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A Review of Circulating Tumor DNA in the Diagnosis and Monitoring of Esophageal Cancer

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Circulating tumor DNA (ctDNA) is a type of cell-free DNA released by tumor cells after necrosis and apoptosis, and it can be actively secreted by tumor cells. Since ctDNA is derived from various tumor sites, it can provide far more comprehensive genomic and epigenomic information than a single-site biopsy. Therefore, ctDNA can overcome tumor heterogeneity, which is the major limitation of a traditional tissue biopsy approach. Noninvasive ctDNA assays allow continuous real-time monitoring of the molecular status of cancers. Recently, ctDNA assays have been widely used in clinical practice, including cancer diagnosis, evaluation of therapeutic efficacy and prognosis, and monitoring of relapse and metastasis. Although ctDNA shows a high diagnostic performance in advanced esophageal cancer, it is far from satisfactory for early diagnosis of esophageal cancer. Monitoring the dynamic changes of ctDNA is beneficial for the evaluation of therapeutic efficacy and prediction of early recurrence in esophageal cancer. It is necessary to establish standards for individualized ctDNA detection in the evaluation of treatment response and surveillance of esophageal cancer and to develop clinical practice guideline for the systemic treatment of patients with "ctDNA recurrence." This review aims to provide an update on the role of ctDNA in the diagnosis and monitoring of esophageal cancer.

Keywords: **Circulating Tumor DNA • Diagnosis • Esophageal Neoplasms • High-Throughput Nucleotide Sequencing • Prognosis**

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Background

Esophageal cancer is one of the most common gastrointestinal tumors. About 604,000 new cases of esophageal cancer and 544,000 deaths occurred worldwide in 2018, placing esophageal cancer as the 10th most frequently diagnosed cancer and the sixth leading cause of cancer-related death, respectively [1]. Esophageal cancer has 2 predominant histological subtypes: esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAD) [2]. Despite progress in diagnosis and treatment in recent years, the prognosis of esophageal cancer is far from satisfactory, with a 5-year survival rate of 20% for all stages combined [3]. This may be due to its characteristic rapid progression and no or atypical symptoms in the early stage [2]. Therefore, early diagnosis and treatment are essential for patients with esophageal cancer [4,5]. Currently, the biggest challenge in cancer diagnosis and treatment is the inability to accurately capture tumors with spatial-temporal specificity. Circulating tumor DNA (ctDNA) provides new opportunities to overcome this problem [6,7]. Since ctDNA detection has convenient, real-time, and noninvasive features, it can monitor the evolution and adaptability of cancer and therapeutic effects in real time, and thus effectively guide individualized treatment and evaluate the prognosis of patients [8]. This review aims to provide an update on the role of the detection of circulating ctDNA in the diagnosis and monitoring of esophageal cancer.

ctDNA

In 1948, Mandel and Métais [9] first discovered the presence of cell-free DNA (cfDNA) in blood. In 1965, Bendich et al [10] found that cancer was associated with circulating cfDNA. In 1977, Leon et al [11] reported for the first time that the serum levels of cfDNA in cancer patients were higher than those in healthy individuals. In 1989, Stroun et al [12] discovered that part of the cfDNA in the plasma of cancer patients originated from cancer cells (termed ctDNA). Subsequent studies have demonstrated that cancer cells release ctDNA fragments not only into circulation, but also into other biofluids such as urine, saliva, and cerebrospinal fluid, while ctDNA carries the unique genomic and epigenomic signatures that are characteristic of the cancer from which they originate [13]. Furthermore, cfDNA is derived from cells that undergo apoptosis, necrosis, or metabolic secretion, and it usually binds with proteins in circulation [14-16]. cfDNA is highly fragmented and its most common fragment lengths (134-145 bp) in cancer patients are shorter than those in healthy individuals (165-167 bp) [17]. Variations in the amount of ctDNA over time in the same patient depend on factors such as tumor stage, anatomical site, and response to therapy [18-22]. cfDNA is rapidly degraded by circulating enzymes such as deoxyribonuclease and factor H, and it is rapidly eliminated by the liver, spleen, and kidneys [23-26]. The half-life of circulating cfDNA depends on various factors, including physical exercise, physiological and pathological conditions (such as pregnancy and cancer), extracellular vesicles, and the status of binding of cfDNA with proteins, and it is estimated to range from several minutes to 2 h, indicating that cfDNA allows quantitative assessment of disease burden, especially for monitoring cancer [16,21,26,27]. It should be noted, however, that there is a remarkable degree of tumor-to-metastasis heterogeneity. Previous studies have demonstrated that intra-tumoral heterogeneity of a primary tumor is higher than that of metastatic sites in some types of cancer, including esophageal cancer [28,29]. Since ctDNA is derived from various tumor sites, it could provide far more comprehensive genomic and epigenomic information than a single-site biopsy, which might overcome spatial and temporal heterogeneity [30].

