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Tissue Biomarker in Colorectal Carcinoma

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Abstract

Globally, colorectal cancer represents one of the most frequently diagnosed malignancies, accounting for approximately 1.9 million new cases in 2022, making it the third most common cancer worldwide. Heterogeneous pathogenetic mechanisms in colorectal cancer give rise to distinct tumor phenotypes, which manifest as specific clinical characteristics, treatment response, and prognostic outcome. Tissue biomarkers provide diagnostic and prognostic information, enabling accurate identification of tumor origin and subtypes as well as guiding personalized treatment strategies. However, despite their clinical importance, tissue biomarkers are not yet optimally implemented in routine practice, potentially limiting the treatment effectiveness. Immunohistochemical patterns of CK7/CK20 are useful for identifying the primary origin of colorectal tumors. The use of CK7/CK20 combined with CDX2 and SATB2 yields a powerful diagnostic panel for colorectal cancer. A profile of CK7-negative, CK20-positive, CDX2-positive, SATB2-positive represents a reliable immunohistochemical signature. Analysis of DNA mismatch repair (MMR) proteins such as MLH1, MSH2, MSH6, and PMS2 serves as an initial screening tool to differentiate hereditary colorectal cancer (Lynch syndrome) from sporadic colorectal cancer. Furthermore, KRAS mutations predict resistance to anti-EGFR agents, including cetuximab and panitumumab, and are associated with worse progression-free survival, whereas tumors harboring the BRAF V600E mutation tend to exhibit aggressive biological features and suboptimal response to standard chemotherapy. Comprehensive evaluation of tissue biomarkers prior to treatment initiation enables molecularly tailored therapeutic approaches, ultimately improving treatment efficacy and overall clinical outcomes.

Keywords: Colorectal Cancer; Tissue Biomarker; Protein Expression; Prognostic Marker; Personalized Medicine

1. Introduction

Globally, colorectal cancer accounted for approximately 1.9 million newly diagnosed cases in 2022 (9.6%) and ranked third in incidence. Additionally, colorectal cancer remains the second-highest contributor to cancer-related mortality worldwide [1]. Given its high burden, accurate and comprehensive diagnostic approaches are essential to ensure appropriate clinical management.

Tissue biomarkers are essential in diagnosing and treating colorectal cancer. A tissue biomarker is a molecule, protein, gene, or structural feature in tissue that yields information relevant to disease diagnosis, prognosis, and therapeutic responses [2]. These biomarkers help identify tumor origin and subtypes, predict disease outcomes, and guide personalized treatment decisions, especially in selecting the most effective therapy for individual patients [3].

Despite the critical importance of tissue biomarker testing, it is still not being implemented optimally in real-world practice. A study involving 1.497 metastatic colorectal cancer patients found that adherence to guideline-recommended biomarker testing was suboptimal, with testing rates of 41% for RAS, 43% for BRAF, and 51% for microsatellite instability/mismatch repair deficiency. This study also showed that among patients treated with anti-epidermal growth

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factor receptor (anti-EGFR), complete biomarker testing in accordance with clinical guidelines was performed in only 28% cases. These gaps suggest that many patients may receive therapies without confirming the tumor's biomarker profile, potentially limiting treatment effectiveness [4].

This narrative review aims to summarize key tissue biomarkers used for colorectal cancer diagnosis and prognosis, highlighting their clinical relevance and implications for patient management.

2. Review Content

2.1. Importance of Tissue Biomarker in Colorectal Cancer

Colorectal cancer represents a biologically heterogeneous malignancy with substantial variations in its molecular characteristics, clinical features, and treatment responses [5]. It is also associated with diverse long-term outcomes and treatment responsiveness [6]. These variations arise from different underlying pathogeneses that ultimately lead to distinct tumor phenotypes. Differences in phenotypes manifest as specific clinical characteristics, treatment response, and prognosis [7]. In this context, tissue biomarkers provide information for appropriate treatment selection for colorectal cancer. For example, anti-EGFR therapy may not be beneficial for patients harboring sarcoma-2 virus (RAS) mutation [8].

