) (Molina-Cerrillo et al., 2020) define CRC subtypes associated with more aggressive behaviour and chemotherapy resistance (Michel et al., 2021; Grothey et al., 2021).

Ribosomal proteins (RPs) play dual roles in cancer, contributing to both oncogenesis and tumour suppression. They influence cellular processes like proliferation, apoptosis, and DNA damage response. Overexpression of certain RPs, including RPS3 and RPS6, has been observed in CRC (Mao-De & Jing, 2007). RPS3 promotes cell proliferation and tumour growth (Alam et al., 2020), while RPS6 is involved in CRC progression through the mTOR pathway (Yi et al., 2021). Moreover, *RPS27A*, which encodes a ubiquitin fusion protein, correlates with poor prognosis by promoting invasion and metastasis via the Wnt/β-catenin signalling pathway and epithelial-mesenchymal transition (Wang et al., 2021). Finally, oncogenic mutations in RP like RPS20 is found to be associated with CRC (Nieminen et al., 2014; Djursby et al., 2020; Tian et al., 2022). As for *uS17* (*RPS11*), while its overexpression in CRC tissues has been noted (El Khoury & Nasr, 2021), its precise role as a prognostic marker in CRC remains to be investigated. The eukaryotic ribosomal protein gene *uS17* encodes ribosomal protein S11, a crucial component of the 40S small subunit of the eukaryotic ribosome. Studies have demonstrated its significant upregulation in human colorectal cancer (CRC) tissues compared to normal tissues (Kasai et al., 2003; Mao-De & Jing, 2007; El Khoury & Nasr, 2021). Its elevated expression is associated with poor prognosis of glioblastoma and hepatocellular carcinoma patients (Yong et al., 2015; Zhou et al., 2020). However, the variation in its expression across different CRC tissues or cell types remains largely unexplored. Hence, its prognostic implications in CRC have yet to be systematically studied, which is why this study aimed to investigate its expression level in two different CRC cell lines (HT29 and HCT116) derived from tissues of varying malignancy.

When comparing CRC cell lines, HT-29 and HCT116 differ significantly in origin, genetic profile, and research utility. HT-29, derived from a female patient with colorectal adenocarcinoma, harbours mutations in *APC* and *p53*, affecting the Wnt signalling pathway (Mouradov et al., 2014). This cell line can differentiate into enterocyte-like cells under specific conditions, making it a useful model for studying intestinal differentiation and epithelial function (Le Bivic et al., 1988). Conversely, HCT116, isolated from a male patient with colorectal carcinoma, possesses wildtype *KRAS* and *p53*, resulting in more aggressive growth and higher tumorigenic potential (Van Erk et al., 2005; Ahmed et al., 2018). This cell line is often used to study KRAS-driven pathways, tumour progression, apoptosis, and drug resistance (Meng et al., 2021). In animal models, HT-29 forms less aggressive tumours, while HCT116 is more tumorigenic (Mittal et al., 2015). Notably, prior to this study, the expression patterns of *uS17* in these two different CRC cell lines had not been thoroughly investigated. We hypothesised that the expression level of uS17 would differ significantly between HT29 and HCT116 cells, with a higher level expected in HCT116, given its more advanced stage of malignancy compared to HT29. Although only two CRC cell lines were analysed, this study serves as a preliminary exploratory investigation into the potential prognostic implications of uS17 in CRC.

2. MATERIALS AND METHODS

The two human CRC cell lines, HT-29 and HCT116, were obtained from the American Type Culture Collection (ATCC, USA). They were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum at 37°C, 95% humidity, and 5% CO_2 until further use. Total RNA extraction from both cell lines was performed using the GENEzol technique, following the manufacturer's protocol. Before adding the GENEzol reagent, the monolayer cell cultures in T25 flasks were washed with cold phosphate-buffered saline (PBS). The cells were then scraped and manually homogenised. The homogenates were transferred into microcentrifuge tubes and incubated at room temperature for 5 minutes. Chloroform (1/5 volume of GENEzol) was added, and the mixture was incubated at room temperature for another 5 minutes. The aqueous and organic phases were separated by centrifugation at 12,000 rpm for 12 minutes at 4°C. RNA from the aqueous phase was precipitated using isopropanol, and the resulting RNA pellet was dissolved in 25 µL of nuclease-free water, then stored at -80°C until needed. The RNA quantity and quality were assessed using agarose gel electrophoresis (AGE) and UV spectrometry.

