

Progress in companion diagnosis of colorectal cancer

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Abstract: The incidence of colorectal cancer has been increasing year by year in China in recent years. This article mainly introduces the current common companion diagnostic techniques for colorectal cancer and the monitoring of minimal residual disease and other relevant research progress in the field of colorectal cancer in vitro diagnosis, and compares them with similar detection techniques. The aim is to provide a reference for summarizing the current research status of molecular diagnosis of colorectal cancer.

Keywords: Colorectal cancer; Companion diagnostic; Molecular diagnostics.

1. Introduction

Colorectal cancer (CRC) is the second most common malignant tumor of the digestive system [1]. In the past 20 years, the incidence of CRC has decreased significantly in developed countries represented by the United States, but it is increasing in China [2]. CRC is asymptomatic or inconspicuous in the early stage, accompanied by intermittent gastrointestinal discomfort, dyspepsia, fecal occult blood and other symptoms, easy to be ignored by patients, resulting in the continued development of cancer, spread and metastasis [3]. Therefore, it is particularly important to perform regular diagnostic screening for CRC as well as gene-directed therapy. In this paper, we review the research progress in the field of molecular diagnosis of CRC.

2. Companion Diagnostics

With the development of precision medicine for tumor genetic testing and pharmacometabolic genomics, the maturity of companion diagnostic techniques for CRC has been promoted. At present, in addition to surgery combined with local chemotherapy, radiotherapy and comprehensive treatment, gene-directed personalized molecular targeted therapy has become an essential method for CRC treatment [4]. The occurrence, development, invasion and metastasis of colorectal cancer are extremely complex mechanisms in which a series of genes are involved in expression and multi-step regulation, and this process is often associated with uncontrolled cell proliferation and escape from apoptosis, involving proto-oncogene activation (common C-myc gene, Ras gene and EGFR, etc.), mismatch repair gene mutation (HMSH1, HLH1, PMS1, PMS2, GTBP), tumor suppressor gene inactivation (common p53 gene, DCC gene, APC gene, etc.) and some risk modifiers (such as COX-2, CD44).

2.1. RAS and BRAF gene mutation and methylation detection

RAS gene family is closely related to the occurrence and development of human tumors [5]. The proto-oncogene RAS gene family includes HRAS, KRAS and NRAS, which are located on chromosomes 11, 12, and 1, respectively, with the highest rate of KRAS gene mutation [6]. Its expression products are all important proteins on RAS-related signaling pathways. The BRAF gene is downstream of the RAS gene and is a key member of the RAS-RAF-MEK kinase pathway,

and BRAF gene mutations are BRAF V600E mutant in 90% of patients with metastatic colorectal cancer [7,8]. Inactivation of tumor suppressor genes is associated with aberrant hypermethylation of CpG islands, resulting in decreased or silent expression of tumor suppressor genes.

Sensitivity and resistance to cetuximab and panitumumab should be tested in CRC patients before treatment, and testing for mutations in the RAS gene family is therefore necessary [9]. The mutated K-Ras protein is able to bypass signaling by its upstream effector epidermal growth factor receptor (EGFR), thereby rendering existing anti-EGFR therapeutics ineffective [10]. BRAF, as an important component of the mitogen-activated protein kinases (MAPK) pathway, can affect the proliferation and differentiation of cells after activation, and BRAF-mtCRC patients can receive a three-drug combination regimen of vemurafenib (BRAF inhibitor), anti-EGFR monoclonal antibodies, and irinotecan [7]. Liu Y [11] et al., used second-generation sequencing technology to detect KRAS gene mutation status, real-time PCR and immunohistochemistry were used to detect the mRNA and protein expression levels of Bcl-2 and caspase-3, respectively, and Spearman method was used to analyze the correlation between KRAS gene mutation and Bcl-2 and caspase-3 protein expression. The results showed that the KRAS gene mutation rate in CRC tissues was 42.2%, Bcl-2 expression was up-regulated and caspase-3 expression was down-regulated in CRC patients with KRAS gene mutation, and the expression of both may be a potential indicator for evaluating the prognosis of CRC patients with KRAS gene mutation. Yang Jiao [12] et al., real-time PCR, ARMS PCR and double-antibody sandwich colloidal gold technique were used to detect KRAS mutation sites of nucleic acid substances in intestinal exfoliated cells in feces and CpG island cytosine methylation and hemoglobin in BMP3 and NDRG4 genes. The positive detection rate of 7 KRAS gene mutations was $\geq 95\%$ when 1% was mutated in the background of 10 ng human genomic DNA, $\geq 95\%$ when 1% was methylated in the background of 10 ng human genomic DNA, and 100% when hemoglobin concentration was 100 ng/ml. This method used fecal DNA combined with fecal occult blood, especially for the detection of advanced CRC tumors, with higher sensitivity and specificity. Xu Jiassen [13] et al developed a real-time PCR-based human SDC2 gene methylation kit that can be used to assist in the diagnosis of precancerous lesions such as adenomas and polyps in CRC. Ye Minling [14] et al. used real-time PCR to verify the performance of human