Approaches for ctDNA Detection

In theory, most of the genetic testing methods can be used to detect ctDNA. However, the biggest obstacle in ctDNA detection is the low concentration of ctDNA in the blood. Highly sensitive and specific techniques are required to identify it against the predominant background of normal DNA for cancer detection and to monitor the response to treatment and early relapse because ctDNA usually composes only a very small fraction (0.01-0.1%) of the total circulating cfDNA and its absolute level changes with tumor burden and response to treatment [31-34]. Previous studies have reported many methods, such as droplet digital polymerase chain reaction (PCR), real-time fluorescence quantitative PCR (qPCR), and high-throughput nucleotide sequencing (next-generation sequencing, NGS), to determine the existence of ctDNA by qualitative and quantitative detection of genetic or epigenetic alterations in circulating cfDNA, each of which has its own advantages and disadvantages [35]. To date, NGS is the most commonly used methods for ctDNA detection [36]. For early diagnosis of cancer, the more sensitive the ctDNA assay, the better it can detect the presence of cancer. Although ctDNA assays can detect mutant-allele frequency (MAF) lower than 0.001% [37,38], some studies have demonstrated that false-positive and false-negative rates are increased for MAF <1% [39-41]. Ultradeep NGS assays can reduce the false-negative rate, whereas the use of white blood cell DNA as a control can reduce the false-positive rate for ultrasensitive NGS assays [42-44]. For example, Spoor et al [45] reported false-positive tumor protein p53 (TP53) variants in ctDNA due to clonal hematopoiesis (CH) in an esophageal cancer patient during long-term follow-up, which was found 30 months after the first NGS detection. TP53 is not only the most frequently mutated gene in esophageal cancer but also the most frequent gene somatically mutated in CH [46]. In addition, patients with low MAF of sensitizing mutations and

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high MAF of resistant mutations had a poorer response than those with high MAF of sensitizing mutations and low MAF of resistant mutations, respectively, when treated with corresponding targeted drugs [47,48]. Given the cost of NGS, the coverage depth of most commercial ctDNA assays is approximately 10 000× in clinical practice in China.

ctDNA Assay Provides Opportunity for Esophageal Cancer

In 2013, Dawson et al [49] reported that noninvasive ctDNA detection can reflect the frequency and pattern of gene mutations in solid tumor tissues, which is an important indicator for evaluation of treatment efficacy and monitoring of prognosis. Therefore, ctDNA detection allows for repeated analyses of evolving tumor molecular profiles at different time points and provides additional information in almost all aspects of cancer diagnosis and treatment, and it thus may help overcome the challenges of intratumoral heterogeneity [50]. After the European Medicines Agency and the US Food and Drug Administration (FDA) approved the plasma epidermal growth factor receptor mutation test, the ctDNA test has been approved for clinical application in lung cancer testing in several countries [51-53]. Given the growing number of variants that need to be analyzed, NGS panels are a very appealing option for ctDNA detection. The FDA has approved several NGS-based multigene diagnostic assays, including Foundation-One CDx (Foundation Medicine, Cambridge, MA, USA), OncomineDx Target Test (ThermoFisher Scientific, Waltham, MA, USA), and MSK-IMPACT (Memorial Sloan Kettering Cancer Center, NY, USA), to identify cancer patients with certain molecular subtypes who might benefit from targeted therapy [54]. Similar assays are expected to be approved for various types of cancer in the near future [55].

