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Research Progress on POLE/POLD1 and Microsatellite Instability in Colorectal Cancer

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Abstract: Colorectal cancer (CRC), one of the most common malignant tumors in the digestive system, is a heterogeneous disease whose pathogenesis and progression involve a complex interplay of environmental, genetic, and other factors [1]. The 2024 V2 edition of the National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines for Colorectal Cancer underwent comprehensive revision, optimizing multiple dimensions from diagnosis to treatment with a focus on advancements in molecular diagnostic technologies, recent achievements in immunotherapy, and adjustments to multimodal treatment strategies. The guidelines emphasize the need for a comprehensive upgrade in molecular testing for patients with suspected or confirmed metastatic adenocarcinoma. In addition to routine testing for RAS and BRAF gene mutations, HER2 gene amplification, and mismatch repair (MMR)/microsatellite instability (MSI) status, mandatory testing should now include rare gene mutations or fusions such as POLE/POLD1, RET, and NTRK. Regarding treatment strategies, the guidelines explicitly recommend prioritizing immunotherapy for patients harboring POLE/POLD1 mutations or defects in mismatch repair (dMMR)/high microsatellite instability (MSI-H), particularly those with unresectable metastases. Furthermore, the guidelines update that adjuvant chemotherapy postoperatively may have limited potential in improving survival rates for dMMR/MSI-H patients [2]. Thus, the discovery of additional molecular biomarkers offers novel insights and methodologies for the diagnosis, treatment, and prognostic evaluation of colorectal cancer. This review summarizes the functions of POLE/POLD1, its relationship with mismatch repair defects/high microsatellite instability (MSI), and the roles of POLE/POLD1 mutations and MSI in the pathogenesis and progression of colorectal cancer.

1. Introduction

Colorectal cancer (CRC) is one of the major malignant tumors threatening human health. Globally, CRC ranks third in incidence (accounting for 10.2% of all cancer cases) and second in mortality (accounting for 9.4% of total deaths) [3]. The 2023 report from the National Cancer

Institute of the United States indicated that there were 153,020 new CRC cases, representing 7.8% of all cancer cases, with a 5-year overall survival rate of 65.0%. However, these figures vary depending on tumor stage; approximately 20% of patients with metastatic CRC have a survival period of less than 2 years [4]. Data [5] show that in 2020, China recorded 4.569 million new cancer cases, among which 559,000 were CRC cases, accounting for 12.2% of all new cancer cases-second only to lung cancer in incidence ranking. According to statistics from the National Cancer Center of China, CRC incidence ranked third among urban cancers and second among rural cancers in 2022 [6]. Additionally, there is a trend toward younger onset of colorectal cancer in China [7]. Projections indicate that by 2030, global annual CRC cases will rise to 2.2 million, with deaths increasing to 1.1 million [8]. Currently, CRC treatment has entered an era of precision and individualization, with molecular biomarker testing serving as the cornerstone of precision therapy and playing an increasingly critical role in clinical diagnosis and management. A nationwide multicenter clinical epidemiological study conducted in 2022 revealed that the detection rates of RAS, BRAF, and microsatellite instability (MSI) in patients with advanced colorectal cancer in China were 41.4%,36.1%, and 28.2%, respectively. The 2024 V2 edition of the "National Comprehensive Cancer Network Clinical Practice Guidelines for Colorectal Cancer" emphasizes that, in addition to the aforementioned molecular markers, testing for rare gene mutations or fusions such as POLE/POLD1, RET, and NTRK should also be included as mandatory examinations. This indicates significant room for improvement in the clinical detection of molecular markers for colorectal cancer in China. This article provides a review of the functions of POLE/POLD1 and MSI, as well as their impacts on the pathogenesis, prognosis, and immunotherapy efficacy of colorectal cancer.

