

Gene expression analysis of specific genes related to genomic stability in colon cancer cell lines - a resource for cancer research

Abstract

Appropriate cell models are the foundation of cancer research as all the cell lines are not same in their ability to mimic primary tumor tissue. Molecular characteristics of various cell lines can help in selecting the right cell model for biomedical studies. This study describes the gene expression profile of nine genes which include the six-protein shelterin complex, tankyrases 1 and 2 and *CTNNB1* in three different models of human colon cancer cell lines. Also, the relative telomere length in each cell line was evaluated. Expression levels of all genes were analyzed by using a real-time polymerase chain reaction. Nearly all test genes were significantly highly expressed in HT-29 as compare to HCT-116 and SW-620. SW-620 record a significantly increased expression of *CTNNB1* gene (P-value < 0.01) while most genes were poorly expressed in HCT-116. Positive correlation between *CTNNB1* and *hTERT* expression was found in HCT-116 and SW-620 but not in HT-29. Telomere was found to be of the same length in all cell. Gene expression data presented in our results can assist in selecting appropriate cell line in colon cancer research. Unique molecular properties of various cancer cell lines should be evaluated before selecting a cell line models for research purpose

Keywords: Gene expression, colon cancer cell lines, telomere, shelterin complex, tankyrases, β -catenin.

Introduction:

Colorectal cancer (CRC) is one of the most occurring types of cancer worldwide. According to GLOBOCAN 2018, CRC is the third most commonly diagnosed cancer with nearly 1.8 million new cases, and the second leading cause of cancer death, accounting for 9.2% of all cancer deaths (1). CRC is a heterogeneous disease with diverse molecular pathways typically due to loss of genomic stability either by genetic or epigenetic alterations. Telomeres play a significant role in maintaining genomic stability and their dysfunction can modulate many cell signals like the Wnt/ β -catenin pathway leading to cancer (2).

The involvement of deregulated Wnt/ β -catenin cascade with colorectal cancer has been well documented (3). One of the most studied and critical Wnt pathways is canonical Wnt signaling, which functions by regulating the amount of the transcriptional co-activator β -catenin that controls the central developmental gene expression program (4). Telomere dysfunction can occur due to impaired telomere length or by disruption of the shelterin complex. Many human diseases especially cancer are linked with mutations in shelterin. Shelterin complex composed of six key individual proteins, the telomere repeat-binding factor 1 and 2 (TRF1 and TRF2), protection of telomeres 1 (POT1), repressor/activator protein 1 (RAP1), TRF1 and TRF2 interacting protein 1 (TIN2) and TIN2-interacting protein 1 (TPP1) (5,6). Telomere is also regulated by tankyrase which is involved in many cellular and molecular processes related to tumor suppressors.

Human cell lines obtained from malignant tumors are being used as a valuable in vitro model in cancer research from decades (7). Cell line based studies are being promoted all over the world to understand the genetic and epigenetic mechanisms involved in many kinds of malignancy (8). Although cell lines are inexpensive and easy to handle in the laboratory,

appropriate selection of proper cell line for a particular study need to be evaluated for best results (9). Cell lines have their origin from tumor tissue samples of a patient (10,11). Sorting the cell for a particular study is a very crucial step as cells in the experiment should truly represent the type of cancer they are supposed to represent. Usually, cell lines represent gene expression patterns the same as their cellular origin (11). Cell lines have their unique genetic characteristics like HCT116 has a mutation in codon 13 of the KRAS proto-oncogene and is mainly used for biomedical studies involving colon cancer proliferation and corresponding inhibitors (12,13). These cells are most suitable for therapeutic research and drug screenings (14). HT-29 has a mutation in the p53 gene (15). These cells are mostly used to study the structural and molecular events involved in cell differentiation and best fit for studies focusing on the anticancer effect of different food compounds (16,17). SW-620 is used to study metastasis processes (18). Physiological properties like cell proliferation rate, drug sensitivities and metabolism depend on the gene expression patterns of particular cell lines (19). HCT-116, HT-29 and SW-620 cell lines are the most commonly used cell lines in colorectal cancer studied but literature reporting the gene expression patterns of some specific genes related to genomic stability in these cells is not available. Therefore, different colorectal cancer cell line models were used, HCT-116, HT-29 and SW-620 to evaluate the telomere length and the expression of the telomere associated proteins; telomerase, TRF1, TRF2, POT1, TPP1, TIN2, TNKL1 and TNKS2 and how they correlate with the transcriptional factor β -catenin expression level.