Reverse transcription of the extracted RNA was carried out using Moloney Murine Leukaemia Virus - Reverse Transcriptase (MMLV-RT). A mixture of RNA and random primers in 15 µL of nuclease-free water was heated to 70°C for 5 minutes, then rapidly cooled on ice for 1 minute. To this mixture, 1.25 µL of 10 mM dNTPs, 5 µL of 5X MMLV reaction buffer, 0.6 µL of Recombinant RNAsin® Ribonuclease Inhibitor, 1 µL of MMLV-RT, and nuclease-free water were added to make a final volume of 25 µL. The final mixture was incubated at 37°C for 1 hour, followed by 15 minutes at 70°C to complete the reaction.

The Polymerase Chain Reaction (PCR) was conducted according to the manufacturer's instructions (Promega, USA). Each 25 µL reaction contained 0.5 µg template cDNA, 1X Green GoTaq® Flexi Buffer, 0.2 mM dNTPs, 1 mM $MgCl_2$, 0.4 mM of each forward and reverse primer, and 1.25 U of GoTaq® Polymerase. The thermal cycling conditions were as follows: initial denaturation at 95°C for 2 minutes; 35 cycles of 95°C for 30 seconds, 51.3°C for 30 seconds, and 72°C for 1 minute; followed by a final extension at 72°C for 5 minutes. A negative control (without template DNA from either cell line) was included. Glyceraldehyde-3-phosphate dehydrogenase gene (*GAPDH*) served as the internal control. Technical replicates were performed for each cell line. The primer sequences for *uS17* were: forward 5'-AGAGGACCATTGTCATCCGC-3', reverse 5'-CGA TCTGGACGTCCCTGAAG-3'; and for *GAPDH*: forward 5'-GTCAGTGGTGGACCTGACCT-3', reverse 5'-CCCTGTTGCTGTAGCCAAAT-3'. The expected amplicon sizes for *uS17* and *GAPDH* were 120 bp and 251 bp, respectively. The PCR products were assessed via agarose gel electrophoresis (AGE), and band intensities were documented and quantified using the ImageQuant Imager (GE Healthcare Life Sciences, USA) and TotalLab Quant software (TotalLab, USA). The amplicons were also verified by sequence analysis.

For data analysis, band intensity values were normalised by comparing the expression of *uS17* to the internal control, *GAPDH*. The calculation of normalised values was based on the formula below.

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

The differential expression of uS17 between the two cell lines was evaluated using a two-tailed unpaired Student's *t*-test, with significance defined as p-value < 0.05. Due to the use of technical replicates and the unavailability of biological replicates during the course of the study, the results of our statistical analysis should be interpreted as indicative of assay precision rather than biological significance.

3. RESULTS AND DISCUSSION

Total RNA extracted from HT-29 and HCT116 cells demonstrated good purity and integrity, as indicated by UV absorbance (A260/A280) ratios of 1.971 and 2.028, respectively. Expression of *uS17* was detected in both HT-29 and HCT116 cell lines (Figure 1, Table 1). The RT-PCR assays produced bands that matched the expected product sizes (Figure 1A). Sequence analysis confirmed the authenticity of the amplicons, identifying them as bona fide PCR-amplified products of the *uS17* gene. Comparison of the forward and reverse sequences with the reference data for uS17 in GenBank (Accession no. NM_001015.5) showed 98% and 100% sequence similarity, respectively (Figure 2). Our results demonstrated that *uS17* expression was generally higher in HT-29 compared to HCT116 (Figure 1B, Table 1). A comparison of the mean normalized band intensity values for the *uS17* gene amplicons between HT-29 and HCT116 revealed a 1.224-fold difference (Figure 1B, Table 1). However, this difference was not statistically significant (p-value = 0.558) based on t-test analysis (Table 1).

