CALIFORNIA STATE SCIENCE FAIR
2007 PROJECT SUMMARY

Name(s)
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Project Number
S0412

Project Title
Selective Amplification of Rare Mutations in Tumor Cells Enriched from Whole Human Blood

Abstract

Objectives/Goals
While the detection of mutated genomic DNA from cancer cells circulating in blood may be an early indicator of metastasis and may improve tumor staging, the task of detecting a few mutated cells in the presence of a large excess of wild-type cells requires a sensitive and selective assay. This study aimed to develop a novel polymerase chain reaction (PCR)-based approach to detect circulating melanoma cells harboring a common point mutation in BRAF, a cytoplasmic kinase in the MAPK (mitogen-activated-protein-kinase) signal transduction pathway.

Methods/Materials
A novel two-step PCR assay was developed to detect BRAF mutations in both cell mixtures and in blood samples spiked with tumor cells. In the first step, primer binding to wild-type BRAF was competitively blocked by a locked nucleic acid (LNA) oligonucleotide. In the second step, the LNA blocking approach was combined with a allele-specific primer that selectively amplified mutant DNA. The assay's sensitivity was tested on cell lines both homozygous and heterozygous for the mutation; both point and tandem mutations were also tested. To determine the clinical utility of this method, human blood was spiked with a defined number of melanoma cells (10 to 10,000 cells/mL blood). After negative epithelial cell enrichment and whole genome amplification were performed, genomic DNA from the enriched tumor cells was amplified by the aforementioned two-step PCR assay.

Results
This novel two-step approach easily detected ten BRAF-mutated melanoma cells mixed with 100,000 wild-type cells. In addition, this approach could readily identify mutant DNA from as few as ten melanoma cells circulating in one mL of human blood. DNA amplification was dose-dependent and highly reproducible.

Conclusions/Discussion
Since this approach facilitates sensitive detection of BRAF-mutated genomic DNA in circulating tumor cells, this assay may predict metastasis early, improve tumor staging, and identify candidate patients for therapeutic agents specifically targeting the MAPK pathway. By utilizing genomic DNA as a direct, inherently stable detection marker, this assay may be a viable alternative to reverse-transcription PCR (RT-PCR) techniques whose clinical applicability is limited by false positives from illegitimate mRNA transcription. Lastly, this highly selective and sensitive approach may be applicable to common point mutations implicated in other cancers.

Summary Statement
This study describes a novel real-time PCR-based assay that can predict metastasis and improve tumor staging by detecting as few as 10 melanoma cells circulating in one mL of human blood.

Help Received
Used lab equipment at Harbor-UCLA Medical Center under the supervision and mentorship of Dr. Michael Kolodney.