SDC2 gene methylation detection kit. The results showed that the total coincidence rate was 92.86%. This method is convenient to sample, has high sensitivity and specificity, and can be used to screen colorectal cancer and precancerous lesions and provide physicians with auxiliary diagnostic basis. Hao Y [15] et al., used polymerase chain reaction and sequencing technology to genotype serum exon mRNA of KRAS and BRAF, and the sensitivity of KRAS and BRAF mutation detection was 73.7% and 75%, respectively, the specificity of mutation detection was 100%, and the overall coincidence rate of KRAS and BRAF mutations was 94.9% and 93.9%, respectively. Several clinical studies have shown that patients with BRAF V600 mutations have a worse prognosis and shorter survival time than other patients [16].

The results showed that RAS, BRAF gene mutation and CpG island methylation detection can be detected by Sanger sequencing, NGS, qPCR and other technologies [17]. In the case of abundant tumor tissue samples or body fluid samples, Sanger sequencing method with low equipment requirements and low price can be used for detection. Methylation can be detected by sequencing, and then primers and probes are designed for real-time PCR. RAS and BRAF mutation sites and mutation types are known, and qPCR assays can be performed directly. Several clinical studies have shown that cetuximab has a significant effect in the treatment of RAS wild-type metastatic colorectal cancer (MCRC), and the survival time of patients can be prolonged. Generally, chemotherapy combined with anti-EGFR monoclonal antibody is used, while RAS mutant patients have no significant benefit and require chemotherapy combined with VEGF monoclonal antibody [18,19]. Patients with BRAF V600E mutated mCRC should be treated with cetuximab + irinotecan + vemurafenib (BRAF inhibitor) or cetuximab + BRAF inhibitor ± MEK inhibitor in the second-line setting, and BRAF gene status has important guiding significance for the treatment and prognostic evaluation of CRC patients [16].

2.2. Microsatellite Instability (MSI) and Mismatch Repair (MMR) in Colorectal Cancer

Microsatellite instability (MSI) is a phenomenon caused by the insertion or deletion mutation of repetitive units in the relevant microsatellite sequences of tumor tissues during DNA replication, resulting in the emergence of new microsatellite alleles. The mismatch repair (MMR) gene can repair the DNA base mismatch phenomenon, and its members include MLH1, MSH2, MSH6 and PMS2 proteins, which can maintain the stability of the genome and reduce the probability of spontaneous mutation and are guardians of biological evolution [20].

For MSI detection, there are currently multiple fluorescence pcr combined with capillary electrophoresis, immunohistochemistry (IHC) to detect MMR protein and NGS platform-based MSI algorithms [21]. Multiplex fluorescent PCR technology is currently accepted as the "gold standard" for MSI detection and is detected at five microsatellite loci BAT-25, BAT-26, D2S123, D5S346, and D17S250 recommended by the National Cancer Institute [22]. IHC was performed using the recognition of specific antibodies to detect MMR protein loss and reflect MSI status [23]. The MSI algorithm based on NGS platform can perform target region sequencing (NGS panel), whole exon sequencing (WES), and whole genome sequencing (WGS), and the detection results are up to 99% consistent with PCR

methods and 92.4% consistent with IHC detection, which can provide information such as tumor mutational burden (TMB), MMR gene germline mutation (Lynch syndrome confirmed), and tumor somatic mutation, and NGS detection simultaneously scans a large number of microsatellite loci for comprehensive MSI evaluation[24]. Because the mismatch repair protein function is normal (pMMR), microsatellite instability will be repaired, thus keeping microsatellite stability (MSS), deletion of mismatch repair protein (dMMR), microsatellite instability cannot be repaired, resulting in MSI phenomenon, MSI phenomenon diffusely forms microsatellite instability (MSI-high, MSI-H). More than two MSI were identified as MSI-H, one MSI locus was identified as low-grade microsatellite instability (MSI-L), and no MSI was identified as MSS. Although most microsatellites are located in non-coding regions, misplaced mutations can lead to frameshift mutations, causing tumor-related gene abnormalities, which in turn induce the development of cancer [25-27].