Tissue biomarkers also assist in determining the origin of colorectal cancer. For example, Cytokeratin 7 (CK7), Cytokeratin 20 (CK20), and CDX2 are commonly used to confirm the colorectal origin [9]. The CK7/CK20 immunophenotype demonstrated a sensitivity of 78.1% and specificity of 92.0% for diagnosing colorectal adenocarcinoma [5]. Additionally, SATB2 (Special AT-rich Sequence-Binding Protein 2) demonstrates high specificity for colorectal carcinoma and is valuable for distinguishing colorectal adenocarcinoma from other adenocarcinomas, making it particularly useful in identifying metastatic lesions of colorectal origin [10, 11].

Tissue biomarkers can also provide important prognostic information in colorectal cancer, helping clinicians assess tumor aggressiveness and estimate patient survival. For instance, the v-raf murine sarcoma b-viral oncogene (BRAF) V600 E mutation, caused by valine-to-glutamic acid amino acid in codon 600 substitutions, is associated with increased tumor aggressiveness as well as poorer prognosis in colorectal cancer. Conversely, deficient DNA mismatch repair (dMMR), which leads to microsatellite instability (MSI), is associated with better prognosis due to lower metastatic potential [12]. Accurate prognostic information provided from these biomarkers is essential, as it can guide more personalized and effective treatment strategies [13].

2.2. Diagnostic Tissue Biomarker in Colorectal Cancer

2.2.1. CK7 and CK20

The intermediate filament proteins Cytokeratin 7 (CK7) and Cytokeratin 20 (CK20) are important diagnostic markers that serve as first-line phenotypic classifiers. CK7 and CK20 exhibit distinct expression profiles across epithelial tissues, reflecting organ specificity. When a tumor is found in the colorectal region, CK7 and CK20 staining are frequently used to help determine whether the tumor originates from the colorectal epithelium or represents a metastasis from another organ. Distinct CK7 and CK20 expression patterns are commonly utilized to assist in identifying colorectal cancer among other adenocarcinomas. The characteristic immunophenotype of colorectal adenocarcinoma is CK7 negative (CK7–) / CK20 positive (CK20+) [14]. This occurs because CK20 is strongly expressed in colonic epithelial cells and is retained in most colorectal tumors, while CK7 is typically absent in colorectal epithelium [15].

CK7/CK20 patterns assist in both identifying the primary origin of a colonic tumor and in determining whether colorectal cancer has metastasized to other organs [9, 16]. For example, in cases where a metastatic lesion is found (e.g., in liver, bile duct, lung, and bone), a CK7–/CK20+ pattern strongly supports metastatic colorectal cancer [9, 17, 18]. CK7–/CK20+ pattern is also commonly seen in Merkel cell carcinoma, and occasionally in appendiceal carcinoma [19]. In addition, the CK7+/CK20– pattern is a characteristic of lung adenocarcinoma and adenocarcinomas from sites like breast, endometrium, ovary (serous), thyroid, salivary gland, pancreas, and biliary tract. The other staining patterns are also useful in determining most metastatic adenocarcinoma. CK7+/CK20+ pattern observed in pancreatic adenocarcinoma, gastric carcinoma, urothelial carcinoma, and ovarian (mucinous). Tumors such as hepatocellular carcinoma, renal cell carcinoma, prostate carcinoma, and adrenocortical carcinoma usually show CK7–/CK20–/ [20]. These patterns do not provide a definitive diagnosis, but serve as an important guide for selecting the next panel of organ-specific markers. When a tumor in colorectal region exhibits CK7–/CK20+ profile, this strongly suggests a colorectal origin and informs the use of the next marker that is highly specific for colorectal adenocarcinoma: CDX2 and

SATB2 [21]. Even in the era of comprehensive molecular testing, CK7/CK20 remain essential screening tools for diagnosing colorectal cancer as well as for prioritizing molecular or genetic assays and guiding targeted use of additional stains [22–24]. However, CK7/CK20 staining also has limitations. It is not sufficiently specific when used alone due to overlapping patterns across tumor types, thus it requires interpretation within a full immunohistochemical panel [15].