The concordance of somatic variants in esophageal cancer tissues and ctDNA differ greatly from the results of different studies [56-60]. Although a study by Maron et al [59] revealed that only 26% (48/183) of variants identified in 34 untreated patients with newly diagnosed stage IV gastroesophageal adenocarcinoma were universally concordant within plasma and primary and metastatic tumor sites, many studies demonstrated superior sensitivity and accuracy of ctDNA detection in monitoring the prognosis of esophageal cancer [46,59,61,62]. Despite the elevated cost of assaying, a combination of tissue and ctDNA NGS testing is helpful to overcome the inherent false-negative rates of either test and increases the sensitivity of somatic variants [59]. ctDNA is a potential biomarker for esophageal cancer, and studies conducted to date have shown promising results (summarized in **Table 1**). To date, ctDNA can be used to diagnose, monitor disease progression, and make treatment decisions for esophageal cancer (**Figure 1**).

Early Screening and Diagnosis

Early-stage esophageal cancer is usually asymptomatic or may present with mild nonspecific symptoms, such as dysphagia and unintentional weight loss. As a consequence, esophageal cancer is usually diagnosed at an advanced stage when the opportunity for optimal treatment has been missed, which seriously affects the quality of life and survival rate of patients [63]. Early intervention can significantly improve the quality of life of cancer patients. Many studies have demonstrated the potential value of noninvasive techniques for the early diagnosis of cancer. ctDNA has great promise as an early detection biomarker that has not yet been accepted as a screening method, especially for cancers such as ovarian, pancreatic, esophageal, and gastric cancers. Some inherent characteristics of ctDNA may strengthen its use as a biomarker for early cancer detection and diagnosis, such as degree of integrity and tumor-specific alterations (point mutations, copy number variations, rearrangements, microsatellite instability, loss of heterozygosity, and DNA methylation) [6,56,64-67].

The occurrence and development of cancers can lead to changes in the amount of ctDNA in the peripheral blood [22,68,69]. There is often insufficient ctDNA in peripheral blood to achieve a sufficiently accurate result for early cancer diagnosis [70]. Previous studies have revealed that the site of esophageal cancer and burden significantly affect ctDNA shedding and consequential ctDNA detection sensitivity [59]. For example, esopageal cancer patients with liver metastases seem to have the highest ctDNA fraction [59,64]. Since the amount of ctDNA in the early stage of esophageal cancer is significantly lower than that in late-stage disease, the sensitivity of ctDNA assays is relatively low in early-stage diseases [59,62,66,71]. Iwaya et al [71] reported that the ctDNA-positive rate in stage I esophageal cancer was 14.3% (1/7), whereas, it was 85.2% (23/27) in stage II or higher. A study by Azad et al [72] revealed that the median proportion of ctDNA in localized esophageal cancer was 0.07%, suggesting that ultrasensitive ctDNA assays are needed for early esophageal cancer detection. Several studies have demonstrated that loss of heterozygosity is observed in the cfDNA of patients with Barrett esophagus and its frequency drops after endoscopic treatment [73,74], suggesting a window of opportunity for early detection of esophageal cancer. Meta-analysis showed that the diagnostic sensitivity and specificity of ctDNA were 71.0% and 98.6%, respectively, for esophageal cancer [75]. Results from previous studies have shown promise in detecting esophageal cancer.

