A Single Ribozyme to Catalyze both Trimming and Transacting Catalysis - Potential Therapeutic for HPV Infection and Cervical Cancer

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Technology description

Summary

This technology relates to a potential therapeutic for treating human papillomavirus (HPV) infection as well as cervical cancer. It is acknowledged that HPV is the primary agent associated with cervical cancer. The life cycle of HPVs progresses with epithelial differentiation and may persist for decades. The E6 and E7 oncogenes are responsible for two viral proteins that target p53 and Rb. The persistence of E6 and E7 in cervical carcinomas has led to them being recognized as the hallmark of cervical carcinomas and makes them excellent targets for therapy. Previously, we reported an engineered hairpin ribozyme (R434) that caused down-regulation of HPV-16 E6/E7 mRNA and inhibited growth of both HPV-16 immortalized cells and tumor cells. To increase efficiency of R434 we constructed a ribozyme expression system (TRL-5) entirely based on cis-cleaving (trimming) hairpin ribozymes (triplex system) that release R434 from long transcripts. Because of the modular structure of the hairpin ribozyme, the catalytic domain B can independently recognize cis or trans targets allowing the use of the same ribozymes for both trimming and therapeutic duties. Thus, this improved system was designed as a three-ribozymes unit in a canonical triplex using an inverted cleavage from one trimming ribozyme.

The Rz434bis system was designed to use a single R434 ribozyme to catalyze both trimming and trans-acting activities. This procedure resulted in a reduced-size triplex system that uses R434 catalytic domain to self-excise itself. RNA from Rz434bis and TRL-5 templates released R434 by a self-processing mechanism thus allowing for the individual activity of multiple trans-acting ribozymes. Both Rz434bis and TRL-5 systems produced an increased cleavage efficiency of HPV-16 target site nt 410 to 445 when expressed from linear or circular templates. Furthermore, duplex Rz434bis and TRL-5 were more efficient in cleaving E6 than duplex single R434. The use of triplex configurations with multi-target ribozymes will ultimately result in better in vivo HPV-16 E6/E7 mRNA degradation.

Advantages

Therefore, implementation of the triplex systems that significantly enhance R434 in vitro activity is offered as an alternative to the antisense oligodeoxynucleotide treatment of cervical cancer.

Institution

NIH - National Institutes of Health