2.2.2. CDX2

Caudal-type homeobox 2 (CDX2) is a nuclear transcription factor characteristic of intestinal epithelial cells from the duodenum to the rectum, which regulates intestinal differentiation [25, 26]. Because of this role, CDX2 is a sensitive immunohistochemical marker indicative of intestinal differentiation. CDX2 provides significant diagnostic value in identifying tumors of gastrointestinal (GI) origin, particularly colorectal adenocarcinoma [27, 28]. CDX2 is positive in approximately 95% colorectal adenocarcinoma, and exhibits higher positivity in predicting colorectal adenocarcinoma compared to the CK phenotype [29]. It is a strong nuclear staining and is generally more consistently positive even when CK20 is lost [9, 28]. When combined with CK7−/CK20+ pattern or SATB2 positivity, CDX2 positivity strongly suggests an intestinal origin, making it useful in confirming metastatic colorectal cancer [21, 29]. CDX2 also retains diagnostic value in poorly differentiated or mucinous colorectal carcinoma, although its expression may be decreased [30]. However, CDX2 is also expressed in other carcinomas showing intestinal differentiation, including small intestine adenocarcinoma and certain oesophageal, ovarian, and pancreatic carcinomas [24, 31]. Therefore, although CDX2 is sensitive, it shows lower specificity for colorectal origin and is less specific than SATB2 [21].

2.2.3. SATB2

Special AT-rich Sequence-Binding Protein 2 (SATB2), a nuclear transcription factor involved in osteoblastic differentiation, is also expressed in colorectal epithelial tissue [32, 33]. SATB2 produces a strong nuclear staining and is highly specific for colorectal epithelium, making it one of the most specific markers for colorectal origin among epithelial tumors [32, 34]. SATB2 effectively distinguishes colorectal carcinoma from gastric cancer, pancreatic cancer, cholangiocarcinoma, ovarian mucinous carcinoma, breast cancer, prostate cancer, and lung cancer, as only few non-colorectal adenocarcinomas express SATB2 [32]. Because of its high specificity, SATB2 is particularly valuable for confirming the colorectal origin of metastatic lesions, especially in liver metastasis [35, 36]. SATB2 expression may be preserved in certain colorectal cancers despite loss of CK20 or CDX2 expression, making it useful in tumors with a nonclassic immunoprofile or poorly differentiated colorectal cancer [37, 38]. Combining CK7/CK20 with CDX2 and SATB2 yields a powerful diagnostic panel for colorectal cancer. A profile of CK7−/CK20+/CDX2+/SATB2+ represents reliable immunohistochemistry (IHC) signatures for colorectal carcinoma [24].

2.3. Prognostic Biomarkers and Predicting Therapeutic Response

2.3.1. MLH1, MSH2, MSH6, PMS2

MutL homolog 1 (MLH1), MutS homolog 2 (MSH2), MutS homolog 6 (MSH6) and post meiotic segregation increased 2 (PMS2) are DNA mismatch repair (MMR) proteins that play essential roles in the DNA repair system [39]. MMR-deficiency (dMMR), resulting from loss of MMR protein expression, is commonly associated with microsatellite instability (MSI) [40, 41]. MSI is a condition characterized by alterations in repetitive DNA sequences with a high number of mutations. This accumulation of mutations contributes to a high tumor mutational burden (TMB). MSI is a key biological and clinical feature in colorectal cancer and is referred to as MSI-high (MSI-H) [42].

Each of the four MMR proteins has a specific function and forms a functional heterodimer that is necessary for DNA mismatch repair. Loss of one protein in a heterodimer leads to secondary loss of its partner on immunohistochemistry, a pattern that helps identify which MMR gene is defective in the tumor. MLH1 pairs with PMS2, forming the MutLα complex, a key component of the DNA mismatch repair pathway. PMS2 is functionally dependent on MLH1. Hence, the loss of MLH1 and PMS2 generally indicates an MLH1 mutation, and the isolated loss of PMS2 generally suggests a PMS2 mutation [43, 44]. Additionally, MSH2 is the primary mismatch recognition protein that partners with MSH6 to form the MutSα complex, and MSH6 similarly requires MSH2 to function. Therefore, loss of both MSH2 and MSH6 expression typically reflects an MSH2 mutation, while MSH6 loss suggests an MSH6 mutation [43, 45]. Recently, initial screening of MMR status using a two-antibody approach with MSH6 and PMS2, rather than the full four-antibody panel, has been studied and shown to demonstrate minimal diagnostic discordance while offering a cost-saving strategy for dMMR detection [46].

