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Review Article

Significance of Microsatellite Instability in Colorectal Carcinoma- A Complete Review

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Abstract

The microsatellite instability (MSI-H) or mismatch repair deficient (dMMR) colorectal tumors recently have been reported that can benefit from immunotherapy, and MSI can be used as a genetic instability of a tumor detection index. Many studies have shown that there are many heterogeneous phenomena in patients with MSI tumors in terms of immunotherapy, prognosis and chemotherapy sensitivity. Here we mainly review the research results of MSI detection methods, its mechanisms, occurrence and its relationship with related tumors, aiming in such a way for brief analysis of the micro satellite instability. Microsatellites (MS) are the repeated sequences of DNA that play an important role in maintaining the tissue morphology. Any mutation of the DNA or chromosomes, lead to the instability of the microsatellites, thereby causing the microsatellite instability. There are three types of microsatellite instability (MSI). High microsatellite instability (MSI-H), low microsatellite instability (MSI-L) and microsatellite stability (MSS). Recent clinical research tends to classify MSS-L and MSS as similar. Microstaellite instability plays an important role in colorectal carcinoma. Based on different molecular mechanisms, MSI in colorectal cancer can be divided into colorectal cancer (CRC) with no obvious family genetic history and Lynch syndrome with non-polyposis with family genetic history. Lynch syndrome is an autosomal dominant disorder and syndrome caused by mutations in MMR strains, and it can also cause tumors in other parts of the colon and rectum. With the recent development of MSI detection technology and immunosuppressant in tumor therapy, researchers found that MSI-H tumors respond well to immunotherapy. There are several methods to detect the microsatellite instability. 1. Next Generation sequencing (NGS), 2. Fluoresence multiplex PCR and capillary electrophoresis. 3. Immunohistochemistry. 4. Single molecule- molecular inversion probes (SmMIP). The main mechanism of MSI includes, Slipped strand mispairing, MMR deficient.

Keywords: Microsatellite, MMR, Immunohistochemistry, lynch syndrome, colorectal cancer.

I. INTRODUCTION

Microsatellite (MS), also called Short Tandem Repeats (STRs) or Simple Sequence Repeat (SSRs), consists of repeated sequences of 1–6 nucleotides. The distribution are different from nucleotides tandem repeats of small satellite DNA. Microsatllite stability are widely distributed and mostly is located near the coding region and may be located others region like intron or non-coding region. Each MS has two parts: the central core and the peripheral flanks, and the specificity of MS is mainly due to the change in the number of core repeating units. The mechanism of MS generation is generally believed to be DNA slippage in the process of replication, or mismatch of the basic group of slippage strand and complementary strand in the process of DNA replication and repair, resulting in one or more of the repeating units missing or insert. The normal tissue DNA repair system, called mismatch repair (MMR), can correct DNA replication errors. The lack of MMR genes in tumour cells, leads to the defect in process of replication repair.

Methods and Mechanisms of MSI

1. Next generation Sequencing Method

The micro samples for MSI detection by NGS can be used for disposable detection to get the acquaintance with MSI and whether MMR—related genes and Tumour mutational burden (TMB) alteration or not. NGS detection is directly targeted to known genes for genome sequencing, to test microsatellite instability in tumour tissues. MSK IMPACT products were approved to detect the microsatellite instability. The accuracy of the method was found to be 92%.

2. Fluorescent multiplex PCR and CE

This method is considered to be the Gold standard in detecting the microsatellite instability. The PCR method is used to detect the tumour loci detection by using the probes. Detection of one loci defect in the tumour tissue was named as (MSI-L) and detection of more than 3 or 4 loci in the tumour tissue is considered to be (MSI- H). The accuracy is found to be 100%. This method can directly reflect the status of MSI. Due to the characteristics of high efficiency, high sensitivity and reliable analysis results, this detection method has become the gold standard for MSI detection.

3. IMMUNOHISTOCHEMISTRY (IHC)-

The method is used to indirectly detect the MMR gene defect in MSI. MMR proteins are broadly divided into hMLH1, hPMS2, hMSH2 and hMSH6. If the result shows that any of the above MMR protein expression is absent, it means MMR deficient (dMMR). The defect in all the four proteins is termed as dMMR, defect in mismatch repair. IHC mainly aims to detect both dMMR and MSI-H. The accuracy of the test is 95%.

4. Single-molecule molecular inversion probes (smMIPs)

Recent method used detect microsatellite instability by using smMIPs, which are accurate. This method can accurately diagnose pan cancer microsatellite instability by single molecule reverse probe capture and high-throughput sequencing. smMIPs can only accurately identify microsatellite instability in colorectal, prostate and endometrial cancers to determine the presence of MSI.