Epigenetic alterations, such as DNA methylation and histone modifications, are one of the early events in carcinogenesis. In particular, alterations in DNA methylation status that frequently occur in the promoter regions of cancer-related genes are one of the most common early molecular events in cancer

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Table 1. Summary of studies involving circulating tumor DNA (ctDNA) and esophageal cancer.

Studies	Methods	No. of patients	No. of sample	Sample types	Purpose
[72]	NGS	45	213	Tissues and plasma	Detection of minimal residual disease
[18]	NGS	21	21	Plasma	Diagnosis
[64]	NGS	30	30	Tissues and plasma	Predicting treatment outcomes
[106]	NGS	56	56	Tissues and plasma	Determining the feasibility of ctDNA assay in advanced gastrointestinal and anal cancers
[107]	NGS	8	8	Plasma	Determining the feasibility of ctDNA assay in advanced gastrointestinal cancers
[88]	qPCR	41	75	Plasma	Diagnosis and surveillance
[61]	NGS	35	116	Tissues and plasma	Surveillance
[62]	NGS, ddPCR	42	121	Tissues and plasma	Predicting response to chemotherapy
[108]	DNA quantification	57	143	Plasma	Monitoring of treatment responses and relapse in advanced ESCC
[66]	NGS	85	449	Tissues and plasma	Diagnosis
[85]	NGS	20	>60	Tissues and plasma	Surveillance
[58]	NGS	5	52	Tissues and plasma	Analyzing the concordance of somatic variants in ESCC tissues and ctDNA
[56]	NGS, Array-CGH	44	88	Tissues and plasma	Analyzing the concordance of copy number alterations in gastroesophageal cancer tissues and ctDNA
[109]	NGS	71	6689	Plasma	Diagnosis
[67]	NGS	100	4077	Plasma	Diagnosis
[34]	NGS	3	20	Tissues and plasma	Predicting response to neoadjuvant therapy
[71]	NGS, ddPCR	35	604	Tissues and plasma	Monitoring clinical outcome during the treatment course of ESCC patients
[46]	NGS	97	245	Plasma	Surveillance
[86]	NGS	25	69	Plasma	Monitoring the prognosis of ESCC patients receiving radiotherapy
[82]	NGS	150	227	Plasma	Diagnosis
[57]	NGS	42	81	Tissues and plasma	Analyzing the concordance of TP53 mutations in ESCC tissues and ctDNA
[110]	NGS, ddPCR	27	>52	Tissues and plasma	Determining the feasibility of ctDNA assay in esophageal cancer
[73]	LOH analysis	40	40	Plasma	Monitoring the neoplastic progression of Barrett's esophagus
[65]	NGS	61	139	Plasma	Predicting treatment outcomes
[83]	NGS	11	55	Tissues and plasma	Determining the feasibility of ctDNA assay in esophageal cancer
[60]	NGS	13	46	Tissues and plasma	Predicting recurrence
[59]	NGS	1630	2140	Tissues and plasma	Evaluate the role of ctDNA in guiding clinical decision-making in gastroesophageal adenocarcinoma
[45]	NGS, ddPCR	1	14	Tissues and plasma	False-positive plasma genotyping
[87]	qPCR	63	115	Tissues and plasma	Predicting recurrence

cfDNA – cell-free DNA; ctDNA – circulating tumor DNA; ddPCR – droplet digital polymerase chain reaction; ESCC – esophageal squamous cell carcinoma; LOH – loss of heterozygosity; qPCR – real-time fluorescence quantitative PCR; TP53 – tumor protein p53.