Assessment of MMR protein expression by immunohistochemistry is the standard component of colorectal cancer testing, as it provides an initial screening tool for detecting Lynch syndrome, a hereditary colorectal cancer, as well as sporadic colorectal cancer. Patterns involving isolated loss of MSH6 or PMS2 are suggestive of a germline mutation, supporting a diagnosis of Lynch syndrome [47]. In addition to detecting hereditary colorectal cancer, MMR IHC is also

useful in identifying sporadic MSI-high (MSI-H) colorectal cancer. The most common sporadic cause is MLH1 promoter hypermethylation, which results in the loss of MLH1 and PMS2 [44, 48]. This pattern is typically associated with BRAF V600E mutation positivity. Therefore, when MLH1/PMS2 loss is observed, BRAF V600E testing is required to distinguish between sporadic MSI-high (MSI-H) colorectal cancer and Lynch syndrome. The presence of BRAF V600E mutation indicates that the tumor is likely of sporadic origin. If absent, MLH1 promoter methylation analysis may be performed to support a sporadic origin [47].

High TMB and neoantigen load in dMMR or MSI-H tumors enhance immune recognition. As a result, these tumors respond remarkably well to treatments that involve T cell activation to attack tumor cells, such as anti-programmed death receptor-1 (anti-PD-1) and anti-cytotoxic T-lymphocyte-associated antigen 4 (anti-CTLA-4) immunotherapies. PD-1 is an inhibitory receptor on T cells that, when bound by its ligand PD-L1, suppresses T cell activity and enables tumors to evade immune detection. Therapies targeting PD-1, such as pembrolizumab and nivolumab, block this interaction to reactive T cells against tumor cells [49]. CTLA-4 also inhibits T cell activation through competition with CD28 for B7 ligands on antigen-presenting cells. Anti-CTLA-4 therapies block this interaction, enhancing T cell activation and proliferation [50]. Therefore, MMR IHC plays an essential role in guiding therapy for colorectal cancer treatment decisions. When a tumor is identified as dMMR/MSI-H, the patient becomes eligible for immunotherapy, and this phenotype is associated with better prognosis in stage II and III colorectal cancer and lower metastatic risk [51, 52]. Additionally, MMR deficiency has been linked to diminished efficacy of 5-fluorouracil (5-FU)-based adjuvant chemotherapy, particularly in stage II colorectal cancer, highlighting the importance of MMR status in guiding chemotherapy selection [53]. Immunotherapy is currently a standard front-line option in metastatic MSI-H colorectal cancer [54]. Conversely, if all four MMR proteins are preserved on IHC, the tumor is considered proficient mismatch repair (pMMR) or microsatellite stable (MSS) [55, 56]. MSS tumors have low mutation burden and produce fewer neoantigens, resulting in reduced recognition by the immune system and generally unresponsive to immunotherapy [51]. In addition, microsatellite instability–low (MSI-L) is defined as instability in only one microsatellite marker within a standard 5-marker panel, which represents minimal genomic instability. MSI-L tumors typically have clinical characteristics similar to those of MSS tumors, therefore, many guidelines consider MSI-L tumors as MSS. Both are considered pMMR in clinical practice [57].

2.3.2. KRAS

Kirsten rat sarcoma viral oncogene homolog (KRAS) mutation analysis provides predictive information regarding the eligibility for anti-EGFR therapy [58]. As a small GTPase, KRAS regulates signaling pathways, particularly those involving the EGFR [59]. Oncogenic KRAS, most commonly in codons 12 and 13 lead to constitutive activation of the RAS–RAF–MAPK pathway [60]. Consequently, cells exhibit continuous proliferation and enhanced survival that occur independently of EGFR signaling, a hallmark of oncogenesis. Therefore, KRAS-mutated colorectal cancer will not benefit from anti-EGFR therapies such as cetuximab and panitumumab. In contrast, anti-EGFR therapy is effective only in KRAS wild-type tumors [61]. For this reason, KRAS mutation testing is important to assess a patient's potential responsiveness to EGFR targeted therapy.