Materials and methods

Cell culture

Three human colon cancer cell lines HCT-116, HT-29 and SW-620 were used in the present study and were kindly provided by Stem Cell Unit, Department of Anatomy at King Khalid University Hospital, Riyadh, KSA. All cell lines were cultured in DMEM medium supplemented with 10% (v/v) fetal bovine serum and 1% (v/v) penicillin-streptomycin (Invitrogen, Carlsbad, CA, USA) at 37°C in a humidified atmosphere containing 5% CO₂.

Nucleic acid isolation

The DNA and total RNA were isolated from each cell line using RNeasy Mini Kit and QIAamp DNA Mini Kit, respectively, according to the manufacturer's instructions (Qiagen). The isolated nucleic acids were quantified spectrophotometrically (NanoDrop 8000, Thermo Fisher Scientific).

Assessment of gene expression via RT-qPCR

Total RNA was reverse-transcribed to cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems), according to the manufacturer's instruction. The expression of each gene was assessed by quantitative PCR using SYBR® Green-based assay (Bio-Rad). Each reaction was carried out in triplicates with a final volume of 10 µl containing 1X SYBR® Green Master Mix, 200 nM forward and reverse primers, and 19.5 ng of cDNA. The Primer sequences and annealing temperature are listed in Table 1. The reactions were performed in ViiA™ 7 Real-Time PCR System (Applied Biosystems) using the following cycling parameters: 1 cycle at 95°C for 5min followed by 45 cycles of amplification (denaturation at 95°C for 15 sec, annealing at 60°C for 30 sec, and extension at 60°C for 30 sec). The relative gene expression was normalized using glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) expression. A non-template negative control was included in each run to check the background signal.

Measurement of relative telomere lengths

Telomere length was determined by a previously described qPCR protocol (42). In brief, two PCR master mixes were prepared, one for telomere reaction with telomere (TEL) primer pair, and the other for single-copy gene reaction with human beta-globin gene (*HBG*) primer pair. The Primer sequences and annealing temperature are listed in Table 1. Each reaction contained 1X SYBR® Green Master-mix (Applied Biosystems, UK), 20 ng of DNA sample, *Tel-1* (300 nM), *Tel-2* (900 nM), *HBG-1*, and *HBG-2* (400 nM). All reactions were performed by Light Cycler 480 II system (Roche Applied Science) using the following PCR cycling condition: initial denaturation at 95°C for 15 minutes followed by 45 cycles of (denaturation at 95°C for 15 sec, indicated annealing temperature for 2 min, and extension at 72°C for 30 sec).

Statistical analysis

All statistical analyses were performed using GraphPad Prism® software (version 8.2.0 GraphPad Inc, USA). The relative gene expression was calculated using the copy number method. Nevertheless, the relative telomere length was calculated using $2^{-\Delta\Delta Ct}$ method (where $\Delta Ct = Ct \text{ single copy gene} - Ct \text{ telomere}$). Unpaired Student's t-test was used to estimate the difference in the gene expression and telomere length between colon cancer cell lines. Pearson's correlation and Linear regression were used to test the association between the different variables. All data are presented as the mean \pm standard error of the mean, and the P-value of less than 0.05 was considered statistically significant.

Results:

Assessment of β -catenin and telomere related proteins expression

The gene expression profile of telomere related proteins (*hTERT*, *TERF1*, *TERF2*, *POT1*, *TIN2*, *TPP1*, *TNKS1*, and *TNKS2*) and *CTNNB1* of all cancer cell lines are presented in Figure 1. SW-620 expressed a significantly higher level of β -catenin as compared to the other two cell lines. Comparing SW-620 and HT-29m there was a significant difference in the expression in all studied genes except *hTERT* and its negative regulator *POT1*. HT-29 showed a significantly high expression of *TRF1*, *TRF2*, *TPP1* and *TNKS2* as compared to SW-620 and HCT-116, where HCT-116 showed the least expression of almost all test genes. The expression level of *hTERT* in all cell lines was found to be the same (Figure 1). Interestingly, *POT1* which functioning in telomere length regulation through controlling telomerase access to the overhang did not show any significant difference in its expression between the selected colon cancer cell lines, as the case in *hTERT*. One notable finding is that the expression pattern of *TERF1*, *TERF2*, *TPP1*, and *TNKS2* was almost identical in all studied cell lines. Furthermore, the level of the previously mentioned genes was significantly low in HCT-116 and high in HT-29, the native and mutated *TP53* models. Person correlation between all the genes of three cell lines is summarized and presented in Figure 3. *CTNNB1* was positively correlated with all studied genes of all cell lines except *POT1* in SW-620 and *TERF1* and *POT1* in HCT-116 it was found to be negatively correlated with its expression. A negative correlation between *hTERT* and *POT1* was found in all three cell lines. In SW-620 and HCT-116, *POT1* was negatively correlated with almost all the genes. Further, genes that were mainly involved in telomere structure regulation (*TERF1*, *TERF2*, *TIN2*, *TPP1* and *TNKS1*) were found to be correlated in HT-29. Figure 2 summarizes the correlation findings.