Table-1: Methods of MSI

Detection method	Test items	Accuracy
NGS	Nearly 100 MS loci	IMPACT™: 92% F1CDx: 94.6%
Fluorescent multiplex PCR and CE	5 MS sites: BAT-26, NR-21, BAT-25, MONO-27 and NR-24	Gold standard, 100%
IHC	The MMR protein: hMLH1, hPMS2, hMSH2, hMSH6	89–95%
smMIPs	DNA from tumor tissue	95.80%

II. MECHANISM

1. Slipped strand mispairing

Microsatellite Stability, can also be caused by slipped strand mispairing (SSM). The process includes DNA replication and synthesis, the allele region of MS repeat sequence between new chain and template chain may be mismatched, resulting in the separation of new chain and template chain or the formation of stable single chain structure by several repeat units. Recently MS sliding mutation rate increased exponentially with the number of repeat units. When slip mutation occurs, MS with small number of repeat units expands more frequently, while MS with large number of repeat units contracts more frequently.

2. MMR deficient

The mismatch repair (MMR), can repair errors during DNA replication. In the above slip mechanism, when the mutated MS is paired with another chain, the redundant structure that may be formed after the slip chain can be restored to the level before replication. MMR deficient (dMMR) makes the errors produced during DNA replication impossible to repair, which leads to nucleotide mutation and changes in the length of simple repeat MS sequence. The most common gene mutation seen in MSI-H is polymerase-E (POLE) gene. There is also loss of the microRNA regulation.

3. MUTATION CHARACTERISTIC OF VARIOUS CANCER

The transmembrane or TGFβ, cell stress response or DNA damage and chromosome or M-phase related molecular functions are abundant in the genes of recurrent MSI, and the occurrence of frameshift MSI in TGFBR2 is more common in Colon adenocarcinoma and Stomach adenocarcinoma.

III. CLINICAL APPLICATION OF MSI

1. Lynch syndrome

The dMMR is common in patients with Lynch syndrome (LS). The patients with MSI-H or dMMR tumors can predict Lynch syndrome through MSI related tests. NCCN guidelines also recommends gene testing for Lynch syndrome, including MMR genes (hMLH1, hMSH2, hMSH6, hPMS2) and EpCAM genes. MMR IHC screening and MSI detection screening are two ways to screen for patients with Lynch syndrome. The IHC test results show that the negative hMLH1 cannot directly rule out the absence of mutation in hMLH1, and the hMLH1 promoter needs to be tested to determine whether there is methylation or BRAF (v-RAF murine

sarcoma viral oncogene homologB1) mutation, so as to exclude Lynch syndrome. EpCAM (Epithelial cell adhesion molecule) gene is still needed to be detected in suspected hMSH2 patients, because LS caused by hypermethylation of hMSH2 which caused by EpCAM body mutation will also lead to the loss of MMR protein expression in IHC detection, but hMSH2 mutation analysis is negative. Only when EpCAM immunostaining is negative, EpCAM abnormality indicates hMSH2 mutation. MSI was detected by nucleotide markers. Recent study states MSI-H was more common in patients over 60 years with LS, and most of them were found in normal adenocarcinoma, villous adenoma and in highly dysplastic adenoma.

2. Colorectal cancer

MSI is closely related to colorectal cancer. Some studies have found MS loci related to CRC. There lies the correlation between high-level microsatellite instability colorectal cancer patients, compared with MSS tumors, tumors are infiltrated with dense cytotoxic T cells. The following NR-21, BAT-26 and BAT-25 markers play an important role in judging MSI status in CRC. The BRAF mutation affects the MMR function of early diseases, and has an important effect on CRC. the prognosis of MSI-H colorectal cancer was good with BRAF mutation in early diseases, while the prognosis of MSS and MSI-L CRC was poor. The lifestyle can affect the molecular pathological types of colorectal cancer and study shows that alcohol consumption is associated with an increased risk of MSI-H colon cancer. MSI can be used as a significant molecular marker for prognosis and adjuvant therapy of CRC. MSI has different effects on the lymph nodes and distant metastasis of CRC in different periods, and on the prognosis of patients.

