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Microsatellite analysis for hereditary non-polyposis colorectal cancer patients

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Abstract

The aims of this study is to establish a radioactive method for the detection of microsatellite instability (MIN). MIN is a characteristic of mutator phenotype in Hereditary Non-Polyposis Colorectal Cancer (HNPCC). For the investigation, we use a radioactive PCR-based method by using denaturing polyacrylamide gel electrophoresis and which is a useful tool for MIN screening in diagnostic pathology.

Key words : Microsatellite. DNA mismatch repair. Hereditary non-polyposis colorectal cancer.

Introduction

Colorectal cancer (CRC) is the second most common cancer and the third most common cause of cancer death in Hong Kong. While the majority of CRC are sporadic, about 15-20% of cases is resulted from inherited predisposition. Hereditary Non-Polyposis Colorectal Cancer (HNPCC) may account for as much as 5-10% of all CRC, and is one of the best studied hereditary CRC syndromes.

HNPCC

Hereditary Non-Polyposis Colorectal Cancer (HNPCC) was initially termed the “Cancer Family Syndrome”. It is a genetic disease resulted from mutations of DNA Mismatch Repair (MMR) genes. Individuals of HNPCC families have a high risk of developing CRC at a relatively young (under 45) age. In addition to CRC, HNPCC patients may also have cancers of the stomach, endometrium, bladder, etc.[1]

During DNA replication, mistakes (such as mismatch error) may occur and these mistakes are usually detected and corrected. The DNA mismatch repair function of a cell is performed by a family of genes called the MMR genes. They include hMSH2 on chromosome 2p, hMSH1 on chromosome 3p, hPMS1 on chromosome 2q, hPMS2 on 7q and GTBP. Germline mutation of a MMR gene is a cause of HNPCC. So far, defects in hMSH2 and hMLH1 have accounted for over 90% of the mutations found in HNPCC families.[2]

Since MMR genes are responsible for correcting mismatch error during DNA replication, mutations inactivating MMR genes will result in accumulation of DNA replication error (RER). As expected, RER is a characteristic feature found in colorectal tumours of HNPCC patients. To facilitate the identification of HNPCC patients, colorectal tumours can be tested for RER at microsatellite loci.

Microsatellites

Repetitive sequences are common in the human genome. The length of each repeat unit may vary from a single nucleotide to several thousand base pairs. Abnormality of repetitive sequences has been implicated in the genesis of a number of human genetic conditions.

Microsatellite are short tandem repeats of one to four nucleotides scattered throughout the genome. Each microsatellite locus usually contains 10 to 30 copies of a mono, di, tri or tetra-nucleotide repeat unit. Since most microsatellites are flanked by unique genomic sequences, they can be amplified by PCR using specific primers. Because of these features of microsatellite and its highly polymorphic nature, PCR based analysis of microsatellite has become a very useful tool in the study of tumour genetics.

Microsatellite Instability

Repetitive sequences including microsatellites are prone to error during DNA replication. DNA polymerase may slip and make a mistake in the number of repeats copied. This will result in an increase or decrease in the number of repeats in the microsatellite. When this mistake is made in a normal cell, it is usually corrected. The mismatched repeat can be recognised by the hMSH2 protein which then assembles a complex of MMR proteins to correct the mistake. But in HNPCC tumours lacking one of the MMR genes, this repair mechanism is not functional (Fig 1 & 2).[3,4]

Microsatellite instability refers to the expansion or reduction of the number of repeats in microsatellites. It is a characteristic of tumours having mutated DNA mismatch repair (MMR) genes, and hence, it is particularly common in tumours of HNPCC patients. Since microsatellite instability can be detected by PCR, analysis using a number of microsatellite locus specific PCR primers is a useful test for HNPCC patients.[5,6]

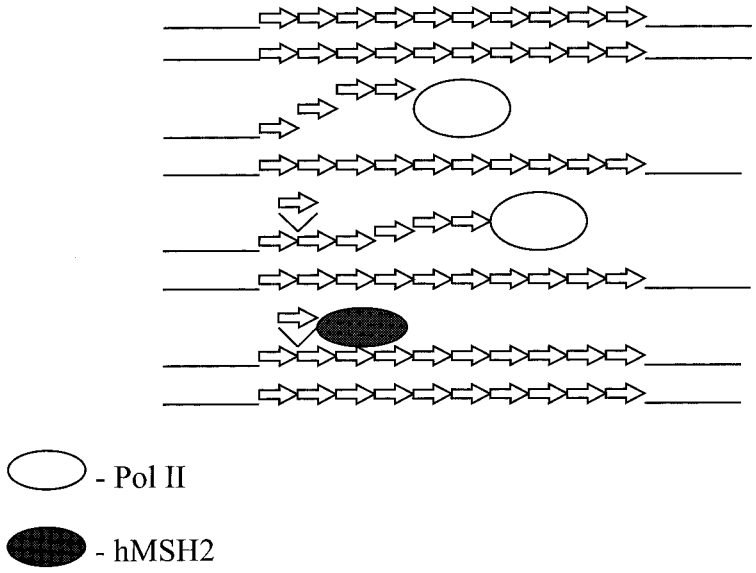

Fig. 1 DNA polymerase slippage increases or decreases the number of repeats. The microsatellite can be recognised by hMSH2.

