

Development of a sensitive, specific and cost-effective T-ARMS PCR assay for the genotyping of R132H of *IDH1* gene in glioma patients

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Abstract

The discovery of isocitrate dehydrogenase isoform 1 (*IDH1*) mutation as a key molecular marker has resulted in a change in glial tumour classification [1]. *IDH1* mutations are commonly in gliomas, particularly in low-grade gliomas and secondary glioblastoma [2]. *IDH1* p.R132H (c.395G>A) accounted for more than 90% of the mutation in *IDH1/2* mutation and had a significant association with clinical outcomes. *IDH1/2* mutations cause gain-of-function resulting in the formation of an oncometabolite, R-2-hydroxyglutarate instead of α -ketoglutarate implying a disruption of oxidative decarboxylation of Kreb's cycle and other cellular mechanisms [3,4,5]. Immunohistochemistry (IHC) and Sanger sequencing is the most common approach used in molecular diagnostics. Nevertheless, both IHC and Sanger sequencing methods have shortcomings. IHC is prone to miss out on the mutation if the tumour samples are of poor quality, and it has also been reported to have low sensitivity when compared to sequencing-based techniques [6,7].

This study aimed to develop a sensitive, specific and cost-effective assay for genotyping *IDH1* p.R132H mutations in glioma patients in order to shorten the time required for confirmatory diagnosis to be made. The tetra primer amplification refractory mutation system polymerase chain reaction (T-ARMS PCR) was used in this study to develop and validate the clinical applicability of the assay. A total of 61 glial specimens were collected and genomic DNA was isolated from all of them. All the samples were subjected to endpoint PCR and Sanger sequencing for mutation detection. T-ARMS PCR was developed and optimized prior to the screening of all the samples, and comparative mutation analysis was carried out.

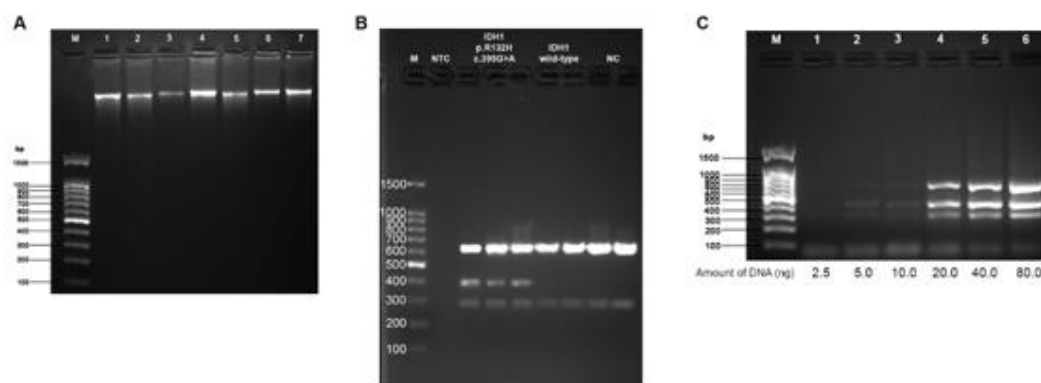


Figure 1: The development and validation of T-ARMS PCR for detection of *IDH1* p.R132H (c.395G>A) mutation and comparative mutation analysis via forest plot. (A) Integrity check of the extracted genomic DNA using 2.0% agarose gel electrophoresis. (B) Validation of T-ARMS PCR in distinguishing *IDH1* p.R132H (c.395G>A) mutation, T-ARMS PCR successful to differentiate *IDH1* p.R132H from wild-type. M: 100 bp DNA ladder; NTC: non-template control; NC: negative control. (C) The limit of detection for T-ARMS PCR assay was at 20ng of genomic DNA.

Overall, *IDH1* p.R132H mutation was found to be 45.90% (n=28/61) prevalent and was found to be significantly associated with gender, tumour subtypes and grading, and location of the lesions ($p < 0.05$). With an F1 score of 0.966, we reported T-ARMS PCR with sensitivity, specificity and accuracy of 100% (95% CI: 87.94-100.00%), 93.94% (95% CI: 80.39-98.92%) and 96.72%, respectively. To compare with published studies, a meta-analysis is T-ARMS PCR detected one case with *IDH1* p.R132C (c.394C>T), howbeit the case could have a double mutation of p.R132C (c.394C>T) and p.R132H (c.395G>A) or single mutation of p.R132Y (c.394_395CG>TA). However, this study was able to detect *IDH1* p.R132G (c.394C>G) mutation via PCR by Sanger sequencing whereas T-ARMS PCR excluded the mutation, suggesting the assay is very specific to *IDH1* p.R132HH (c.395G>A) only.

Table 1 Diagnostic accuracy analysis of T-ARMS PCR assay

Parameter	Value
Sensitivity	100.00%
Specificity	93.94%
Positive predictive value	93.33%
Negative predictive value	100.00%
Accuracy	96.72%
F ₁ score	0.966

Hereby, the performance of the T-ARMS PCR assay sheds a light that can be adapted for preoperative or intraoperative diagnosis.

Keywords

Glioma, *IDH1*, T-ARMS PCR

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