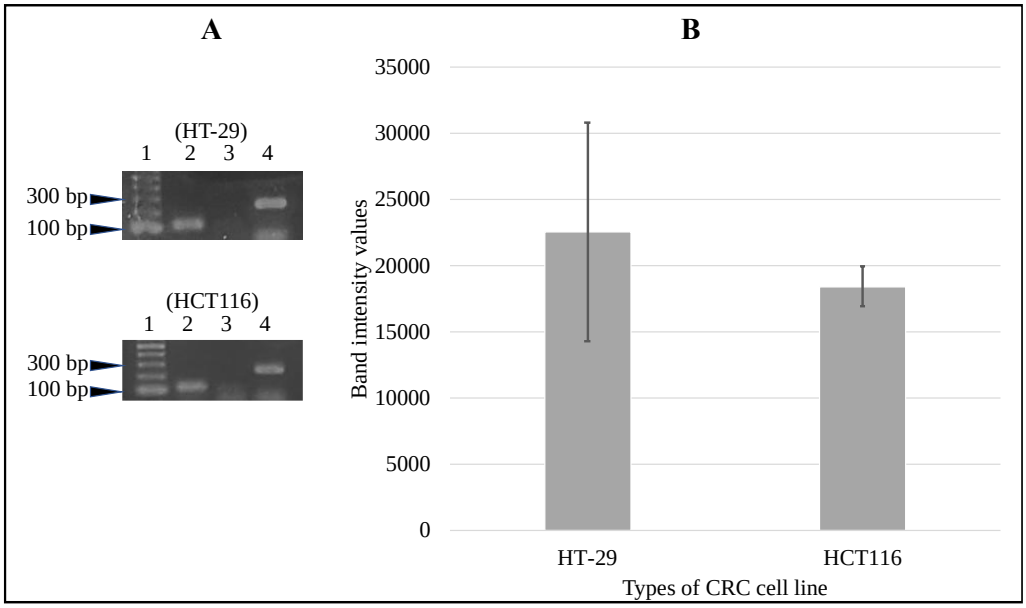

Figure 1. RT-PCR results of *uS17* expression in colorectal cancer cell lines: (A) Representative AGE diagrams of amplicon bands in HT-29 and HCT116 cells where Lane 1 is the DNA ladder, Lane 2 is the PCR products of *uS17*, Lane 3 is the negative control, and Lane 4 is the PCR products of *GAPDH*; and (B) bar graph of the mean normalized band intensity values with the associated standard deviation error bars.

Table 1. Band intensity values of *uS17* amplicons in colorectal cancer cell lines and the *t*-test result

Cell lines	Replicate	Band intensity values				Standard deviation	*t*-test (*p* value)
		uS17	GAPDH	Normalized (uS17)	Mean normalized		
HT-29	1	28865.907	42848.735	28415.131	22583.018	8247.853	0.558
	2	16485.170	41510.467	16750.905			
HCT116	1	20590.066	24268.409	19510.477	18455.969	1491.299	
	2	16438.572	21723.501	17401.461			

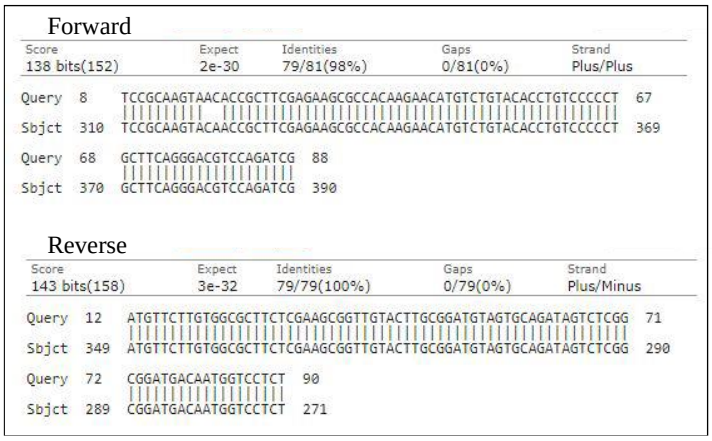

Figure 2. DNA sequence comparison between *uS17* amplicon (forward and reverse sequence) with reference *uS17* gene entry data (Accession no. NM_001015.5) from the GenBank database.