2. POLE/POLD1

2.1 Function and Pathogenesis of POLE/POLD1

Among numerous highly promising biomarkers, DNA polymerase ε (POLE) [9] and DNA polymerase δ1 (POLD1) [10] stand out. Both belong to the DNA polymerase B family, with POLE playing a central role. POLE and POLD1 are crucial in DNA repair, performing various functions such as base excision repair, nucleotide excision repair, mismatch repair, and double-strand break repair. Due to the high homology and similarity between the exonuclease domains of POLE and POLD1, most studies analyze both genes simultaneously [11]. During DNA replication, the exonuclease domains of POLE and POLD1 act like "quality control inspectors," continuously monitoring and removing mismatched bases to maintain genetic sequence accuracy. However, when mutations occur in the POLE/POLD1 exonuclease domains (POLE/POLD1-EDM), the "quality control system" fails, and cells lose their proofreading and correction capabilities. The erroneous base was not promptly removed., leading to the accumulation of mutated genes within cells, disrupting normal biological processes and becoming a major contributor to cellular pathogenesis. Given that eukaryotic DNA undergoes an average of one error per 1 to 10 billion nucleotide copies during replication, POLE and POLD1 are not only key participants in DNA replication but also critical factors ensuring replication accuracy and maintaining genomic stability [12]. Somatic or germline mutations in POLE have been identified in various human tumors, such as non-melanoma skin cancer, endometrial carcinoma (EC), and colorectal cancer (CRC) [13]. Wang et al. conducted an analysis of 47,721 patients with various cancer types, and the data demonstrated that the incidence rates of POLE/POLD1 somatic mutations were 2.79% and 1.37%, respectively. Additionally, POLE/POLD1 germline mutations were identified as significant susceptibility factors increasing the risk of colorectal cancer (CRC), endometrial cancer (EC), ovarian cancer, and brain tumors [14].

2.2 Mutation types of POLE/POLD1:

When pathogenic mutations occur in the exonuclease domain of POLE/POLD1, the proofreading mechanism during DNA replication is impaired, leading to a dramatic increase in mutation frequency-up to 10 to 100 times higher than under normal conditions. Extensive studies have demonstrated that patients with tumors harboring POLE/POLD1 mutations exhibit significantly higher tumor mutation burden (TMB) values compared to those without such mutations across various cancer types. Notably, the specific mutation sites within the POLE gene substantially influence tumor phenotypic characteristics and clinical prognosis. However, not all mutation sites in the exonuclease domain are pathogenic; the five most representative high-frequency mutation sites-P286R, V411L, S297F, A456P, and S459F-are collectively referred to as hotspot mutations [15]. Additionally, approximately 3%-4% of patients with colorectal cancer (CRC) and endometrial cancer (EC) are found to carry non-exonuclease domain mutations in POLE/POLD1 [11]. Although sufficient evidence remains lacking to confirm the pathogenic role of these non-exonuclease domain mutations, several studies have demonstrated that mutations such as the V1368M mutation in the POLE gene can disrupt normal protein function or structure, thereby classifying them as pathogenic [16]. Furthermore, research by Garmezy et al. has identified pathogenic missense or frameshift mutations in the POLD1 gene outside its exonuclease domain [17]. POLE/POLD1 mutation detection can be performed using PCR or Sanger sequencing techniques; however, next-generation sequencing (NGS) is typically employed in clinical practice. NGS technology enables simultaneous detection of other genetic alterations. Additionally, large-panel NGS testing can calculate tumor mutational burden (TMB) levels, which can be used to corroborate the results of POLE mutation detection.

