Summary and Conclusions
Telomerase is overexpressed in most of the cancers and it provides proliferative advantage to the cells by maintaining telomere length as well as its contributions in tumorigenesis by unknown mechanism. Telomerase reverse transcriptase (hTERT) and telomerase RNA component both play role in maintenance of telomere length and associated cellular signaling events. As cancer is a disease caused by accumulated mutations and deregulated signaling and cell cycle events. Restricting its population doublings would be one of the effective strategies for combinatorial therapies. Targeting telomerase and its RNA component could prove to be an effective approach in suppressing cancer specific events and it would be appropriate to evaluate the global gene expression effects on targeting telomerase in cancer cells.

One of the approaches is using ribozyme to knockdown gene expression and targeting telomerase RNA by ribozyme would be one of the strategies in cancer therapeutics. Ribozymes are catalytic RNA molecules that can be directed to base pair with the specified target sequence and can cleave the transcript in to functionally inactive fragments that are further degraded by endogenous RNase digestion.

Hammerhead ribozyme was designed using Zuker’s M Fold programme by energy minimization and the conserved secondary structures of hTR were compared at multiple foldings. We selected 180th GUC of pseudo knot region of hTR for targeting.

Ribozyme coding oligos were annealed and cloned in pStu I vector and confirmed with sequencing. The positive clones were taken for cleavage assays. For in vivo expression ribozymes were cloned in pCI Neo mammalian expression vector and screened by colony blotting and further sequencing for confirmation of intactness of conserved sequence.

Human telomerase RNA was reverse transcribed and cloned in pGEM-T vector and sequenced to confirm the presence of target sequence. The ribozyme and hTR was in vitro transcribed and in vitro cleavage reaction was analyzed for the designed ribozyme. The ribozyme cleaves the target with relatively low turn over. Therefore it is necessary to use more than stochiometric concentration of ribozymes while a catalyst is characterized by non stochiometric relation with the substrate.

The evaluated ribozymes were cloned in mammalian expression vector (pCI-NEO) and transfected to HeLa cells and maintained as stable transfectants for 120 days. The ribozyme expressions were monitored and confirmed with sequencing for intactness
of its sequences. Telomerase RNA levels were found to be reduced in ribozyme expressed cells compared to vector only and mutant ribozyme transfected cells.

Telomerase activity was also found to be reduced in ribozyme transfected cells, but at higher concentration of cellular extracts, telomerase activity was found also in ribozyme transfected cells suggesting only partial inhibition of telomerase by ribozyme. The telomere length was reduced in ribozyme expressing cells.

The cell sorting analysis didn’t show any change of cellular profiling between active ribozyme and mutant ribozyme transfected cells, revealing there is no change in cell cycle and cell proliferation and division cycles are not disturbed 45 days post transfection.

Morphology of cells was modified in ribozyme transfected cells compared to vector and mutant transfected cells. The ribozyme expressing cells showed extending morphology that is typical to differentiation phenotype. Most of the cells have shown such extended morphology in ribozyme transfected cells, though some similar cells were also observed in mutant ribozyme expressing cells.

Transcription profiling of vector, ribozyme and mutant ribozyme transfected cells reveals the disappearance of certain transcripts in ribozyme expressed cells compared to vector and mutant ribozyme transfectants. Disappeared transcripts were cloned and identified by sequencing and found to be coiled coil domain containing protein 5 (HEI-C), retinoblastoma binding protein -2 (RBP2), methionine s-adenosyl transferase (MAT-2B), ribosomal binding protein 23 and 35 (RPL23 and RPL 35) and Sterol isomerase.

Proteomic profiling of vector and ribozyme transfected cells reveals change in profiling and ribozyme transfected cells found to have appearance of spots which are absent in cells transfected with vector only. The proteins identified were IL12R from vector only transfected, and ATRIP, keratin10, β transducin repeat containing isoform and hCG from ribozyme transfected cells.

The proteomic and transcriptome of ribozyme expressing cells provide hints on crosstalk of telomerase, and telomerase RNA component with other cellular genes. From the existing evidences and from our studies, it seems that during proliferation, phosphatidyl Ianositol-3 kinase pathway plays role along with MAP kinase pathway and suppression of hTR inhibits these pathways and activates the TGF β pathway.
The following objectives were accomplished

- Ribozyme was designed against human telomerase RNA by comparing conserved secondary structures.
- Designed ribozyme was cloned in pStu I and pCI neo vector.
- Human telomerase RNA's cDNA was cloned in to pGEM-T vector.
- Cleavage assay of ribozyme reveals designed ribozyme cleaves the target but less efficiently.
- Expression of ribozyme in mammalian cells shows the expression of intact ribozymes and reduction of telomerase RNA and reduction of telomerase activity in ribozyme transfected cells.
- Discernible reduction of telomere length was observed in ribozyme transfected cells compared with vector and mutant transfected cells.
- There is no change in cell cycle profiling in transfectants but change in morphology observed in ribozyme transfected cells.
- Transcript profiling reveals there is change in profiles in ribozyme transfected, mostly disappearance of transcripts in ribozyme compared to vector and mutant ribozyme transfected cellular RNAs.
- Proteomic profiling reveals the expression of certain peptides in ribozyme transfected compared to vector only transfected cells.
- The transcriptome and proteomic profiling reveals the possible cross talks of telomerase RNA with other cellular genes and opening new avenues for extracurricular activities of telomerase and proves that telomerase can be effective target in cancer therapeutics.