Our findings on the expression of the *uS17* transcript in colorectal cancer (CRC) cell lines align with previous studies (Kasai et al., 2003; Mao-De & Jing, 2007; El Khoury & Nasr, 2021), which have associated this gene with CRC. In contrast to earlier research that focused on tissue samples, we utilized cell lines and observed no significant difference in *uS17* expression between the two CRC cell lines studied, HT-29 and HCT116. In our earlier work on two other ribosomal protein genes, *eL14* and *uS19*, we identified significant differential expression between HCT116 and another CRC cell line, SW480 (Sim et al., 2020). Both HT-29 and SW480 are non-metastatic, but they differ in disease stage—HT-29 corresponds to Duke's stage C (Ahmed et al., 2013), while SW480 is Duke's stage B (Leibovitz et al., 1976). Thus, while *eL14* and *uS19* exhibit differential expression between CRC cell lines of varying types (grades) and stages (Sim et al., 2020), *uS17* does not show a significant change in expression between the two different CRC cell lines studied. However, it is important to note that our results should be interpreted with caution. In the absence of biological replicates, the statistical comparisons presented are limited to assessing assay precision and cannot be extrapolated for biological generalisation. Further investigations are therefore necessary, including the incorporation of biological replicates, a broader panel of CRC cell lines (alongside a normal control), and colorectal cancer tissues representing different grades, stages, and anatomical sites. Such future efforts will be essential to accurately determine the diagnostic and prognostic significance of uS17 in CRC oncogenesis.

4. CONCLUSION

The expression of the ribosomal protein gene *uS17* did not differ significantly between the two CRC-derived cell lines, HT-29 and HCT116. Given that our analysis was limited to two colorectal cancer cell lines and relied solely on technical replicates, no definitive conclusions can be drawn regarding the diagnostic or prognostic value of *uS17* in CRC tumorigenesis.

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CONFLICT OF INTEREST

We declare no conflict regarding the publication of the study

REFERENCES

Advani, S. M., Swartz, M. D., Loree, J., Davis, J. S., Sarsashek, A. M., Lam, M., Lee, M. S., Bressler, J., Lopez, D. S., Daniel, C. R., Morris, V., Shureqi, I., Kee, B., Dasari, A., Vilar, E., Overman, M., Hamilton, S., Maru, D., Braithwaite, D., & Kopetz, S. (2021). Epidemiology and molecular-pathologic characteristics of CpG Island methylator phenotype (CIMP) in colorectal cancer. *Clinical Colorectal Cancer*, *20*(2), 137-147. https://doi.org/10.1016/j.clcc.2020.09.007

Ahmed, D., Eide, P. W., Eilertsen, I. A., Danielsen, S. A., Eknaes, M., Hektoen, M., Lind, G. E., & Lothe, R. A. (2013). Epigenetic and genetic features of 24 colon cancer cell lines. *Oncogenesis*, *2*(9), e71. https://doi.org/10.1038/oncsis.2013.35

Alam, E., Maaliki, L., & Nasr, Z. (2020). Ribosomal protein S3 selectively affects colon cancer growth by modulating the levels of p53 and lactate dehydrogenase. *Molecular Biology Reports*, *47*(8), 6083-6090. https://doi.org/10.1007/s11033-020-05683-1

Djursby, M., Madsen, M. B., Frederiksen, J. H., Berchtold, L. A., Therkildsen, C., Willemoe, G. L., Hasselby, J. P., Wikman, F., Okkels, H., Skytte, A. B., Nilbert, M., Wadt, K., Gerdes, A. M., & van Overeem Hansen,

