Ultra-sensitive and multiplex mutation detection of tert and idh mutaionts in glioma patients

Abstract Submitter: Lei Chen, Sweden*

Co-Authors: Tomas Edgren, Malgorzata Trybula, Malgorzata Lysiak, Annika Malmström, Peter Söderkvist

*Rarity Bioscience AB

Abstract

Background & Objectives*

Molecular characterization on TERT promoter and IDH1 mutations is critical for glioblastoma diagnosis, prognosis, and treatment stratification. Traditionally, these mutations are identified in tissue biopsies; however, liquid biopsies offer a less invasive alternative but severely limited by the BBB.The GC-rich regions in the TERT promoter further limit the detection sensitivity.

Methods*

cfDNA dervived from plasma and genomic DNA from tissue was analysed with superRCA assay. Sequence of interest are enriched by targeted PCR amplification and converted to DNA circles that are subjected to rolling-circle amplification. Padlock probes specific for mutant or wild-type sequences are then applied with exquisite specificity through major voting mechanism that suppress background noise, followed by RCA of the circularized probes. The large DNA clusters are referred to as superRCA products. The reactions are then analyzed using conventional flow cytometry, providing the mutant allele frequency as the number of particles originating from mutant- vs. wild-type sequence.

Results

TERT C228T/C250T superRCA were designed and verified on high VAF genomic DNA samples confirmed by Sanger sequencing as well as low VAF spike-in samples derived from the patient genomic DNA sample to confirm the LoD for TERT C228T/C250T is at 0.00X%.which is 100X more sensitive than conventional ddPCR assay (LoD=0.5-1% level).

A total of 20 glioma patients, with matched tissue and plasma samples from diagnosis were analysed against TERTC228T/C250T and IDH1 p.R132H . gDNA input were 66 ng, while the average plasma volume was 1.55 mL (0.5-6mL) yielding an average of 32.88ng (4.83-66ng).

The gDNA analysis from tissue 100% match the Sanger sequencing data, with 13, 6, 2 patients being positive for TERT C228T, TERT C250T, and IDH1 p.R132H respectively. In plasma samples, 4 patients had matched TERT and IDH1 mutations, follow-up with VAF range 0.022%-27.09%.

Conclusion

This study demonstrated that it is possible to detect TERT and IDH mutation in multiplex from a limited amount of ctDNA derived from plasma. The superRCA further demonstrated high sensitivity in detection the targeted mutations, with higher genomic DNA input demonstrating sensivity below the input levels of the ctDNA samples, suggesting that larger volumes of plasma would increase detection efficiencty.

Do you have any conflicts of interest?

No, I do not have a conflict of interest.