Review Article

Review of Plasma Exosomal DNA for Detecting EGFR Mutations in Non-Small Cell Lung Cancer (NSCLC)

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Abstract

This review systematically evaluated the literature on detecting epidermal growth factor receptor (EGFR) mutations in non-small cell lung cancer (NSCLC) using plasma exosomal DNA by analyzing data from eight studies selected from a comprehensive literature search (PubMed, Embase, Web of Science; 2010–2024). The findings revealed a wide range of EGFR mutation prevalence (10%–26.8%) across studies, with most mutations located in exons 19 and 21. Comparative analysis highlighted the potential of plasma exosomal DNA (exDNA) as a non-invasive alternative to tissue biopsy, although significant heterogeneity in sensitivity and specificity was observed across liquid biopsy methods (including circulating tumor cells and exDNA analyses). This heterogeneity underscores the need for standardization and further validation to optimize the clinical utility of plasma exDNA in detecting EGFR mutations, monitoring treatment response, and identifying resistance mechanisms in NSCLC.

Keywords: EGFR mutations, exosomal DNA, non-small cell lung cancer (NSCLC)

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Introduction

Lung cancer is one of the deadliest cancers and causes 1.8 million deaths worldwide. Lung cancer is divided into several subtypes based on the type of cells. Non-small cell lung cancer (NSCLC) accounts for approximately 85% of lung tumor cells.[1-3] NSCLC is the most common type of lung cancer. However, most patients with NSCLC are diagnosed at an advanced stage and thus lose the chance to be eligible for surgery.[4,5]

The current diagnostic techniques for EGFR mutations have limitations, especially at early stages of the disease, where circulating cell-free DNA (cfDNA) might be insufficient. Exosomes, carrying cancer-specific genetic signals, is a potential biomarker for early detection. Nonetheless, there are advantages and disadvantages to using exosome detection methods for clinical use.[6]

Evidence shows that the etiology of NSCLC can be multifactorial; however, it is known that the expression of epidermal growth factor receptor (EGFR) is increased in NSCLC cells.[6,7] Numerous genetic alterations, including gene amplification and activating mutations in various genes, have been studied in NSCLC. These alterations are valuable for diagnosis, treatment selection, monitoring treatment response, and prognosis prediction. Important known mutations in NSCLC mostly happen in the EGFR gene. EGFR is a membrane protein expressed in lung cells.

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The most important part of EGFR is a tyrosine kinase domain, where tyrosine amino acids are phosphorylated after ligand binding to this receptor. These phosphotyrosines recruit other proteins, ultimately leading to the stimulation of intracellular signaling pathways and cell proliferation. Somatic mutations in this domain of the gene mainly occur in exons 18 to 21, leading to continuous receptor activation. This causes uncontrolled cell division and proliferation, angiogenesis, adhesion, invasion, tumor progression, and tumor cell migration (metastasis).[8,9] By examining samples obtained from tumor biopsies, approximately 100 mutations in the EGFR gene have been identified. Nevertheless, tumor biopsy has several limitations, including its invasiveness and the risk of false-negative results due to tumor heterogeneity, the small number of tumor cells obtained, the possibility of new genetic changes occurring between biopsy and the start of chemotherapy, the risk of infection in patients, severe chest pain, and the substantial financial burden on the patient and the healthcare system.[10]

Recent studies have shown that EGFR mutations, due to their increased prevalence, can be one of the main factors in the pathogenesis of NSCLC.[11,12]

Based on recent studies, the occurrence of EGFR mutations can be one of the main factors in the pathogenesis of NSCLC owing to its increased prevalence. For this reason, it has been determined that the (c.2369C>T) p.T790M mutation and many other mutations can occur in the *EGFR* coding gene.[13,14] Evidence shows that the use of different methods can have different sensitivities and characteristics for mutation detection. Besides sensitivity and specificity, availability and no need for complex equipment can be among the key options for selecting the methods to detect mutations.[15]

EGFR is a receptor tyrosine kinase that plays a pivotal role in cellular signaling pathways that regulate proliferation, survival, and differentiation. Mutations within the *EGFR* gene, particularly in exons 18 through 21, are often associated with a subset of NSCLC characterized by enhanced sensitivity to EGFR-targeted therapies. These mutations facilitate continuous activation of the receptor, promoting tumor growth and resistance to apoptosis. The most common mutations include deletions in exon 19 and the L858R point mutation in exon 21, which collectively account for a substantial proportion of patients with EGFR-driven NSCLC.[16]

Plasma exosomal DNA (exDNA) holds significant promise as a minimally invasive biomarker for cancer detection and monitoring. Its accessibility and relative stability compared to cfDNA make it an attractive target for liquid biopsy approaches.[17] NSCLC, a leading cause of cancer-related mortality worldwide, frequently harbors EGFR mutations that significantly impact treatment selection and prognosis. Traditional methods for detecting these mutations, such as tissue biopsies, are invasive and may not accurately reflect the tumor's heterogeneity. Recent breakthroughs with the advent of next-generation sequencing (NGS) applications have markedly improved the sensitivity and specificity of the detection of oncogenic drivers in the complex landscape of patients with NSCLC.[18] The understanding of molecular and genomic features has changed the way of diagnosing and treating this heterogeneous disease, allowing for the selection of more effective and personalized treatments in several subsets of patients with EGFR-mutated NSCLC.[19]

This review will examine the current literature on the use of plasma exDNA for the detection of EGFR mutations in NSCLC, analyzing its sensitivity, specificity, and clinical utility compared to existing methods. We will explore the challenges and limitations associated with this approach, including standardization of extraction and detection methods, and discuss future directions in this rapidly evolving field.

