Detection of Mutated Tumor DNA From Colorectal Cancer Using Real Time-PCR

Erik L. Anderson DO, PhD[1,2]; Naveed Ahmed DO[1,2]; Sofia Tortora MS, DDS[2]; Laura Martello-Rooney PhD[2], Manuel Martinez MD[1,2]

[1]Brooklyn VA NY Harbor Healthcare, Brooklyn, NY, [2]SUNY Downstate Medical Center

Introduction

Colorectal cancer (CRC) is the third leading cause of cancer related death in the United States. Screening for CRC has long been identified as an effective approach to prevent and manage advanced disease which decreases cancer mortality. However, despite screening guidelines currently in place, many Americans are not screened for the disease. Current technologies to analyze DNA mutations, one hallmark of cancer, in patient samples are labor intensive and have a high cost as they require special machinery and downstream data analysis. The objective of our study was to determine if mutated DNA can be detected in colon tissues and serum from colon cancer patients using a standard molecular biology technique, polymerase chain reaction (PCR).

Methods

We used a SYBR Green real-time PCR method to detect a common point mutations in DNA from serum from colorectal cancer. Briefly, we used primers complementary to the mutated DNA with the sequence ending at the point mutation in the 3' terminal in both directions (mutated primer). To inhibit amplification of wild type DNA, we used modified replication deficient primers that were complementary to the wild type sequence (WT blocking primer). This method was verified with genomic DNA isolated from human cancer cell lines with known mutations and those without the mutation. Serum cell free DNA was purified using Qiagen blood mini kit.

Results

Graphical Abstract

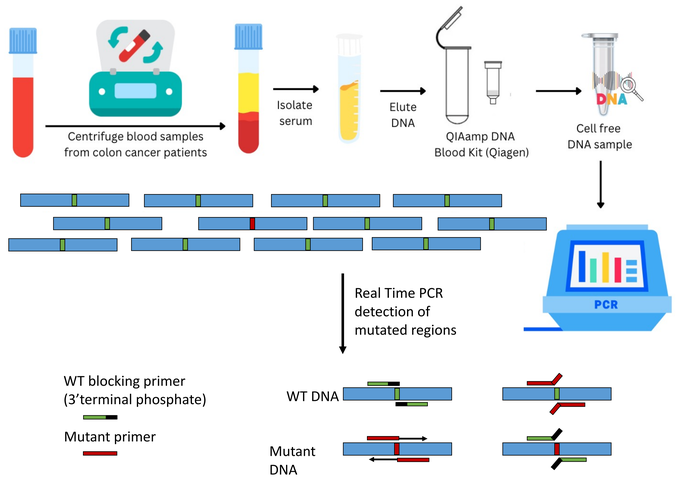


Figure 1: Verification of assay in cell lines with known mutations

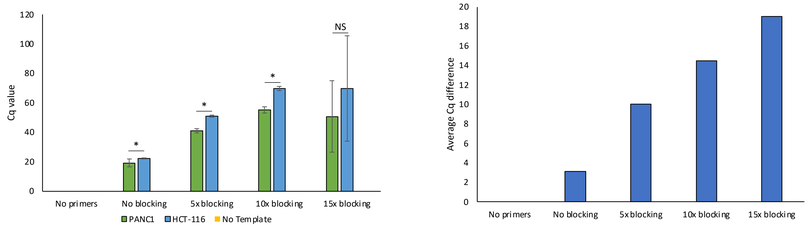


Figure 2: Analysis of patient samples

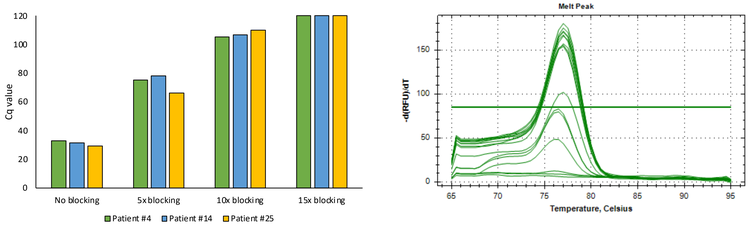
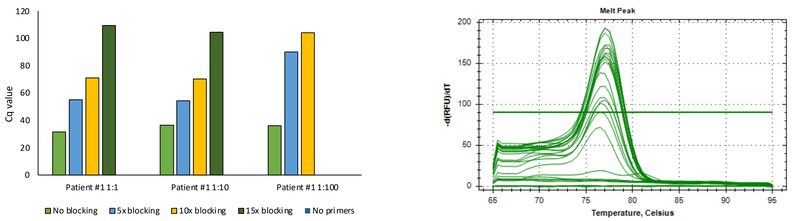


Figure 3: Reduction of signal with sample dilution



Real-Time PCR of human cell lines genomic DNA *p<0.05, n=4 (**Figure 1**), purified cell free DNA (cfDNA) from sera from colon cancer patients (**Figure 2**), and increasing dilutions of purified cfDNA from patient sera (**Figure 3**)

Summary

- RT-PCR use in combination with blocking primers is a valid method to detect point mutations in cell lines with known mutations.
- Purification and testing of patient serum allows detection using the same assay.
- Dilution of purified sample causes reduction in signal of assay.

Future directions

- Gene sequencing of patient DNA to determine what patients harbor mutations.
- Experiments with HLPC purified oligos instead of desalted oligos for possibly better separation.
- Compare Taqman assay to SYBR Green methods.
- Obtain different oligos to test assay on other point mutations.

Conclusion

Point mutations, such as TP53 818 G>A are detectable using RT-PCR in cfDNA in colon cancer patients' sera. This method can be modified to any point mutation or single nucleotide polymorphism and potentially can be used to determine a molecular diagnosis in a cost-effective manner.

Support

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Contact: Erik Anderson
Brooklyn VA NY Harbor Healthcare System
Email: erik.anderson@downstate.edu