DEVELOPING A REAL-TIME PCR PROCEDURE FOR DETECTING D842V MUTATION OF THE *PDGFRA* GENE IN GASTROINTESTINAL STROMAL TUMORS (GIST)

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INTRODUCTION

Mutations in *PDGFRA* exon 18, especially D842V, are a major cause of resistance to Imatinib in GISTs. A reliable, cost-effective diagnostic tool for detecting the D842V mutation is needed in clinical practice. Real-time PCR (qPCR) offers a feasible solution due to its efficiency, reliability, and high sensitivity. This study aims to: (1) optimize primer design; (2) create a positive control plasmid; and (3) establish a SYBR Green qPCR protocol to detect the D842V mutation. The results will help clinicians select appropriate treatments, particularly Gleevec (Imatinib), and may improve outcomes for GIST patients.

MATERIAL & METHODOLOGY

15 cases with a confirmed pathological diagnosis of GIST HCMCOH from January 2021 to June 2023.

Specific primers targeting the mutation at position 842 (D to V) were designed using primer-BLAST (NCBI) ***(Figure 1)***, and primer concentration and annealing temperature were optimized.

A plasmid carrying the D842V mutation of the *PDGFRA* gene was created and used as a positive control to validate the quality of the SYBR Green real-time PCR assay.

The developed assay was applied to the 15 samples, which were previously assessed by Sanger sequencing.

Figure 1. *The optimal results of the primer for detecting the D842V mutation in exon 18 of the PDGFRA gene*

RESULTS & DISCUSSION

A successful protocol was established to detect the D842V mutation in exon 18 of the *PDGFRA* gene using Sanger sequencing and SYBR Green real-time PCR, creating a valuable tool for the diagnosis and treatment of GIST.

Constructing a DNA plasmid with the D842V mutation as a positive control helps ensure the sensitivity, specificity, and accuracy of the real-time PCR assay. ***(Figure 2)***.

The analysis of 15 GIST FFPE samples to detect the D842V mutation in exon 18 of the *PDGFRA* gene using SYBR Green real-time PCR showed negative results, consistent with data from the Sanger sequencing method ***(Table 1, Figure 3)***.

Result	SYBR Green qPCR	Sanger sequencing
Mutation	0	15
No Mutation	15	0
Total	15	15

Table 1. *The distribution of SYBR Green real-time PCR results and Sanger sequencing analysis.*

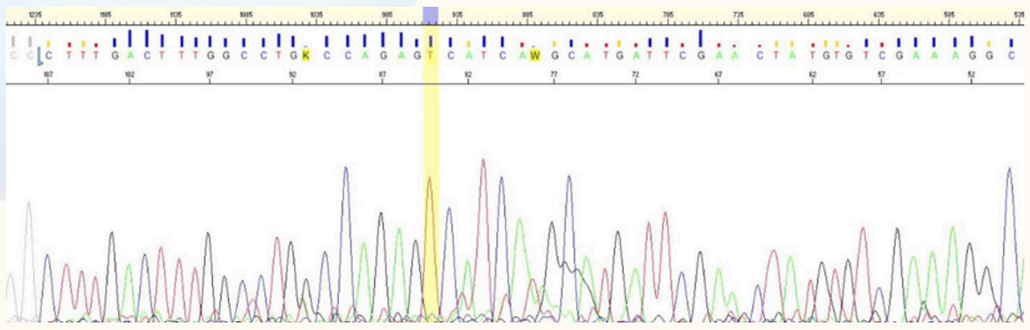

Figure 2. *The Sanger sequencing results of the DNA plasmid containing the D842V mutation, used as a positive control.*

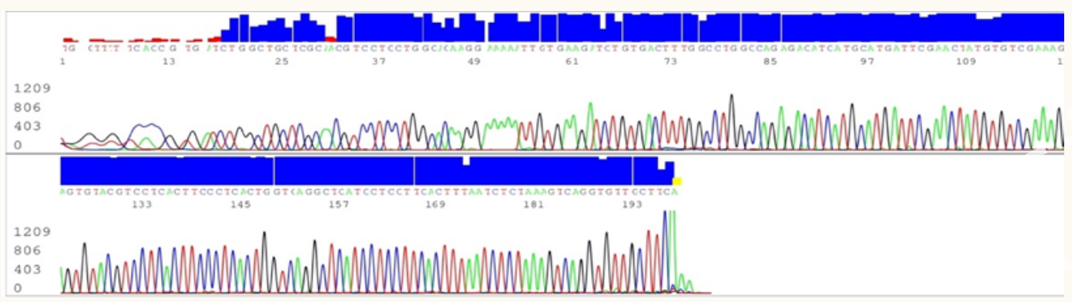

Figure 3. *PDGFRA exon 18 sequence with good signals and no mutation.*

CONCLUSION

The SYBR Green real-time PCR assay for detecting the D842V mutation of the *PDGFRA* gene in GIST patients can be routinely applied to assist clinicians in determining appropriate Gleevec (Imatinib) treatment for GIST patients.

REFERENCE

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