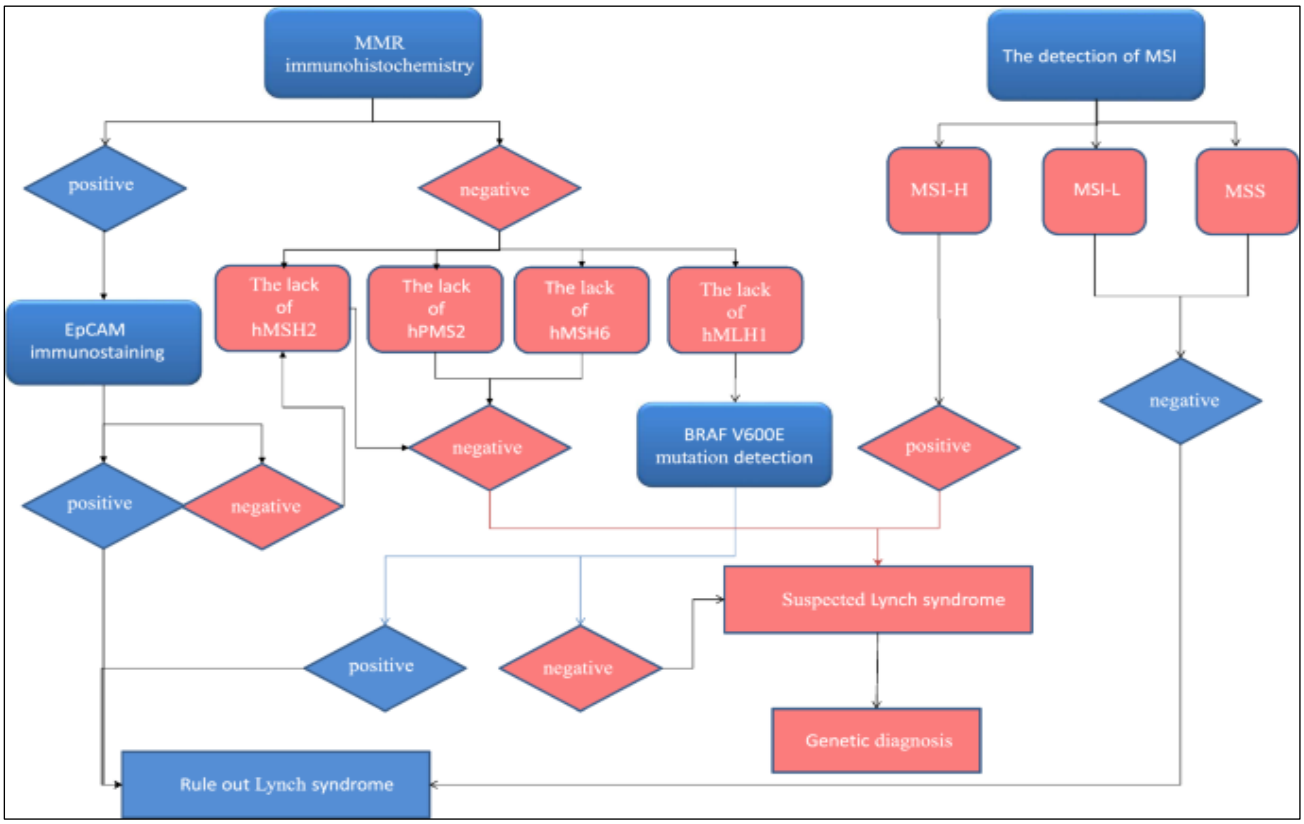

Fig-1

3. Other Cancer application

MSI also plays an important role in the detection of the mutation of various genes of stomach adenocarcinoma, bladder cancer, pancreatic ducatl adenocarcinoma, ovarian malignancy and endometrial carcinoma.

IV. RELATION BETWEEN MSI and TMB-

1. MSI is due to MMR deletion or gene replication process deletion or error, leading to changes in the length of MS.
2. TMB refers to tumor mutational burden, representing the number of mutations per million bases. Both MSI and TMB represent the production of new antibodies. Recent studies show many patients have high MSI-H also have high TMB levels. The MSI-H adrenocortical carcinoma and cervical squamous cell carcinoma have high mutation. Tumors with high mutation rates may respond well to checkpoint inhibitors (CPI).

V. CONCLUSION

A large number of studies have shown that MSI is closely related to tumor. The development of fluorescence multiplex PCR and CE and IHC promote the development of MSI detection. The smMIPs detection plays an important role in clinical application of MSI detection. The main limitations include, for the detection of chromatic cancer, prostate cancer. Hence NGS can be considered despite IHC and PCR are still widely used. Early diagnosis of MSI is of great significance to the prognosis and treatment of MSI. There are certain correlation between MSI related tumor and clinical phenotype. The early diagnosis can help to take preventive measures for its diseases, such as Lynch syndrome and in patients with hMSH2 and hMLH1 gene changes is of great significance to reduce the risk of cancer related to Lynch syndrome. In general, the detection method of MSI, the mechanism of MSI and its relationship with related tumors have made progress. However, it is still necessary to detect MSI in rare tumors, and improve the number of MSI related tumors and the classification of tumors. It is believed that with the more accurate detection technology of MSI and the clearer relationship between the mechanism of MSI and MSI-related tumors, MSI will open up a new field for the diagnosis, prevention and treatment of diseases.

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