Relative telomere length (RTL) measurement and correlation with gene expression

Telomere was found to be of the same length in all cell lines as shown in Figure 3. The correlation analysis was performed with Pearson's correlation test to demonstrate the association between the gene expression of the telomere related proteins and *CTNNB1* with telomere length (Figure 4). Interestingly, HT-29 and SW-620 show a counter result. While the telomere length in HT-29 shows a positive correlation with the expression of all studied genes, the telomere length of SW-620 on other hand was negatively correlated with the expression of all genes. In the case of HCT-116, no notable correlation or pattern was found between telomere length and the expression of all genes. Overall, the analysis showed that

among the studied genes, no significant correlation was determined between telomere length and their expression

Discussion:

Studies have found that telomere dysfunction is associated with chromosomal instability, a hallmark of cancer and causative of the acquisition of genetic lesions that are involved in the stepwise progression of cells into malignancy. Indeed, different factors and pathways may play a role in the regulation of telomere related proteins and thus telomere biology. Previous studies have been documenting that Wnt/ β -catenin pathway is involved (20). The present work aims to estimate the relative telomere length and the expression level of the telomere related proteins (*hTERT*, *TERF1*, *TERF2*, *POT1*, *TIN2*, *TPP1*, *TNKS1* and *TNKS2*) using q-PCR in most commonly used colon cancer cell lines and how they correlate with the transcriptional factor β -catenin expression level. The mRNA level of β -catenin and *TNKS1* was significantly higher in SW-620 as compared to the other two cells. SW620 is metastatic and poorly differentiated as compared to HT-29 and HCT116 which suggests that β -catenin may play an important role in metastasis. Lai *et al* explored the molecular marker for metastasis in human cancer tissue. He reported an elevated level of β -catenin expression in a poorly differentiated tissue. Similar results are reported in human hepatoma cell line HA22T which has high proliferation activity (21). Many human cancers are reported with mutations and abnormal expression of β -catenin which is correlated with tumor invasion and metastasis (22). Remarkably, it has been found that SW-620 owns a mutated *APC* (23,24) and wildtype *CTNNB1* status (24). Previous studies have reported that both *TNKS1* and *TNKS2* reducing β -catenin degradation by destabilizing the levels of Axin (25); a key effector in the Wnt pathway and has been identified as a tumor suppressor and therefore hyperactivation Wnt/ β -catenin signaling (26,27). This finding is compatible with a previous study which found that the Wnt/ β -catenin pathway is activated during CRC progression and accompanied by enhanced cell invasive behavior (28). This makes the Wnt/ β -catenin pathway become a potential therapeutic target for inhibiting CRC metastasis. Therefore, due to the role of tankyrase in Wnt/ β -catenin signaling, Tankyrase 1 inhibitors may become a promising therapeutic target for CRC through suppresses Wnt signaling and tumor growth.

hTERT is one of the main catalytic subunits of the telomerase enzyme and is expressed in almost 90 % of human cancers. The expression level of *hTERT* in all cell lines was found to be the same in the presented data. Our results are well supported by Kawanishi-Tabata *et al* (2002) results where he proved that the expression level of *hTERT* was not correlated with

cancer progression in colon carcinoma (29). Furthermore, copy number gain of the *hTERT* gene is highly correlated with *hTERT* expression found in neuroblastomas and lung cancer, but no such correlation has been found in colorectal carcinomas (30).