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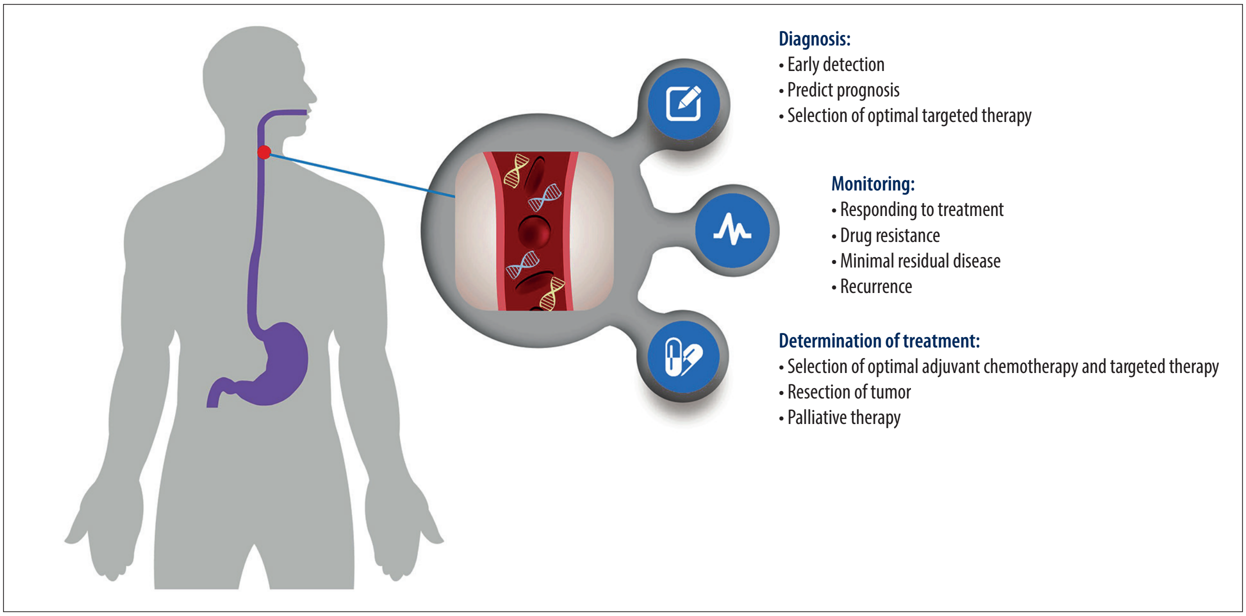

Figure 1. Clinical utility of circulating tumor DNA (ctDNA) for esophageal cancer.

and thus have potential utility as biomarkers for early cancer detection [76]. DNA methylation signatures can predict the tissue of origin and cancer subtypes [77-79]. It is speculated that ctDNA methylation signatures may be more sensitive and specific than somatic mutation signatures in patients with early-stage cancer [80]. One recent study by Klein et al [81] focused on the development of a noninvasive cfDNA-based multicancer detection assay using whole genome bisulfite sequencing (30×) for methylation and reported a sensitivity of 63% for esophageal cancer (n=19). Another study by Tian et al [82] using the nano-hmC-Seal method reported a sensitivity of 93.75% and specificity of 85.71% for esophageal cancer (n=150). Qiao et al [66] identified 921 differentially methylated regions that showed promising potential as diagnostic biomarkers of esophageal cancer (n=168), whereas the sensitivity was only 58.8% for stage 0-II. The Circulating Cell-Free Genome Atlas study (CCGA) also showed similar results with a sensitivity of 85.0% for all esophageal cancer (n=100), 12.1% for stage I (n=8), and 64.7% for stage II (n=17) [67]. Further studies are required to validate these findings. Applications of artificial intelligence and machine learning can help to enhance the ability to decode DNA methylation patterns in esophageal cancer and improve diagnostic tests for clinical applications.