Methods

This review systematically evaluated the literature on the detection of EGFR mutations in NSCLC using plasma exDNA. A comprehensive search of PubMed, Embase, and Web of Science databases was conducted using a combination of keywords, including "exosomes," "exosomal DNA," "EGFR," "mutations," "non-small cell lung cancer," "sensitivity and specificity," and "detection". The search was limited to English-language publications and studies published between 2010 and 2024. The inclusion criteria were studies that: (1) used plasma exDNA for EGFR mutation detection in patients with NSCLC; (2) reported sensitivity and specificity data; and (3) provided sufficient detail on methods for exosome isolation, DNA extraction, and mutation detection. Exclusion criteria included studies that: (1) focused solely on circulating cfDNA, circulating tumor DNA (ctDNA), RNAs, and liquid biopsy; (2) did not report quantitative data on EGFR mutation detection; (3) involved animal models or *in vitro* studies only; and (4) were reviews or editorials; and (5) focused on therapeutic features of this method. Two reviewers independently screened titles and abstracts, and full texts of potentially eligible studies were reviewed for inclusion. Data extraction included study characteristics (sample size, patient demographics, exosome isolation methods, mutation detection techniques), analytical performance metrics (sensitivity, specificity, positive predictive value, negative predictive value), and clinical outcomes (if reported). The quality of included studies was assessed using a standardized checklist focusing on methodological rigor, reporting bias, and potential confounding factors. Data synthesis was primarily narrative owing to the heterogeneity of study designs and reported outcomes.

Refining keyword combinations for literature review

Initial searches were performed using broad terms related to my topic. This preliminary step allowed us to assess the scope of available literature and identify common terminologies used in the field. Then, the key concepts were extracted, and a list of keywords, including synonyms and variations, were generated.

To enhance the specificity of searches, the controlled vocabularies were utilized from established databases, particularly PubMed. By employing Medical Subject Headings (MeSH) terms, it was possible to standardize the terminology relevant to the study, ensuring a more precise and effective literature search.

Literature search strategy

("exosomal"[All Fields] OR "exosomes"[MeSH Terms] OR "exosomes"[All Fields] OR "exosome"[All Fields] OR "exosomic"[All Fields]) AND ("DNA"[MeSH Terms] OR "DNA"[All Fields]) AND ("sensitivity and specificity"[MeSH Terms] OR ("sensitivity"[All Fields] AND "specificity"[All Fields]) OR "sensitivity and specificity"[All Fields] OR "sensitivity specificity"[All Fields]) AND "receptors"[All Fields]) OR "EGFR"[All Fields]) AND ("mutate"[All Fields] OR "mutated"[All Fields] OR "mutates"[AllFields]OR"mutating"[AllFields]OR"mutation"[MeSH Terms] OR "mutation"[All Fields] OR "mutations"[All Fields] OR "mutations"[All Fields] OR "mutational"[All Fields] OR "mutator"[All Fields] OR "mutators"[All Fields]) AND ("detect"[All Fields] OR "detectabilities"[All Fields] OR "detectability"[All Fields] OR "detectable"[All Fields] OR "detectables"[AllFields]OR"detectably"[AllFields]OR"detected"[All Fields] OR "detectible"[All Fields] OR "detecting"[All Fields] OR "detection"[All Fields] OR "detections"[All Fields] OR "detects"[All Fields]) AND ("carcinoma, non-small cell lung"[MeSH Terms] OR ("carcinoma"[All Fields] AND "non-small cell"[All Fields] AND "lung"[All Fields]) OR "non-small-cell lung carcinoma"[All Fields] OR "NSLC"[All Fields] OR "nsclc s"[All Fields] OR "nsclcs"[All Fields].

Data extraction

Data were extracted from each selected study, focusing on:

- Author(s) and year of publication
- Study design and sample size
- Population characteristics
- Methodological details, including exosome isolation techniques (e.g., ultracentrifugation, commercial kits)
- DNA extraction methods
- Detection techniques for EGFR mutations (e.g., PCR-based methods, next-generation sequencing, digital droplet PCR)
- Reports of sensitivity, specificity, and overall accuracy.

Quality assessment

The quality of the included studies was assessed using established criteria such as the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool, which evaluates the risk of bias and applicability for diagnostic accuracy studies. Two independent reviewers performed the quality assessment, with discrepancies resolved through consensus.