On examining the expression level of the six protein components of the shelterin complex we found that HT-29 showed significantly high expression of *TRF1*, *TRF2* and *TPP1* as compared to SW-620 and HCT-116, where HCT-116 showed the least expression of almost all components of shelterin complex. Many reports have shown overexpression of *TRF1* and *TRF2* in many kinds of cancer tissues (31). HT-29 is a mutant for the p53 gene. Shelterins protect the chromosomes and prevent their ends but any kind of genotoxic stress or mutation can induce tumor (32). The p53 protein is known for its tumor suppressor properties and plays a major role in the maintenance of genome integrity (33). Mutation in p53 in HT-29 can be related to the high expression of these shelterin proteins as p53 protein enhanced telomere stability even in presence of DNA damage. Some studies have also reported the inhibition of *TRF2* in the induction of apoptosis in a p53 dependent manner. Impressively, there was a significant correlation between the expression of *TERF1* and *TERF2*, *TPP1* and *TNKS1*. Likewise, *TERF2* expression was significantly correlated with the three previously mentioned genes. However, *TPP1* has correlated with *TERF1* and *TERF2* expression but not tankyrases. These findings suggest that these telomeric proteins may play a role in cancer by enhancing the genomic instability through controlling DNA damage response (DDR) and manipulating the telomere structure but not length since we found that all the three colon cancer cell lines were almost having the same length. The suggestion is consistent with published data that found a significant correlation between *TERF1* and *TERF2* expression and demonstrates an inverse relation between *TERF1* and *TERF2* level with DDR activation (34).

Tankyrase proteins having poly(ADP-ribose) polymerase activity are the main components of the human telomeric complex among which Tankyrase-1 and 2 are most important. Change of expression level of these two proteins is identified in many cancers tissues. The expression level of the Tankyrase-1 and Tankyrase 2 were found to be significantly high in HT-29 followed by SW-620 whereas HCT-116 showed the least expression. The expression level of the Tankyrase protein depends on the tumor stage (35). Cell lines used in the study were different in their ability to differentiate as HT-29 has an intermediate capacity to differentiate while SW-620 and HCT-116 are highly aggressive cell lines with the least differentiate. Our results agree with Abdulaziz, et al (35), who found high expression of *TNKS1* in the early stages than late stages of CRC development. Also, Gelmini et al. (2006) (36), reported an inverse relation of TNKS with colon cancer stages.

As shown in Figure 2.C, *CTNNB1* was significantly positively correlated with *hTERT*, while the last one was significantly correlated with *TERF2*. This finding is compatible with a study that found that the *hTERT* is a target of the Wnt/ β -catenin pathway, and induction of β -catenin expression leads to increased *hTERT* expression while silencing endogenous β -catenin expression by β -catenin gene-specific shRNA effectively decreased *hTERT* expression (37). Altered expression of β -catenin is found to be correlated with poor prognosis, aggressive phenotype and metastasis (38–40) as clearly shown in our results of SW-620 cell lines where we found a strong correlation of *CTNNB1* and with other genes as compared to other two cells. Our results showed a strong correlation between β -catenin and *hTERT* concerning the different capacities of differentiation among the cell lines. The expression status of β -catenin significantly correlates with the de-differentiation process and prognosis in many human cancers tissues (41).