KRAS mutation status also provides prognostic information of colorectal cancer, as KRAS-mutant colorectal cancer is linked to reduced progression-free survival. KRAS mutations are linked to more aggressive tumor behavior, leading to a higher likelihood of metastasis. It is also linked with poorer outcomes in metastatic colorectal cancer [61]. Among patients undergoing metastasectomy, those with KRAS-mutant tumors exhibit a higher risk of recurrence and reduced survival compared with patients who have wild-type KRAS [62]. However, in early-stage colorectal cancer (stages I-III), the prognostic significance of KRAS mutations has conflicting results. Studies indicate that KRAS mutations may not substantially affect overall survival or recurrence rates in non-metastatic colorectal cancer. In stage III colorectal cancer, however, KRAS mutations have been associated with poorer overall survival, suggesting that the prognostic impact of KRAS mutations becomes more clinically relevant as the disease advances. The prognostic value of KRAS mutation also varies by tumor location. KRAS-mutated right-sided colon cancers typically exhibit less favorable outcomes compared to left-sided tumors, underscoring the importance of incorporating tumor location when evaluating clinical outcomes [63].

2.3.3. BRAF

BRAF mutation testing—especially for the V600E mutation—is a standard component in the colorectal cancer diagnosis. It is a key factor in guiding prognosis assessment and treatment decision-making in individuals with colorectal cancer [64]. As mentioned previously, BRAF mutation testing helps distinguish sporadic MSI-H colorectal cancer from Lynch Syndrome. The BRAF V600E mutation is detected in sporadic MSI-H tumors caused by MLH1 promoter methylation, but is almost never found in Lynch syndrome [47]. BRAF V600E mutation also predicts poorer clinical outcomes, especially in metastatic colorectal cancer. BRAF V600E-mutated colorectal cancers exhibit aggressive

biological behavior, including poor response to standard chemotherapy [65]. Abnormal MAPK pathway activation due to this mutation promotes uncontrolled cell proliferation and survival without reliance on normal growth factor stimulation [64].

BRAF V600E mutations are predominantly observed in tumors arising in the proximal or right-sided colon [66]. These tumors also exhibit distinct metabolic gene expression profiles, including elevated expression of glycolytic enzyme enolase 2 (ENO2), which supports tumor progression and contributes to treatment resistance. Despite advances in combination therapies, treatment response in BRAF-mutated colorectal cancer remains suboptimal, and some responses are short-lived [67, 68]. This underscores the necessity for a comprehensive understanding of tumor mechanisms and the establishment of effective predictive biomarkers.

In conclusion, BRAF testing is essential for guiding therapeutic decisions in colorectal cancer. The presence of BRAF mutation, especially the V600E variant, predicts poor response to anti-EGFR monotherapy. Therefore, anti-EGFR agents should not be used alone in BRAF-mutant colorectal cancer. Instead, treatment regimens may include combinations such as an EGFR inhibitor with BRAF inhibitor, an EGFR inhibitor with both BRAF inhibitor and MEK inhibitor, or triplet chemotherapy (FOLFOXIRI) with bevacizumab. For metastatic colorectal cancer harboring the BRAF V600E mutation, Food and Drug Administration (FDA)-approved targeted therapy includes the combination of encorafenib and cetuximab [68]. BRAF testing directly guides the selection of appropriate targeted therapies and plays a crucial role in optimizing treatment outcomes.

3. Conclusion

Tissue biomarker testing plays a critical role in the diagnosis and prognostic assessment of colorectal cancer, as well as determining the most effective therapeutic strategies. Evaluating tissue biomarkers before initiating therapy ensures that patients receive treatment tailored to the molecular characteristics of their tumors, thereby improving treatment efficacy and overall clinical outcomes.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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