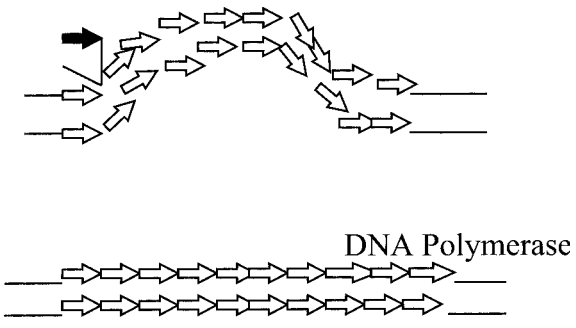

Fig. 2 Model of mismatch repair. hMSH2 binds to the mismatch repeats by assembly a complex. After excision of the strand containing the mismatch nucleotides, the gap is filled by polymerase II.

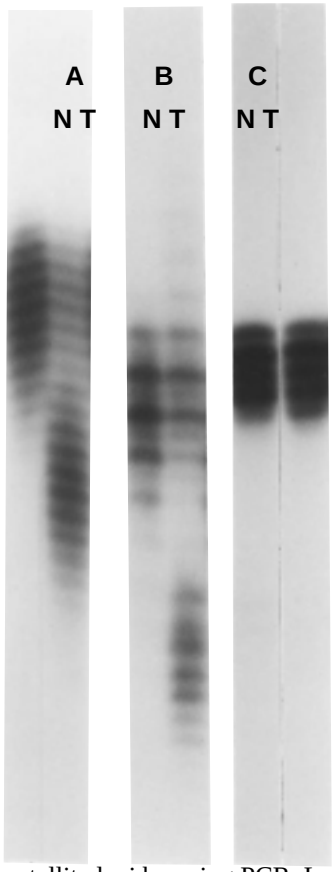

Fig. 3 Analysis of three different microsatellite loci by using PCR. Lane A and B show microsatellite instability: an electrophoretic mobility shift of PCR products between pairs of constitutional normal and tumour tissue at two different microsatellite loci. Lane C shows no electrophoretic mobility shift of PCR products

Procedure of microsatellite analysis

1. Choice of material
DNA should be extracted from both normal and tumour tissue from the same patient. Formalin fixed paraffin embedded tissue can be used. Only sections containing more than 60 % of tumour should be used because higher percentage of tumour cells would increase the chance of detecting RER in tumour tissue.

2. DNA extraction
The paraffin sections (50-75 micron) should be dewaxed and digested by proteinase K until the tissues are completely dissolved. The samples can then be purified by phenol-chloroform extraction and ethanol precipitation.

3. Quality of the extracted DNA
A control PCR should be performed to confirm that the purified DNA could be amplified by PCR.

4. PCR for microsatellite marker
After testing the DNA, PCR for at least 4 microsatellite loci should be performed. The PCR condition for each locus should be optimised. ^{32}P nucleotide incorporation can be used to label the PCR product.

5. Gel electrophoresis
The PCR products can then be resolved by using denaturing polyacrylamide gel electrophoresis. After fixing and drying the gel, the result can be visualized by autoradiography.

Data interpretation

Instability of a microsatellite is identified if there is an electrophoretic mobility shift of its PCR product from tumour DNA when compared with constitutional normal DNA. Cases showing shifts in at least 2 loci are classified as having the RER phenotype. Examples of microsatellite instability are shown in

Fig 3. This picture shows the autoradiograph of 3 different microsatellite loci. The left lane is the DNA extracted from the normal (N) tissue and the right one is from the tumour (T) tissue. When comparing the PCR products from normal and tumour tissues, extra bands are observed in the tumour DNA for 2 of the 3 loci analysed. This mobility shift indicates microsatellite instability.

Conclusion

Although 10-15% of sporadic CRC may show RER, over 90% of HNPCC tumours exhibit microsatellite instability. Therefore, identification of RER positive tumours can guide the clinician to offer the appropriate surveillance and counseling to members of the HNPCC family.

Apart from detecting RER, microsatellite analysis can also be used to detect Loss of Heterozygosity (LOH) of nearby tumour suppressor gene. The detection of microsatellite LOH can sometimes be used to confirm the presence of tumour cells in a clinical specimen.

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