T. (2020). New pathogenic germline variants in very early onset and familial colorectal cancer patients. *Frontiers in Genetics*, *11*, 566266. doi: 10.3389/fgene.2020.566266

El Khoury, W., & Nasr, Z. (2021). Deregulation of ribosomal proteins in human cancers. *Bioscience Reports*, *41*(12), BSR20211577. https://doi.org/10.1042/BSR20211577

Grothey, A., Fakih, M., & Tabernero, J. (2021). Management of BRAF-mutant metastatic colorectal cancer: a review of treatment options and evidence-based guidelines. *Annals of Oncology*, *32*(8), 959-967. https://doi.org/10.1016/j.annonc.2021.03.206

Jørgensen, K. H., et al. (1982). "Cell kinetics of a human colonic carcinoma cell line (HT-29) in nude mice." Virchows Archiv B, 40(3), 297-307. doi:10.1007/BF02899711.

Kasai, H., Nadano, D., Hidaka, E., Higuchi, K., Kawakubo, M., Sato, T. A., & Nakayama, J. (2003). Differential expression of ribosomal proteins in human normal and neoplastic colorectum. *Journal of Histochemistry & Cytochemistry*, *51*(5), 567-573. https://doi.org/10.1177/002215540305100502

Le Bivic, A., Hirn, M., & Reggio, H. (1988). HT-29 cells are an in vitro model for the generation of cell polarity in epithelia during embryonic differentiation. *Proceedings of the National Academy of Sciences*, *85*(1), 136-140. https://doi.org/10.1073/pnas.85.1.136

Leibovitz, A., Stinson, J. C., McCombs III, W. B., McCoy, C. E., Mazur, K. C., & Mabry, N. D. (1976). Classification of human colorectal adenocarcinoma cell lines. *Cancer Research*, *36*(12), 4562-4569. ISSN 1538-7445

Liebl, M. C., & Hofmann, T. G. (2021). The role of p53 signaling in colorectal cancer. *Cancers*, *13*(9), 2125. https://doi.org/10.3390/cancers13092125

Mao-De, L., & Jing, X. (2007). Ribosomal proteins and colorectal cancer. *Current genomics*, *8*(1), 43-49. https://doi.org/10.2174/138920207780076938

Markowitz, S. D., & Bertagnolli, M. M. (2009). Molecular origins of cancer: Molecular basis of colorectal cancer. *New England Journal of Medicine*, *361*(25), 2449-2460. https://doi.org/10.2174/138920207780076938

Meng, M., Zhong, K., Jiang, T., Liu, Z., Kwan, H. Y., & Su, T. (2021). The current understanding on the impact of KRAS on colorectal cancer. *Biomedicine & Pharmacotherapy*, *140*, 111717. https://doi.org/10.1016/j.biopha.2021.111717

Michel, M., Kaps, L., Maderer, A., Galle, P. R., & Moehler, M. (2021). The role of p53 dysfunction in colorectal cancer and its implication for therapy. *Cancers*, *13*(10), 2296. https://doi.org/10.3390/cancers13102296

Mittal, V. K., Bhullar, J. S., & Jayant, K. (2015). Animal models of human colorectal cancer: Current status, uses and limitations. *World Journal of Gastroenterology*, *21*(41), 11854. https://doi.org/10.3748/wjg.v21.i41.11854

Molina-Cerrillo, J., San Román, M., Pozas, J., Alonso-Gordoa, T., Pozas, M., Conde, E., Rosas, M., Grande, E., García-Bermejo, M. L., & Carrato, A. (2020). BRAF mutated colorectal cancer: new treatment approaches. *Cancers*, *12*(6), 1571. https://doi.org/10.3390/cancers12061571

Mouradov, D., Sloggett, C., Jorissen, R. N., Love, C. G., Li, S., Burgess, A. W., Arango, D., Strausberg, R. L., Buchanan, D., Wormald, S., O'Connor, L., Wilding, J. L., Bicknell, D., Tomlinson, I. P., Bodmer, W. F., Mariadason, J. M., & Sieber, O. M. (2014). Colorectal cancer cell lines are representative models of the main molecular subtypes of primary cancer. *Cancer Research*, *74*(12), 3238-3247. https://doi.org/10.1158/0008-5472.CAN-14-0013

