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Diagnosis

Liquid Biopsy and Colorectal Cancer

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Abstract

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The majority of patients with colorectal cancer (CRC) will ultimately develop metastasis. Identifying specific molecular characteristics in them can help optimize their management in a personalized manner. This requires a noninvasive method for frequent sampling. Liquid biopsy provides such an option that is gaining increasing importance in most tumor types. We present the current status of liquid biopsy in CRC with respect to early diagnosis in high-risk population, screening, follow-up of patients on treatment, early identification of progression, and value of serial sampling. We will also discuss the potential for liquid biopsy to help identify changes related to microbiota, specific tumor-causing bacteria, and testing for ribonucleic acid associated with exosomes.

Keywords

- molecular
- driver mutations
- monitoring
- minimal residual disease
- biomarker

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Introduction

In colorectal cancer (CRC), approximately 25% present with metastatic disease and more than half will ultimately require treatment for metastatic disease.[1] While prognosis remains poor for the majority, a significant number of such metastatic CRC patients can be long-term survivors with the use of cancer-directed systemic therapy.[2] We use tumor tissue characteristics for prognostic and predictive significance. Biomarkers are a valuable addition to the armament. We know that early identification of the high-risk subset among CRC patients is the path toward early intervention that would lead to better overall survival (OS), save treatment costs, and perhaps cure a larger portion.

Precision medicine and personalized oncology is dependent on identifying specific genetic alterations that can identify high-risk subset and enrich the population that is most likely to benefit from targeted therapy.[3–5] Unfortunately, routine tissue biopsy has several limitations. Some of the well-known ones include intratumoral heterogeneity, limited tissue sample size, challenge of accessing difficult sites of disease, patient discomfort, and the inability to repeat invasive procedures.[6]

Liquid biopsy circumvents most of these problems and has been gaining increasing importance in several cancers, including CRC. Liquid biopsy is the term used for a diagnostic sample obtained from a patient's body fluid (most commonly blood) that can be tested for specific cancer-related aberrations.[7,8] Its value and application in the routine care of CRC has gained increasing acceptance.[9–13]

Methods

Liquid biopsy for CRC can consist of three types of analysis—circulating tumor cells (CTCs), circulating tumor deoxyribonucleic acid (ctDNA), and/or circulating noncoding ribonucleic acids (RNAs) (inside or outside exosomes).[14]

Initial efforts were focused on the identification of CTCs. These could have reached the blood circulation from either the primary tumor or from a metastatic site. Isolation of CTCs was based on the principle that they are epithelial cells. Initial studies were promising. Unfortunately, we now know that CTC assays are difficult to standardize and are positive in only approximately 30% of patients with advanced CRC.[15] Their concordance with molecular changes in the primary tumor were also limited, seen in 50 to 70% of cases.[16]

Focus quickly shifted to ctDNA. These could also represent either shedding from the primary tumor or the metastatic site. But detection techniques were more robust and consistent—polymerase chain reaction (PCR) or next-generation sequencing (NGS). Concordance with tumor biopsy samples was also found to be higher.[17] For instance, Yu et al did a study in 150 CRC patients with metastatic disease. They showed a concordance rate of as much as 92% when plasma ctDNA digital PCR was compared with primary tumor tissue DNA for common BRAF and *KRAS* mutations.[18] Some studies have also shown a lower concordance rate, but that is likely to be due to the two samples having been obtained at different time intervals.[19] Evaluation of three advanced methodologies (COLD-PCR, microarray, and droplet digital PCR [ddPCR]), for identifying the mutational status by liquid biopsies in metastatic CRC patients is gaining momentum.

Recent attention has also been on circulating noncoding RNAs. One large study of 326 CRC patients showed good correlation between exosomal miR-21 from liquid biopsy specimen as compared with that from CRC tumor tissue.[20]

When the strengths and weaknesses of the above three methods are evaluated, ctDNA is a clear winner. It is well-established and widely studied methods (e.g., NGS, ddPCR, Amplification Refractory Mutation System [ARMS]), has high sensitivity and consistent correlation, can predict drug response/resistance, and helps in monitoring of CRC patients—especially for early detection of progressive disease.