2.3 Significance of POLE/POLD1 in Colorectal Cancer

Zhu et al. demonstrated that POLE mutations increase the risk of gastrointestinal tumors, and POLE lineage mutations may serve as effective molecular markers for predicting survival and metastasis [18]. Palles [19] and colleagues detected L424V and POLE S478N lineage mutations in multiple colorectal adenomas and CRC. L424V is a conserved site within the exonuclease domain of the DNA polymerase B family; lineage mutations reduce polymerase proofreading fidelity, increase the mutagenic rate, and promote tumor development, predisposing individuals to multiple colorectal adenomas and CRC. The POLD1 S478N mutation exhibits largely similar colorectal tumor characteristics to the POLE L424V mutation and demonstrates high conservation. Rohlin et al. identified the POLE Asn363Lys lineage mutation in another hereditary tumor family and concluded that it is more carcinogenic than the L424V mutation, potentially contributing to various tumor types [20]. In populations with microsatellite stable (MSS)/ mismatch repair intact (pMMR) features, approximately 2%-8% of colorectal cancer cases harbor somatic POLE pathogenic variants, whereas somatic POLD1 pathogenic variants are exceedingly rare [21][22]. Kane et al. identified the POLE P286R somatic mutation in human CRC, and this mutation impair the exonuclease domain's DNA-binding and proofreading functions [23]. In colorectal cancer (CRC), POLE mutations are predominantly observed in males, the right colon, and early-stage patients [24]. Studies have demonstrated that patients carrying POLE/POLD1 mutations exhibit a favorable prognosis, often characterized by an extremely high tumor mutational burden (TMB)>100 mutations per megabase (mut/Mb) [25][26]. The research team led by Wang Feng at Sun Yat-sen University analyzed a cohort receiving immune checkpoint inhibitor (ICI) therapy and found that patients with POLE/POLD1 mutations had significantly better prognoses compared to those with wild-type POLE/POLD1. Similar results were observed in the overall survival analysis of POLE/POLD1-mutated tumors that were not MSI-H. Although dMMR-positive CRC is suitable for

ICI therapy, the prospects for immunotherapy in most CRC patients remain limited [27]. Currently, few studies have investigated the correlation between POLD1 and ICI efficacy, with most focusing on POLE mutations. Wang et al. [14] analyzed genetic data from 47,721 patients with various cancers and identified POLE/POLD1 as an independent risk factor for immunotherapy benefit through multivariate Cox regression analysis (after adjustment for MSI status and tumor type). A study involving 1,278 advanced cancer patients receiving ICI therapy with low/medium TMB levels revealed that patients harboring POLE missense mutations outside the exonuclease domain often achieved better overall survival rates. Therefore, the discovery of POLE/POLD1 mutations not only enhances our understanding of the molecular mechanisms underlying CRC pathogenesis but also facilitates the identification of more patients who may benefit from ICI therapy [28]. Future research directions on POLE/POLD1 mutations primarily include: investigating whether the favorable prognosis of POLE or POLD1-mutated CRC is independent of adjuvant chemotherapy; and exploring whether inhibitors targeting these molecular targets can serve as novel therapeutic strategies to improve treatment efficacy in a broader patient population.

3. Microsatellite instability

3.1 Pathogenesis of Microsatellite Instability (MSI)

DNA mismatch repair (MMR) is a critical DNA damage repair mechanism capable of correcting base mismatches, insertions, deletions, and errors induced by exogenous damage during DNA replication and genetic recombination. Microsatellites, a type of satellite DNA with shorter repeating units than small satellite DNA, were identified in the late 1980s by James (Marshfield Medical Research Foundation) and Wis (Oregon Health & Science University) and colleagues [29]. They consist of repetitive DNA sequences of 2-5 base pairs, typically occurring 10-60 times in both coding and non-coding regions of the genome. Due to their repetitive structure, microsatellites are particularly prone to errors during replication; insertions or deletions in DNA coding regions can induce frameshift mutations, leading to protein truncation [30][31]. Under normal conditions, the MMR system identifies and repairs errors in these microsatellite regions, maintaining genomic stability. However, when MMR function is defective (deficient mismatch repair, dMMR)-characterized by the loss or deficiency of key MMR proteins such as MLH1, PMS2, MSH2, and MSH6-these errors cannot be effectively repaired, resulting in length alterations and high microsatellite instability (MSI-H). MSI typically manifests in three states: microsatellite instability-high (MSI-H), microsatellite instability-low (MSI-L), and microsatellite stability (MSS) [32]. Among these, the dMMR/MSI-H phenotype accounts for approximately 15% of all colorectal cancers and represents one of the critical molecular pathways involved in colorectal cancer pathogenesis [33][34]. Extensive studies have confirmed [35] that MSI is closely associated with the initiation and progression of various tumors and plays a pivotal role in tumor gene regulatory networks. Currently, microsatellite instability serves as a clinical marker for guiding disease diagnosis, treatment, and prognosis assessment.