Nieminen, T. T., O'Donohue, M. F., Wu, Y., Lohi, H., Scherer, S. W., Paterson, A. D., Ellonen, P., Abdel-Rahman, W. M., Valo, S., Mecklin, J. P., Järvinen, H. J., Gleizes, P. E., & Peltomäki, P. (2014). Germline mutation of RPS20, encoding a ribosomal protein, causes predisposition to hereditary nonpolyposis colorectal carcinoma without DNA mismatch repair deficiency. *Gastroenterology*, *147*(3), 595-598. https://doi.org/10.1053/j.gastro.2014.06.009

Olkinuora, A. P., Peltomäki, P. T., Aaltonen, L. A., & Rajamäki, K. (2021). From APC to the genetics of hereditary and familial colon cancer syndromes. *Human Molecular Genetics*, *30*(R2), R206-R224. https://doi.org/10.1093/hmg/ddab208

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* (1), 31-39. https://doi.org/10.55230/mabjournal.v49i1.1652

Tian, Y., Babaylova, E. S., Gopanenko, A. V., Tupikin, A. E., Kabilov, M. R., Malygin, A. A., & Karpova, G. G. (2022). Changes in the transcriptome caused by mutations in the ribosomal protein uS10 associated with a predisposition to colorectal cancer. *International Journal of Molecular Sciences*, *23*(11), 6174. https://doi.org/10.3390/ijms23116174

Wang, J., Deng, Z., Lang, X., Jiang, J., Xie, K., Lu, S., Hu, Q., Huo, Y., Xiong, X., Zhu, N., & Zhang, W. (2022). Meta-Analysis of the Prognostic and Predictive Role of the CpG Island Methylator Phenotype in Colorectal Cancer. *Disease Markers*, *2022*(1), 4254862. https://doi.org/10.1155/2022/4254862

Van Erk, M. J., Krul, C. A. M., Caldenhoven, E., Stierum, R. H., Peters, W. H., Woutersen, R. A., & Van Ommen, B. (2005). Expression profiling of colon cancer cell lines and colon biopsies: towards a screening system for potential cancer-preventive compounds. *European Journal of Cancer Prevention*, *14*(5), 439-457. 10.1097/01.cej.0000174781.51883.21

Yamamoto, H., Watanabe, Y., Maehata, T., Imai, K., & Itoh, F. (2020). Microsatellite instability in cancer: a novel landscape for diagnostic and therapeutic approach. *Archives of toxicology*, *94*, 3349-3357. https://doi.org/10.1007/s00204-020-02833-z

Yi, Y. W., You, K. S., Park, J. S., Lee, S. G., & Seong, Y. S. (2021). Ribosomal protein S6: a potential therapeutic target against cancer? *International Journal of Molecular Sciences*, *23*(1), 48. https://doi.org/10.3390/ijms23010048

Yong, W. H., Shabihkhani, M., Telesca, D., Yang, S., Tso, J. L., Menjivar, J. C., Wei, B., Lucey, G. M., Mareninov, S., Chen, Z., Liau, L. M., Lai, A., Nelson, S. F., Cloughesy, T. F., & Tso, C. L. (2015). Ribosomal proteins RPS11 and RPS20, two stress-response markers of glioblastoma stem cells, are novel predictors of poor prognosis in glioblastoma patients. *PLoS One*, *10*(10), e0141334. doi: 10.1371/journal.pone.0141334.

Zhou, C., Sun, J., Zheng, Z., Weng, J., Atyah, M., Zhou, Q., Chen, W., Zhang, Y., Huang, J., Yin, Y., Mao, H., Zhang, Z., Yi, Y., & Ren, N. (2020). High RPS11 level in hepatocellular carcinoma associates with poor prognosis after curative resection. *Annals of Translational Medicine*, *8*(7), 466. doi: 10.21037/atm.2020.03.92.