Utility in CRC

Today, it is possible to predict risk of tumor metastasis using genome-wide sequencing along with bioinformatics tools like biological pathways, receptors, and protein network as well as artificial intelligence/machine learning. For patients with advanced CRC, it is the genomic instability and additional molecular alterations occurring at the metastatic site that determines patient prognosis and guides further therapy.[21–23] One study of 47 CRC patients in early or late cancer stages and found that stage IV patients had significantly higher ctDNA concentrations than stage I patients.[24] Another study of 97 metastatic CRC patients demonstrated that those with higher ctDNA burden had shorter OS. This was also true for those with BRAF mutations as compared with the BRAF wild-type cohort.[25] A third study looking at pretreatment ctDNA confirmed that patients with increasing levels while on first-line chemotherapy (CT) had poorer outcome.[26] Tie et al showed that falling ctDNA levels (more than 10-fold reduction) after first-line CT predicted longer progression-free survival (PFS).[27] The same group also showed that in surgically resected patients, if ctDNA was detectable post-surgery, there was a higher 5-year recurrence risk (38.6% vs. 85.5%) and lower OS (64.6% vs. 89.4%).[28,29] In addition, a recent study in 23 patients of CRC with deficient mismatch repair/microsatellite instability-high monitored serial ctDNA during programmed cell death protein 1 (PD-1) blockade. It was successful in predicting responses weeks earlier than standard imaging studies—thus indicating the role of ctDNA in predicting early tumor response to immunotherapy.[30] In a multicenter study involving 265 patients with nonmetastatic CRC (stage I–III), serial ctDNA testing was able to detect minimal residual disease (MRD) 8 months earlier (range, 0.56–21.6 months) than radiologic evidence of relapse.[31] The IDEA-France phase III trial, gave valuable insight into positive ctDNA results being an independent prognostic marker—in fact it helped select ctDNA positive patients for longer treatment (6 months) to provide outcome that was similar to ctDNA negative group (treated for 3 months).[32] Not surprisingly, meta-analysis has confirmed the prognostic and predictive value of pretreatment ctDNA levels in the management of CRC.[33] Measuring ctDNA has a

role in selecting patients that do not require adjuvant CT as well.[34] This large study included 455 patients with stage II colon cancer, of which 302 were managed based on ctDNA results whereas 153 received standard treatment and were the control arm. The ctDNA-guided arm received less adjuvant CT (15%) as compared with the standard therapy arm (28%; relative risk of1.82 with a 95% confidence interval [CI] of 1.25–2.65). The recurrence-free survival at 2 years was identical in the two groups (93.5% in the ctDNA-guided arm and 92.4% in the standard arm). This strategy allows a significant number of stage II colon cancers patients to avoid unnecessary adjuvant CT without increasing the risk of recurrence. Use of ctDNA for serial RAS mutational evaluation can help in deciding right rechallenge strategy for selected patients.[35] A phase 2 single-arm multicenter study involved patients who were previously treated with irinotecan and cetuximab in the first line as well as oxaliplatin and bevacizumab in the second line. The ctDNA showed RAS mutations in 12/25 evaluable patients (48%) at second relapse. When rechallenged with irinotecan and cetuximab, the group with wild-type RAS mutations on ctDNA had better PFS (median PFS 4.0 vs. 1.9 months).