Monitoring Treatment Response and Recurrence

ctDNA has a short half-life, and the fast turnaround time allows for a dynamic assessment of tumor status within an interval of only a few hours [16,26]. Rapid clearance of ctDNA enables more accurate monitoring of the tumor burden dynamics, which is helpful in detecting minimal residual disease after treatment and evaluating the prognosis of cancer patients. The dynamic change of ctDNA is consistent with tumor burden and closely correlates with resistance to therapy, disease progression, and relapse [34,46,71,83,84]. Esophageal cancer patients with detectable ctDNA at any posttreatment time point usually have a worse prognosis than those without detectable ctDNA after completion of therapy, whereas, relative ctDNA concentration (variant allele fraction) decreases or becomes undetectable when patients have a good response to surgery, chemoradiotherapy, and targeted therapy [46,59,60,62,71,72,85,86]. Boniface et al [34] found that ctDNA levels are closely correlated with the response to neoadjuvant therapy in EAD patients. Luo et al [83] reported that after surgery, mutations in the plasma of ESCC patients disappeared or their variant allele fractions decreased, revealing the feasibility of ctDNA assays for monitoring treatment effects. Two recent studies by Ococks et al [46,85] revealed that postoperative ctDNA-postive EAD patients had a high risk of recurrence and death. A study by Komatsu et al [87] showed similar results, with the frequency of cyclin D1 (CCND1) amplification in ctDNA also decreasing after surgery in patients with superficial ESCC. Andolfo et al [88] found that copy number of erbb2 receptor tyrosine kinase 2 (ERBB2) in ctDNA of esophageal cancer patients was higher than those of healthy individuals, which was correlated with an adverse prognosis. A recent study by Fujisawa et al [62] reported that the ctDNA dynamics before and after an initial cycle of chemotherapy can predict responses at the end of chemotherapy with high accuracy. The response rate of first-line anti-HER2 therapies in gastroesophageal junction cancer is lower than 50% [89]. Maron et al [59] reported that ctDNA NGS assay enhanced the predictive utility

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of standard single-lesion tissue-based HER2 testing. Kim et al [65] found that the fragment ratio score of ctDNA was associated with treatment response and survival time and therefore may be a relatively simple and inexpensive biomarker to predict treatment response after chemoradiotherapy. In addition, Ling et al [90] reported that 76% of patients with aberrant mutS homolog 2 (MSH2) methylation in ESCC tissues displayed the same ctDNA alteration, which was not observed in healthy individuals. Patients with high MSH2 methylation had a poorer prognosis compared with those with MSH2 unmethylation after surgery. Therefore, monitoring the dynamic changes of ctDNA will be beneficial for the evaluation of treatment effects and prognosis prediction in esophageal cancer.

The ability to detect minimal residual disease (MRD) allows early recognition of cancer relapse after treatment, which could facilitate early intervention and thereby improve therapeutic outcomes. MRD is a major source of ctDNA after surgery and treatment. ctDNA drops to undetectable levels when cancer treatment is effective and successful. The presence of ctDNA can identify patients who may be at a high risk of relapse, and patients with persistently detectable ctDNA after treatment have a high risk of relapse. Some studies have demonstrated that ctDNA assays can precede clinical and imaging evidence of cancer relapse by at least several months [71,72,85,91-93]. Ococks et al [85] used serial personalized tumor-informed ctDNA assay to detecting MRD in 20 patients with resected EAD. Five patients that replased had ctDNA-positive assays at baseline. ctDNA assay preceded radiologic and clinical evidence of recurrence with a median lead time of almost 1 year. A study by Azad et al [72] showed that detectable ctDNA levels after chemoradiotherapy in patients with localized esophageal cancer were correlated with relapse and poor prognosis and ctDNA assay preceded imaging evidence of tumor progression by an average of 2.8 months. Iwaya et al [71] reported that the continuous decline in ctDNA levels after chemotherapy followed by the maintenance of a ctDNA-negative state indicated extended survival time in ESCC patients. Routine ctDNA monitoring can predict clinical recurrence with a median lead time of 5 months compared with radiological evidence. Komatsu et al [87] found that ESCC patients with CCND1 amplification had shorter relapse-free survival than those without CCND1 amplification, which may be an independent risk factor for relapse. In addition, it is possible to determine the site for cancer metastasis by detecting the tissue-specific methylation patterns of cfDNA in the future [67]. Taken together, as a potential biomarker for monitoring tumor relapse and metastasis, ctDNA can effectively improve the long-term survival of esophageal cancer patients. Prospective clinical trials are necessary to establish the clinical utility of ctDNA assays for assessing MRD.