3.2 Detection Methods for MSI

Currently, MSI detection methods primarily include immunohistochemical staining (IHC) and polymerase chain reaction (PCR) [36-38]. (1) The "2021 China Expert Consensus on Clinical Detection of Molecular Markers for Colorectal Cancer" indicates that polymerase chain reaction combined with capillary electrophoresis (PCR + CE) is the most reliable method for detecting microsatellite instability (MSI). The consensus recommends using the NCI Panel 2B3D to analyze five key loci: BAT-25, BAT-26, D5S346, D2S123, and D17S250. The specific diagnostic criteria are

as follows: if two or more of the five loci are unstable, the condition is classified as highly unstable microsatellite (MIS-H); if only one locus is unstable, it is classified as lowly unstable microsatellite (MSI-L); if all loci remain stable, the status is microsatellite stable (MSS) [39]. (2) Immunohistochemistry (IHC) detects the expression levels of four MMR proteins (MLH1, MSH2, MSH6, and PMS2) to determine the presence of MMR dysfunction (dMMR). Clinically, when all four MMR proteins are positively expressed, it indicates intact mismatch repair function (Proficient Mismatch Repair, pMMR); conversely, the absence of expression for any one or more of these proteins is defined as defective mismatch repair (Defective Mismatch Repair, dMMR) [40]. Typically, dMMR corresponds to the MSI-H phenotype, whereas pMMR is associated with MSI-L or MSS phenotypes [41]. Compared to PCR, IHC is widely used in general pathology laboratories and also serves as a guide for genetic testing [42].

3.3 Significance of Microsatellite Instability in Colorectal Cancer

The correlation between microsatellite instability (MSI) and colorectal cancer (CRC) has garnered increasing attention in the academic community. Numerous studies have demonstrated that MSI plays a significant role in the pathogenesis and disease progression of CRC. CRC cases with different MSI statuses exhibit notable differences in clinical pathology, disease prognosis, and treatment outcomes. Therefore, accurate detection of MSI status holds substantial value for clinical treatment planning and prognostic evaluation in CRC patients. Regarding prognosis, Gryfe et al. first reported that under identical chemotherapy regimens, MSI-unstable CRC exhibited better prognoses compared to MSI-stable CRC, with lower rates of lymph node metastasis and distant metastasis, particularly in MSI-H cases [43][44]. This finding was further corroborated by a meta-analysis conducted by Guastadisegni C et al. involving 12,782 CRC patients [45]. Similar results were also reported by Chen Xi [46], Kim C.G. [47], and Zhang Chaofan [48]. Early tumor staging is associated with MSI-H; the earlier the stage, the higher the MSI-H positivity rate. Patients with MSI-H CRC at stage II have favorable postoperative outcomes and a lower incidence of liver metastasis [49][50]. Additionally, studies have shown that MSI-type CRC at stages I-II exhibits higher disease-free survival rates compared to MSS-type CRC, whereas in stage III, MSI-type CRC demonstrates lower disease-free survival rates than MSS-type CRC [51]. The relationship between MSI and the prognosis of stage IV CRC remains unclear; however, some studies suggest that MSI is a factor associated with poor prognosis in stage IV CRC [52]. MSI is also a favorable prognostic factor for stage N1 colon cancer, with MSI-H patients in stage N1 exhibiting better overall survival (OS) compared to MSS patients. However, MSI-H has no impact on the prognosis of stage N2 patients [53]. Regarding adjuvant therapy, the findings of Sargent et al. [54] indicate that not all colon cancer patients benefit from adjuvant therapy with 5-fluorouracil (5-FU). Although patients with stage II MSI-H CRC have a better prognosis than those with MSI-L/MSS, they do not respond to conventional chemotherapeutic agents such as 5-FU [55]. In contrast, Hewish et al. demonstrated that patients with MSI-H who exhibit microsatellite instability have a better prognosis when treated with adjuvant 5-FU [56]. The 2024 V2 edition guidelines have updated the postoperative chemotherapy regimen for dMMR/MSI-H colorectal cancer: patients with stages 0-IIb do not require postoperative chemotherapy and may be managed with observation; patients with stage IIc may opt for observation or low-risk chemotherapy regimens, as studies have shown that adjuvant chemotherapy improves survival in stage IIc patients by less than 5% [2]. In the field of immunotherapy: Extensive studies have demonstrated that immune checkpoint inhibitors (ICI) exert antitumor effects by enhancing T-cell activity through inhibition of immune checkpoints represented by programmed cell death protein 1 (PD-1) and cytotoxic T lymphocyte-associated protein 4 (CTLA-4). Deficient mismatch repair (dMMR)/high-level microsatellite instability

