The effect of hTERT repression on the TERRA expression and telomere length in gastric cancer

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Background: Telomeres play a vital role in maintaining the integrity of the genome. Mammalian telomeres are certainly transcribed into telomeric repeat-containing RNA (TERRA). This Long non-coding RNA participates in the regulation of telomere length and telomerase activity. As reported, IncRNAs play important roles in gastric cancer progression. Telomerase and its major catalytic subunit (hTERT) are upregulated in most cancers, including gastric cancer. RNA interference (RNAi) has been proven to be a powerful tool for gene knockdown and hold good promise for the treatment of human diseases including cancer. In this study, we investigated the effect of hTERT repression on the TERRA expression and telomere length in multiple passages.

Methods: AGS gastric cancer cell line was treated with hTERT FlexiTube siRNA and Hyperfect transfection reagent. Cell viability was examined by MTT assay. A DAPI staining method was used to analyze cell cycle by Flow Cytoometry. Real-time PCR was used to evaluate the expression level of hTERT and TERRA and assessment of telomere length.

Results: The MTT assay showed that increasing the exposure time to 48 hours decreased cell viability below the 50% viability mark. The flow cytometry cell cycle analysis showed a significant increasing in the number of cells in G1 phase and decreasing in the number of cells in S phase. The results of the qRT-PCR analysis demonstrated that siRNA transfection decreased the hTERT expression, although has no significant effect on TERRA expression in early passages. However, upregulation of TERRA expression in the passage of 20 compared to the control cells has been shown. Also, telomere length measurement in each passage was decreased after hTERT siRNA treatment.

Conclusions: The significant downregulation of hTERT mRNA inhibited the cell viability of AGS cells and cell cycle arrest. This study provides the fact that downregulation of hTERT expression had no effect on TERRA expression level in early passages of AGS cell line. Also, showed a direct link between decreasing in telomere length and downregulation of hTERT expression.

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