Current Status of ctDNA Testing in Diagnosis and Monitoring Esophageal Cancer

Currently, cancer diagnosis still depends mainly on imaging examination and histopathological biopsy. Endoscopy examination is the most important diagnostic procedure for esophageal cancer. However, it is unlikely to be suitable for population-wide esophageal cancer screening due to the invasive, inconvenient, and time-consuming process [94]. Recently, many efforts have been made to explore the feasibility of ctDNA assay in the diagnosis and monitoring of esophageal cancer. Although somatic variant-based ctDNA assays exhibit a better performance in monitoring esophageal cancer than routine clinical examination [72,85], there is still a lack of systematic and large-scale study of somatic variant-based ctDNA assays in the early diagnosis of esophageal cancer to date. A meta-analysis by Chidambaram and Markar [75] revealed a higher diagnostic performance of ctDNA in esophageal cancer, whereas there was no independent result of ctDNA in early-stage esophageal cancer. The sensitivity of ctDNA assay in the early diagnosis of esophageal cancer is speculated to be possibly lower because of low ctDNA shedding [59,62]. Ultradeep NGS assay can partly solve the problem, whereas it is a major challenge for how to interpret the clinical impact of these detected somatic variants. For example, Nasrollahzadeh et al [57] found 5 controls with TP53 mutations in ctDNA among 39 controls, one of whom was subsequently found to have ESCC 6 months after enrollment. The other 4 controls had no malignancy tumor during 15 years of follow-up. Kuderer et al [39] found that cancer-like TP53 somatic variants were observed in 11% (n=225) of individuals without cancer after excluding CH variants. Furthermore, the high cost of ultradeep NGS assay will limit its broad application as screening tool in high-risk populations. Methylation-based ctDNA assay also has lower sensitivity for early-stage esophageal cancer [66,67]. Further large-scale prospective studies are warranted to explore the feasibility of ctDNA assay in the early diagnosis of esophageal cancer.

Although a few targeted and immune therapies are currently available for esophageal cancer patients, some patients with advanced cancer can benefit from targeted drugs for other types of cancer and from experimental targeted drugs, such as afatinib (EGFR amplification), crizotinib (MET amplification), and AMG 337 (MET amplification) [95-98]. Except for ERBB2 (anti-Her2 drugs for esophageal cancer: trastuzumab, trastuzumab deruxtecan) and cyclin-dependent kinase inhibitor 2A (CDKN2A) (experimental drugs: palbociclib and ribociclib), the mutation frequencies of 66 anticancer drug-related genes are low in 1543 esophageal cancers in cBioPortal (**Figure 2**) [99-101]. On the other hand, bespoke individualized panels can increase the sensitivity of ctDNA assays in monitoring therapeutic efficacy and recurrence [44]. Several studies have also revealed that

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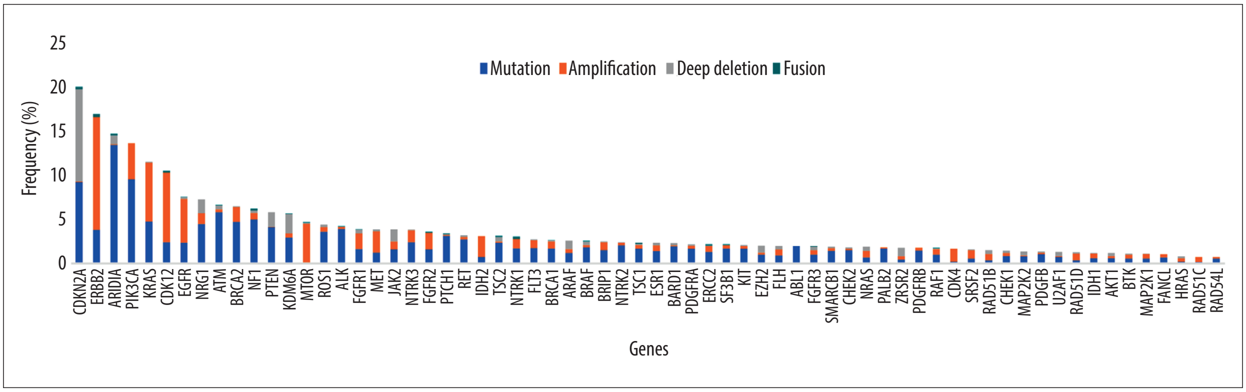