Compared with PCR, IHC is more than 90% in agreement, but IHC requires higher experience of physicians, protein expression interpretation errors may occur in clinical practice, and some MMR protein function defects but still retain the original antigenicity, which easily leads to false negatives (normal protein expression) [28]. Compared with PCR, NGS-based MSI detection has higher molecular diagnostic efficiency, less sample consumption, and covers a wider range of microsatellite loci, but in order to distinguish microsatellite loci from their own algorithms and microsatellite loci selection, it is difficult to capture and sequence repeats and there are many interfering factors [29].

Clinical studies have shown that Lynch syndrome is due to MMR germline mutations that predispose individuals to CRC, and MSI testing for people with a family history of cancer or patients with premature tumors helps to reduce tumor morbidity and mortality. However, MSI-H is an indicator of better prognosis in patients with stage II and III CRC, and its prognosis is generally better than that in patients with MSS. At the same time, patients with MSI-H are insensitive to fluorouracil (5-FU) monotherapy, so adjuvant chemotherapy is not recommended in patients with MSI-H, while immune checkpoint inhibitors have better efficacy in patients with MSI-H/dMMR[30,31]. In addition to chemotherapy, MSI can also respond to targeted agents in patients with mCRC, and it has been found that patients with MSI-H can benefit from bevacizumab, rather than cetuximab, and the risk of treatment death is reduced by 87% [32]. Andre T [33] et al showed that there were many mutations in MSI-H/dMMR tumors and patients had extensive immunogenicity, and thus responded well to PD-1/PD-L1 inhibitors, while patients with MSI-H/dMMR used pembrolizumab drugs when they were dying, with an objective response rate of 43.8%, while standard chemotherapy methods only 33.1%. Therefore, MSI/MMR testing is recommended for CRC patients and is of great help in diagnosis, drug therapy, and prognosis recovery.

2.3. TMB tumor gene mutation burden

Tumor mutation burden is an index of the number of mutations in the genome of tumor cells and assesses the total number of substitutions and insertion/deletion mutations per megabase in the coding region of gene exons. Currently, the detection of TMB is used to evaluate the benefit of immune checkpoint inhibitors (PD-1) in patients [34]. The more mutated genes in tumor tissue, the more abnormal proteins are

produced and the probability of being recognized by the immune system is greater, thus activating the anti-cancer immune response of the human body, so the better the efficacy of cancer immunotherapy. It is generally believed that more than 20 mutations per million bases of TMB are high, and less than 10 mutations per million bases are low. Studies have found that patients with MSI-H generally have high TMB, while PD-L1 expression is not related to TMB levels, and the combined detection of the two can better predict the efficacy [35]. Currently, the TMA test is still in the experimental stage, and it is not recommended to perform TMB test in routine examination.

2.4. TMB tumor gene mutation burden

At present, there are still many CRC-related molecular markers that are still in the research stage and perform well, but the incidence of these molecular markers in the development of CRC is relatively low, and the clinical significance remains to be studied in depth, but it has been confirmed that there is a corresponding link, such as HER-2, PIK3C4 mutation, NTRK fusion, etc., which can be used as a new direction for the detection of CRC patients who have failed standard treatment[36-38].

3. Minimal Residual Disease Monitoring

CRC patients need to be reexamined regularly after surgery to detect minimal residues of pathogens in the body, most of which are produced by residual tumor cells, and capturing these signals will greatly improve the survival rate of patients and reduce the recurrence of CRC [39]. However, there are often few residual cancer cells, and residual molecular markers are difficult to be detected, so it requires extremely high sensitivity and specificity, and (multiplex) real-time PCR techniques are commonly used for detection. Tracking ctDNA as a noninvasive biomarker to detect minimal residual disease to assess the risk of recurrence and stage the patient's condition has been well confirmed in the clinical application of CRC [40,41].

Tarazona N et al showed that 152 cases of ACT were evaluated for preoperative ctDNA status, the preoperative ctDNA detection rate was 90%, 9.2% (14/152) were found to be MRD positive, 78.5% (11/14) eventually recurred, 10.1% (14/138) were MRD negative, the median number of MRD detected by continuous ctDNA was 9.08 months (0.56 ~ 16.5 months), the sensitivity was 79.1%, and the specificity was 99% [42].

4. Perspectives

Colonoscopy is the gold standard for colorectal cancer screening, but it is not indicated for regular early screening tests. Fecal immunochemical testing has been recognized for its excellent clinical performance and generalizability. Fecal immunochemical detection, (multiplex) real-time PCR and immunohistochemistry should be used to detect fecal protein markers, ctDNA mutations and methylation, carcinoembryonic antigen and other tumor molecular markers. Early multidimensional noninvasive screening for colorectal cancer and gene-directed individualized targeted therapy are the direction of future development.

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