Detection of Mutations in Heterogeneous Tissues

Background

The detection of point mutations in genes from heterogeneous biological samples is often complicated by the presence of both mutated and wildtype cells. Methods currently in use such as DNA chips, single strand conformation polymorphism (SSCP), and Denaturing Gradient Gel Electrophoresis (DGGE), are expensive, cumbersome, not amenable to high throughput analyses, and involve the performance of electrophoresis. Other methods such as immunohistochemistry and Western blotting approaches used to detect the mutant protein, although useful, require diagnostic antibodies. Thus, there is a long felt need for techniques that can rapidly distinguish a normal allele from a disease causing allele in a specific and sensitive manner.

The present invention relates to a diagnostic strategy for the detection of mutant alleles in a heterogeneous tissue sample.

Description of the Technology

UMDNJ researchers have developed a method to detect point mutations in genes derived from heterogeneous biological samples containing both mutated and wildytpe cells. Normally, point mutations introduce new restriction site(s) in genes, which could then be identified using restriction enzymes that cut PCR products at specific sites of the DNA. Thus, the ability to detect these mutations depends on the presence of restriction sites within the site of mutation, as well as the sensitivity of detection techniques such as gel electrophoresis and southern blotting. However, the three most commonly found point mutations (Q61K, Q61R N-Ras and V599E B-Raf) in the N-Ras and B-Raf components of the MAPK pathway do not introduce restriction sites. Based on this observation, a strategy that introduces restriction sites via site directed mutagenesis has been developed to enable the detection of as low as 100 copies/µl of the mutant mRNA. The feasibility of this approach in the detection of point mutations has been validated using melanoma tissue specimens.

Advantages

- Many different mutations may be identified in the same specimen.
- Multiple specimens may be screened simultaneously.
- The assay requires only small amounts of the specimen and may used on archival tissues as well.
- Compared to immunohistochemistry and Western blotting, this assay is inexpensive to perform.
- The assay is rapid, highly sensitive, and specific.
- The assay can be easily modified to detect newly identified mutations.

Applications

- For the diagnosis of cancers and genetically determined diseases.
- For the screening of disease carriers.
- To stratify patients during clinical trials depending on the type of mutations.
- To predict patient outcome and plan therapy.

. • To clarify diagnosis: Certain cancers are difficult to diagnose based on histological examinations. This assay may be used to clarify diagnosis.

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Patent Status

•PCT Application filed. (Application No.: PCT/US04/19618)

Licensing Opportunity

•This technology is available for non-exclusive or exclusive license.

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