(MSI-H) is the first established molecular marker for predicting ICI efficacy. Research has confirmed that ICI exhibits higher antitumor activity in solid tumors with dMMR/MSI-H [57]. However, dMMR/MSI-H is observed in only 10-15% of colorectal cancer cases, and the percentage decreases with more advanced tumor staging [58]. Although immune checkpoint inhibitors (ICIs) have achieved significant breakthroughs in treating metastatic tumors with microsatellite instability (MSI), in the field of colorectal cancer (CRC), 85% of cases remain microsatellite stable (MSS) tumors with low tumor mutation burden (TMB), and these patients often fail to respond to ICI therapy. In 2017, the U.S. Food and Drug Administration (FDA) approved PD-1 for the treatment of patients with unresectable or metastatic, previously treated solid tumors exhibiting dMMR or MSI-H.This drug is the world's first antitumor agent whose indication is determined based on biomarkers, breaking away from the traditional treatment approach defined by tumor location and establishing a novel therapeutic concept of "focusing on biomarkers rather than tumor location." These findings collectively demonstrate that dMMR/MSI-H serves as a robust predictive indicator for immunotherapy efficacy in colorectal cancer. Routine microsatellite instability testing should be performed clinically to determine the optimal immunotherapy regimen.

4. Correlation between POLE/POLD1 mutations and TMB and MSI.

The pathogenic mutations in POLE are closely associated with high TMB, and tumors harboring dMMR/MSI-H or POLE mutations typically exhibit elevated TMB [59]. MSI-H is predominantly observed in tumors with a TMB range of 10-100 mut/Mb, whereas POLE/POLD1 mutations can result in TMB exceeding 100 mut/Mb, referred to as hypermutational status [60], which is correlated with microsatellite stability (MSS). The Cancer Genome Atlas (TCGA) identifies POLE mutations and MSI as two critical molecular markers for tumor classification, a perspective supported by numerous studies. In colorectal cancer (CRC), Carethers et al. classified tumors with POLE mutations and MSI as exhibiting a hypermutational phenotype [61]. In a large-scale study involving 6,004 CRC patients, 5% demonstrated both MSI and TMB-H. Although MSI and TMB are interrelated, 2.9% of CRC patients with MSS were found to exhibit TMB-H along with a higher frequency of POLE mutations [62]. Recent advances in research on the association between genetic mutations and microsatellite instability (MSI) in CRC include proteomic studies from 2019, which revealed significant enrichment of mismatch repair pathway alterations and POLE/BRAF gene mutations in highly MSI-H CRC cases [63]. In 2020, research conducted by the Mo team demonstrated that colorectal cancer patients carrying POLE-EDMs exhibited a higher probability of developing MSI-H, and all these patients displayed elevated tumor mutation burden (TMB), with an average value of 200.8 mut/Mb [64]. Meanwhile, Hwang et al. found that functional defects in the MSH6 and MSH2 genes occurred earlier than POLE gene mutations. Based on these findings, they proposed that POLE mutations are triggered by dMMR, which, by impairing POLE gene function, significantly elevates the TMB levels in tumors coexisting with both dMMR and POLE mutations [59]. Additionally, He et al. classified tumors according to the clonal proportion of POLE gene mutations. The results showed that tumors with POLE gene mutations were more prone to developing MSI-H compared to tumors with wild-type POLE genes, providing a novel perspective for understanding the pathogenesis of colorectal cancer [65]. Furthermore, the study revealed that TMB-H CRC patients may respond to immune checkpoint inhibitors (ICIs) such as PD-1/PD-L1 independently of MSI status [66]. In summary, current evidence indicates that POLE mutations serve as a driver of dMMR in tumors coexisting with POLE mutations and MSI-H, ultimately leading to the development of MSI-H. Although the incidence of POLE/POLD1 gene mutations in CRC ranges at a moderate level of 1%-10%, given that the global incidence of colorectal cancer has risen to rank third among all cancers-with 1.93 million new cases reported worldwide in 2020-this