ctDNA methylation has also been shown to be a good biomarker. One study involving five methylation genes (ITGA4, *EYA4*, *GRIA4*, MSC, and *MAP3K14-AS1*) predicted better response and PFS.[36] There is also value in quantifying the methylated circulating DNA. For instance, one recent study showed that the median DNA methylation levels of TMEM240 promoter hypermethylation for CRC is 0.0021 while it remained undetectable (0.0000) in healthy subjects.[37] Another study on cfDNA (cell-free DNA) looked at three tumor-specific DNA methylation markers (chromosome 9 open reading frame 50 [C9orf50], CAP-Gly domain containing linker protein family member 4 [CLIP4], and potassium voltage-gated channel subfamily Q member 5 [KCNQ5]) showed the ability to distinguish stage in CRC patients with a specificity of 99%. The sensitivity was 80% for stage I, 85% for stage II, 89% for stage III, and 88% for stage IV.[38,39] Another study involved 299 patients having stage I to III CRC. Of the 296 patients in whom preoperative samples were available, 232 (78.4%) had tested positive for at least one of the six ctDNA methylation markers [33a]. One month after surgery, ctDNA-positive patients had 17.5 times higher risk of relapse (hazard ratio [HR], 17.5; 95% CI, 8.9–34.4; $p < 0.001$). After adjuvant CT, ctDNA-positive patients had shorter recurrence-free survival (HR, 13.8; 95% CI, 5.9–32.1; $p < 0.001$). And sequential ctDNA analysis showed that ctDNA-positive patients had poorer recurrence-free survival (HR, 20.6; 95% CI, 9.5–44.9; $p < 0.001$). Discovery of methylated circulating DNA biomarkers for comprehensive noninvasive monitoring of treatment response in metastatic CRC is also of value in the follow-up of CRC patients who do not have driver mutations.

Beyond Circulating Tumor Biomarkers

This article would not be complete without a mention of use of liquid biopsy for (1) oral microbiota related bacterial network, (2) gut microbiome-associated serum metabolites, and (3) P-element Induced WImpy testis (PIWI)-interacting RNAs (piRNAs), microRNA(miRNAs), long noncoding RNAs (lncRNAs), and circular RNAs (circRNAs).[40]

Saliva and stool matched samples from patients with CRC patients, when compared with those from healthy controls, have identified four bacterial species found in abundance predominantly in CRC patients only—they are *Solobacterium moorei*, *Streptococcus anginosus*, *Streptococcus koreensis*, and *Peptostreptococcus stomatis*[41] Other organisms of interest are *Fusobacterium nucleatum* and *Streptococcus gallolyticus* subspecies gallolyticus (Sgg). Measurements of anti-*F. nucleatum* immunoglobulin A levels could serve as a robust biomarker for CRC and might find its place in screening high-risk persons in the community.[42] We also know that Sgg promotes the development of CRC. Liquid biopsy measurement of type VII secretion system (T7SS; called $SggT7SS^{T05}$) is also known to identify persons with colonization by Sgg and hence high risk for the development of CRC.[43]

Conclusion

Liquid biopsy is quickly cementing its crucial role in the management of patients with CRC. Its advantages include being of noninvasive nature, ability to do frequent sampling, help as additional tool in diagnosis, have prognostic and predictive significance as a reliable biomarker, and guide personalized therapy by monitoring patient disease status several weeks ahead of other diagnostic modalities. In India and South Asian countries, their utility shall require access to reliable laboratories that have demonstrated consistent validation of sample handling/transport, wet laboratory processing, and robust bioinformatics that allow quick turnaround time. In the future, ctDNA could also be used as a surrogate endpoint for response rate, PFS, and even OS. If this concept stands the test of time and proves to be a robust marker, patients would be the ultimate beneficiary in many ways. We end with a word of caution. If using ctDNA for MRD results in a significant false negative rate (which has been reported to be as much as 15% in some publications), it could lead to undertreatment. Similarly, patients who are false positive could receive additional therapy that was unnecessary.[44] We need to remember that currently ctDNA as a companion diagnostic test is not considered standard of care. So, if a CRC patient is identified solely by ctDNA to have RAS mutations or BRAF V600E use of targeted therapy guided by liquid biopsy should be used with caution.[45]

Conflict of Interest
None declared.

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