Conclusion

The selection of appropriate cell lines for molecular-based cancer studies is a very critical step to interpret and understand cancer biology. The gene expression findings provided in the present study can help researchers to choose the most appropriate cell line models for cancer studies and clinically related therapies.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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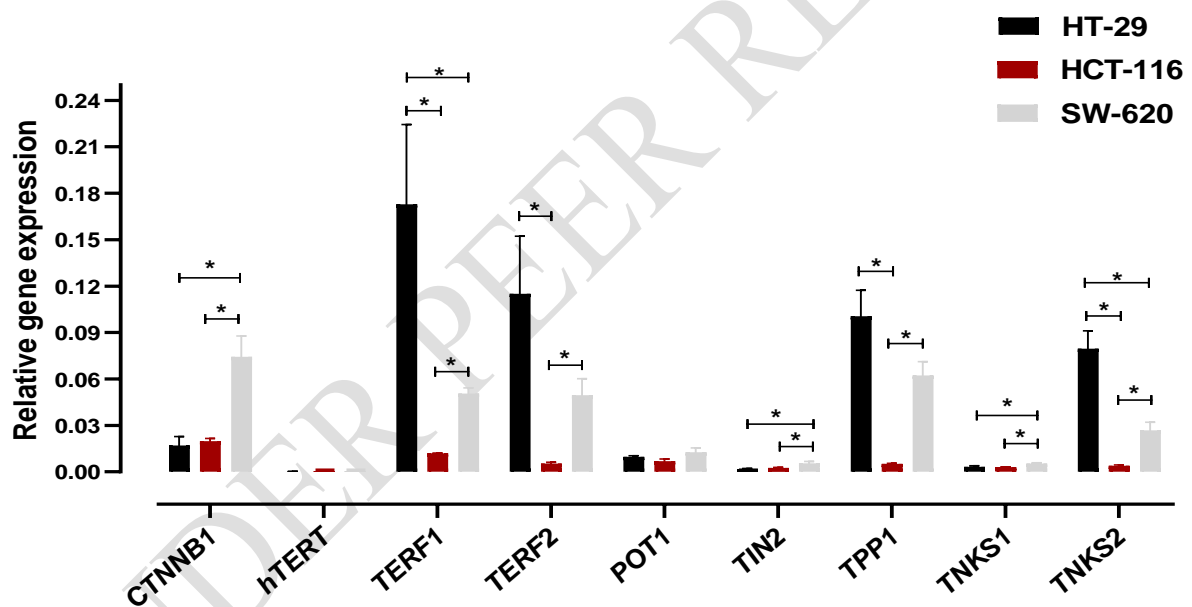
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* for P-value < 0.05

Figure 1. Expression of *CTNNB1* and telomere-related proteins in three types of human colon cancer.