novel biomarker, POLE/POLD1, may potentially influence treatment strategies for thousands of CRC patients worldwide.

5. Summary and Outlook

Current research on POLE and microsatellite instability (MSI) in colorectal cancer has yielded numerous achievements, providing critical insights into the pathogenesis, clinicopathological characteristics, and prognostic evaluation of colorectal cancer. Some studies have elucidated the intrinsic relationship between POLE mutations and MSI status in colorectal cancer. Although these two mechanisms differ at the molecular level, both contribute to genomic instability and collectively promote tumorigenesis. Clinically, patients with POLE mutations or MSI-H colorectal cancer typically exhibit distinctive pathological features, such as high differentiation and fewer lymph node metastases, and generally demonstrate better prognoses, offering essential references for clinicians in disease assessment and treatment planning. In terms of treatment response, patients with POLE mutations or MSI-H colorectal cancer exhibit higher sensitivity to immunotherapy, offering new therapeutic options and improved survival prospects. However, current research still has limitations. Firstly, sample sizes and study scopes are limited, with most studies focusing on specific regions or populations, potentially affecting the generalizability and reproducibility of findings across different ethnicities and geographic backgrounds. Secondly, the synergistic mechanisms between POLE and MSI in colorectal cancer progression remain incompletely understood, necessitating further exploration of their mutual regulatory molecular pathways and key nodes to fully elucidate their mechanisms of action. Furthermore, in terms of clinical application, existing detection methods require improvement in accuracy, sensitivity, and specificity, and there is a lack of unified detection standards and protocols. This may lead to discrepancies in test results, thereby compromising the accuracy of clinical decision-making.

Looking ahead, on one hand, large-scale, multicenter, and cross-racial studies are required to obtain more representative data and conduct in-depth investigations into the distribution patterns, interrelationships, and clinical-pathological and therapeutic implications of POLE and MSI across different populations. On the other hand, leveraging emerging multi-omics technologies such as transcriptomics, proteomics, and metabolomics to integrate and analyze multidimensional data will enable systematic elucidation of the molecular regulatory networks of POLE and MSI in colorectal cancer, thereby identifying potential therapeutic targets and biomarkers. Concurrently, the development of more precise, convenient, and standardized detection technologies will enhance diagnostic accuracy and reliability, providing robust support for early clinical diagnosis, prognostic assessment, and personalized treatment. Furthermore, building upon the findings of POLE and MSI research, prospective clinical trials should be conducted to explore novel therapeutic strategies and combination therapies, which may further improve treatment outcomes and quality of life for colorectal cancer patients.

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