Figure 2. The frequencies of somatic variants of 66 anticancer drug-related genes in 1543 esophageal cancers in cBioPortal.

individualized ctDNA assays exhibit a better performance in monitoring therapeutic efficacy and recurrence in esophageal cancer [46,59,61,71,85]. Therefore, individualized ctDNA analysis using tumor sequencing after treatment is conducive to early detection of recurrence, even in the absence of clinical and radiological evidence, which enables patients with a low burden of metastatic disease to receive treatment and have a better prognosis compared with those with radiologically detectable disease [46,59,61,71,85,102]. It seems more appropriate to use a large NGS panel to select patients who are likely to benefit from anti-HER2 therapies, immune checkpoint inhibitors, TRK inhibitors, or other drugs. This may be helpful in developing a more sensitive individualized ctDNA assay for subsequent monitoring. There is a need for well-designed clinical trials to standardize individualized ctDNA detection methodology and optimal time points for ctDNA detection and to develop clinical practice guidelines for the systemic treatment of patients with "ctDNA recurrence."

Challenges for ctDNA Assay

As an important class of liquid biopsy, ctDNA assays play an increasingly important role in different stages and aspects of the diagnosis and treatment of esophageal cancer. It is an opportunity as well as a challenge, but there are still many problems to be overcome. First, although many technologies are used in ctDNA assays, there is still a lack of industry standards. Any clinical assay should provide high specificity, sensitivity, and stability. It is crucial to establish integrated, easy-to-use, robust, and reproducible workflows covering the requirements for the clinical setting. Ultrasensitive ctDNA assays have been developed recently, but it will take time to apply ctDNA for early cancer screening, especially in a broad population requiring precise specificity. Somatic mutations gradually accumulate with aging, even in healthy individuals [103]. The vast majority of somatic mutations in cfDNA may be a result of CH, which may lead to false-positive results of ctDNA [43,45,104]. Previous studies have demonstrated that the prevalence of CH variants increases with age [43,46,104]. The ctDNA detection rate is decreased after excluding CH variants. Given that the majority of CH variants are individual specific, a combination of cfDNA and matched white blood cell sequencing should be performed to accurately interpret the ctDNA assay results. Second, although highly standardized analysis pipelines for basic NGS data processing and downstream analysis have been established, reliable data analysis and interpretation of results are still challenging [105]. There is a strong need to develop easy-to-use bioinformatics tools to generate comparable results that integrate the latest progress in basic and clinical research as well as guidelines. Third, the cost of ultrasensitive ctDNA assays is relatively expensive at present, which may hinder its widespread clinical application. To mitigate the financial burden of cancer patients, it is necessary to continuously improve the technology for detecting ctDNA and reduce its costs. Finally, the extensive application of ctDNA assays in clinical practice is an inevitable trend, whereas large-scale clinical trials still need to be conducted, especially for prospective research.

Conclusions

Although there are relatively few studies on ctDNA in esophageal cancer compared with other types of cancer such as lung cancer, the existing results have fully demonstrated the great potential of ctDNA in monitoring treatment response and recurrence in esophageal cancer [46,59,60,62,71,72,85,86]. The future roles of ctDNA include the practical integration of this method into the diagnostic and surveillance pathway for esophageal cancer patients.

Declaration of Figures' Authenticity

All figures submitted have been created by the authors who confirm that the images are original with no duplication and have not been previously published in whole or in part.

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