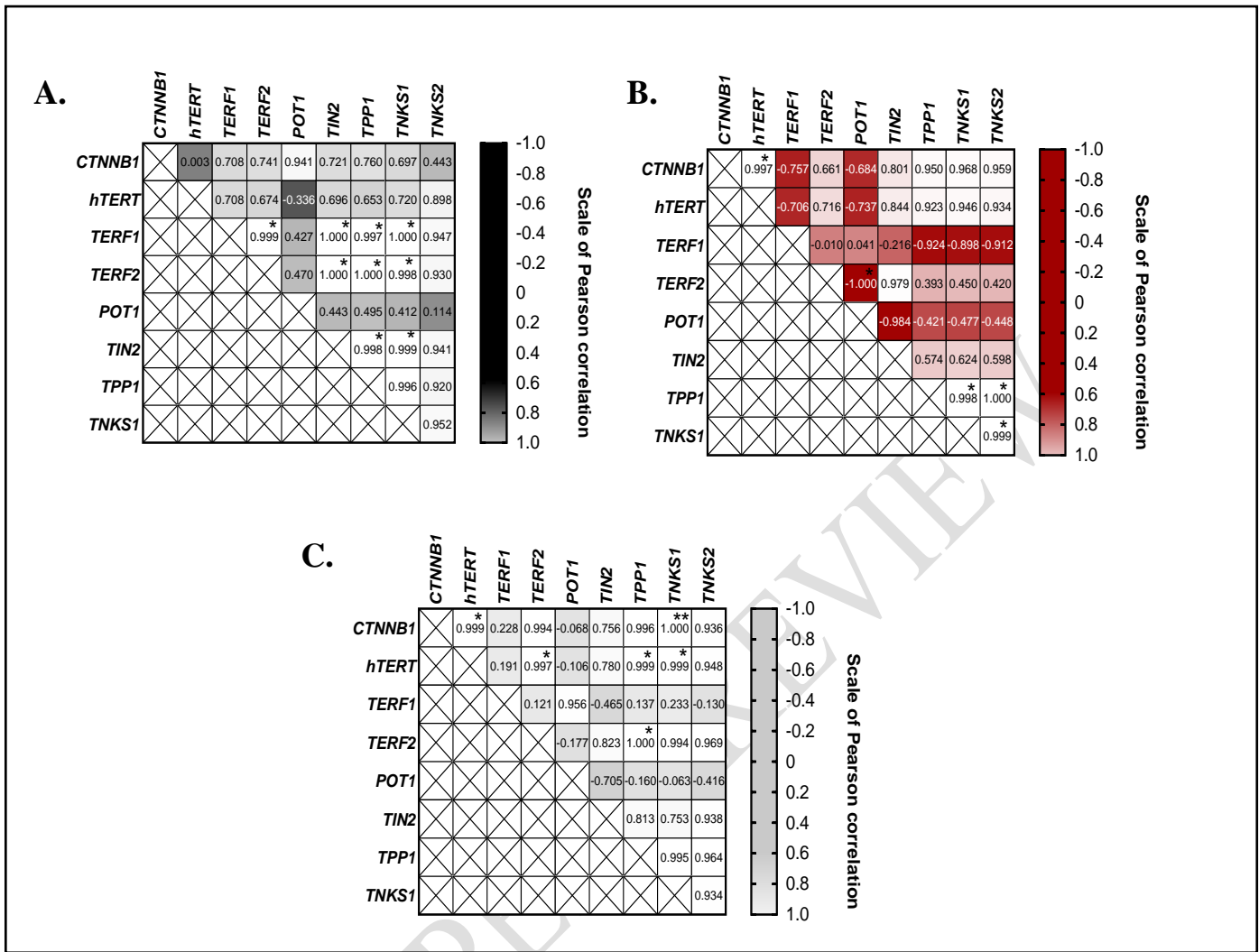


Figure 2. Correlation between the expression of the interest genes in different model of human colorectal cancer, A. HT-29, B. HCT-116, C. SW-620, * for P-value < 0.05, ** for P-value < 0.01

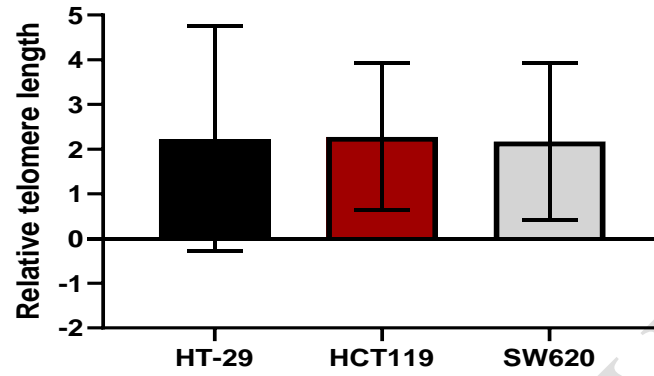


Figure 3. Relative telomere length (RTL) in three different colorectal cancer cell lines, all three models show almost the same telomere length.

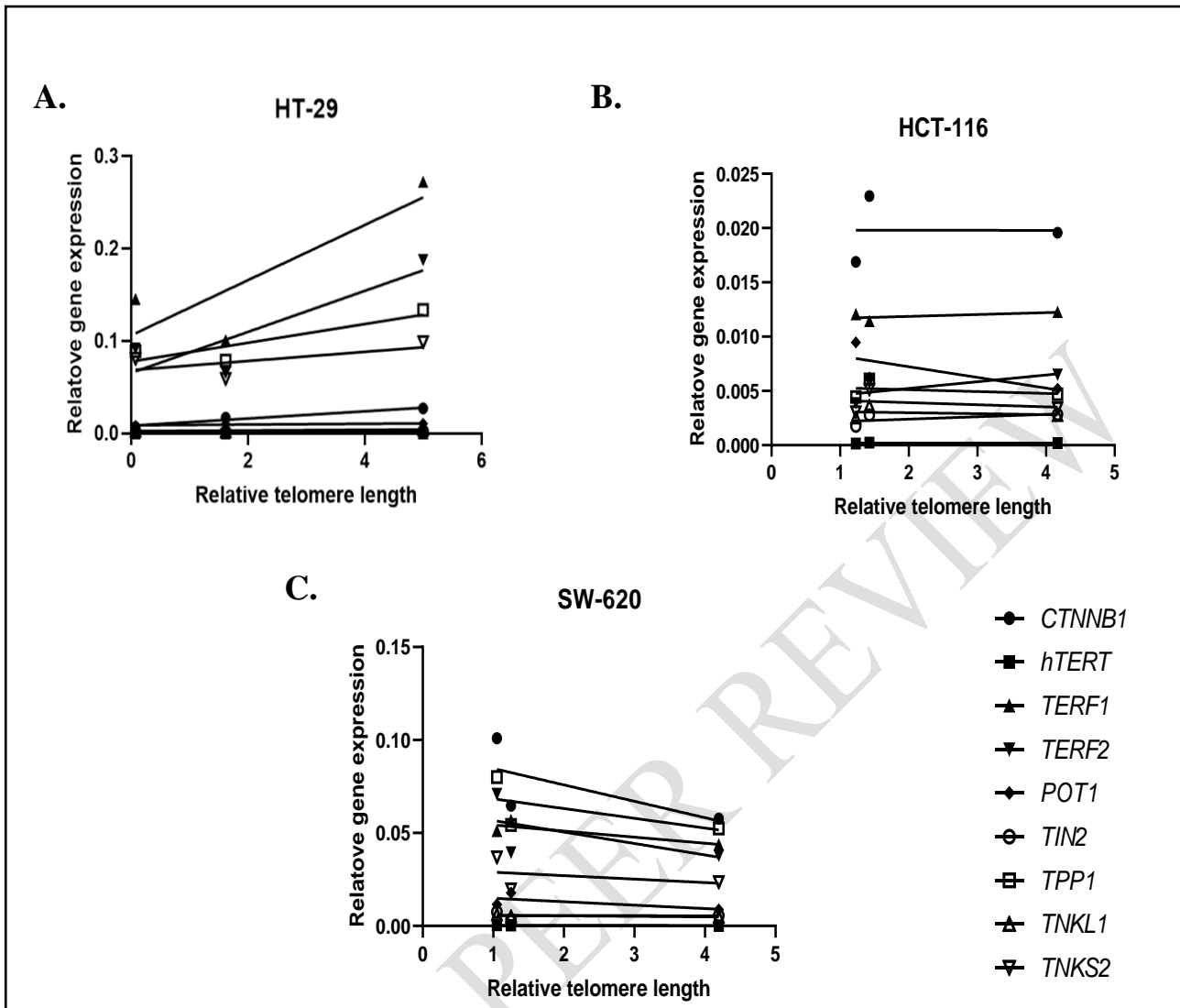


Figure 4. Pearson's correlation between gene expression of telomere related proteins and relative telomere length in the studied colorectal cancer cell lines, A. in HT-29 telomere length is positively associated with the expression of all interests genes, B. no notable results were recorded and C. SW-620 shows a negative correlation between the telomere length and the expression of all genes.

Table 1. Primers sequence used to evaluate gene expression using real-time q-PCR and relative telomere length.

Gene	Primer sequence (5' - 3')	Ta (°C)	Reference
<i>GAPDH</i>	For: GGT ATC GTG GAA GGA CTC ATG AC Rev: ATG CCA GTG AGC TTC CGT TCA GC		(43)
<i>CTNNB1</i>	For: TCT GAG GAC AAG CCA CAA GAT TAC A Rev: TGG GCA CCA ATA TCA AGT CCA A		(44)
<i>hTERT</i>	For: CGT CCA GAC TCC GCT TCA TC Rev: GAG ACG CTC GGC CCT CTT		(45)
<i>TERF1</i>	For: CCA CA TGA TGG AGA AAA TTA AGA GTT AT Rev: TGC CGC TGC CTT CAT TAG A		(46)
<i>TERF2</i>	For: TCA ATC GCT GGG TGC TCA A Rev: GTA CCG GCT ACC CCG AAA G		(47)
<i>POT1</i>	For: GAA GTG GAC GGA GCA TCA TT Rev: TTT GTA GCC GAT GGA TGT GA	60	(48)
<i>TPP1</i>	For: GCG TGA CGT CTC ACA TCG Rev: ATC GTG ATG GTT CTG CCT TC		(49)
<i>TIN2</i>	For: GGC CTA AAG GCC AAG GTG GTG Rev: GGC CAA ATC CAC AGG AGC CTC		(50)
<i>TNKS1</i>	For: GTC CCT GAC AGC CTA GAA ATA AG Rev: GGG AAC AGT AGC AGT TGA GTA TG		(51)
<i>TNKS2</i>	For: TCC GGA CAA CAA GGT CTT AAC Rev: CTC TTC CTC CAC AGA CTG AAA C		(51)
<i>TEL-1</i>	GGT TTT TGA GGG TGA GGG TGA GGG TGA GGG T	55	(52)
<i>TEL-2</i>	TCC CGA CTA TCC CTA TCC CTA TCC CTA TCC CTA		
<i>HBG-1</i>	GCT TCT GAC ACA ACT GTG TTC ACT AGC	60	(52)
<i>HGB-2</i>	CAC CAA CTT CAT CCA CGT TCA CC		

*For: forward, Rev: reverse, Ta: annealing temperature.