Data synthesis

A narrative synthesis was employed to summarize the findings across studies. Highlights of the comparative performance of plasma exDNA against conventional tissue biopsy methods for detecting EGFR mutations were emphasized. Additionally, the review discussed the potential of plasma exDNA as a non-invasive biomarker in clinical practice, particularly in monitoring treatment response and identifying resistance mechanisms in NSCLC.

Totally, 176 citations were retrieved; after removing the duplicates and evaluation of the full text of related ones, eight articles were included [Figure 1].

Results

This review analyzed eight studies investigating the use of plasma exDNA and related methodologies for detecting EGFR mutations in NSCLC. The studies employed diverse methodologies, including reverse strip assay (RSA), real-time PCR (RT-PCR), nested PCR, and various NGS approaches, with sample types ranging from pleural effusions to plasma-derived exosomes and cfDNA for comparison. While one study using cfDNA[20] showed comparable sensitivity to traditional biopsy in detecting EGFR mutations (26.8% mutation prevalence, with common mutations in exons 19 and 21), the majority of studies focused on exosomes or CTCs. A meta-analysis of circulating tumor cells (CTCs)[21] demonstrated high pooled sensitivity (0.82, 95% CI: 0.50–0.95) and specificity (0.95, 95% CI: 0.24–1.00) for detecting EGFR mutations, although significant heterogeneity was observed across subgroups based on factors such as blood volume and testing methodology. Studies directly evaluating exDNA[22,23] demonstrated the feasibility of detecting EGFR mutations using this approach, highlighting its potential for minimally invasive diagnostics. Nonetheless, these studies often lacked large sample sizes and detailed comparisons to established methods. Review articles[24,25] emphasized the advantages of monitoring EGFR mutations using exosomes over traditional methods for assessing treatment response and revealing tumor heterogeneity. Overall, although evidence suggests the potential of plasma exDNA for EGFR mutation detection in NSCLC, further research is needed to standardize methodologies, validate findings in larger prospective studies, and compare exDNA approaches directly with established methods. In particular, studies are needed that establish robust sensitivity and specificity metrics compared to current gold standards. Beaufaller's study analyzed the prevalence of rare EGFR mutations, specifically in exon 18 and exon 20, across 10,117 patients with NSCLC as part of the French ERMETIC-IFCT network. Among 1,047 identified EGFR-mutated samples, exon 18 mutations were notably low in frequency, observed in less than 4% of lung cancer patients with EGFR mutations; the study highlights the heterogeneous nature of these rare mutations and their differing sensitivity to EGFR–TKIs.[26] Table 1 shows the comparison of sensitivity and specificity metrics of exDNA versus cfDNA and CTCs. The choice between exosomal DNA, cfDNA, and CTCs should be guided by the clinical context, stage of cancer, and specific information needed by the healthcare provider. exDNA appears to combine strong

sensitivity and specificity, making it a promising biomarker for cancer diagnosis and monitoring. CTCs are highly sensitive but may present challenges in isolation and detection, while cfDNA remains a valuable marker, especially for systemic evaluations. Table 2 provides a concise overview of the key studies related to exDNA and EGFR mutation detection in NSCLC, highlighting methodologies, findings, and clinical implications. Each study contributes to the understanding of how non-invasive methods can enhance mutation screening and monitoring in lung cancer, ultimately aiming to improve patient management and outcomes.

Study overview

Safa *et al*. (2024) conducted a comprehensive investigation measuring the prevalence of EGFR mutations in a cohort of 306 patients with NSCLC and found that 26.8% exhibited detectable mutations. The study utilized both RSA and RT-PCR on biopsy samples and cfDNA. Notably, exons 19 and 21 were identified as the most common mutation sites, with a significant 70.2% of samples showing no detectable mutations.[20]

Hu *et al*. (2018) performed a meta-analysis focusing on CTCs and their ability to reflect EGFR mutation status. This study underscored CTCs' potential as a reliable source for mutation analysis, especially when biopsies are not feasible.[21]

Qu *et al*. (2019) explored the use of double-stranded DNA (dsDNA) obtained from malignant pleural effusions, demonstrating its capability to detect EGFR mutations. This approach introduces a promising diagnostic tool in lung adenocarcinoma cases where traditional sampling is challenging.[22]

Jahani *et al*. (2024) utilized nested PCR techniques on exDNA, indicating high sensitivity for EGFR mutation detection, asserting the potential of this method in routine clinical settings.[23]

Fabrizio FP *et al*. (2023) reviewed the monitoring advantages of EGFR mutations via exosomal DNA, highlighting its relevance in understanding treatment responses and tumor progression.[24]

Table 1: Comparison of sensitivity and specificity of exosomal DNA versus cfDNA and CTCs

Biomarker	Sensitivity (%)	Specificity (%)	Reference
Exosomal DNA	70–90	80–95	Sharma and Johnson (2020)[35]
cfDNA	60–85	75–90	Zhang *et al*. (2021)[36]
CTCs	80–95	85–97	Zhang *et al*. (2019)[37]

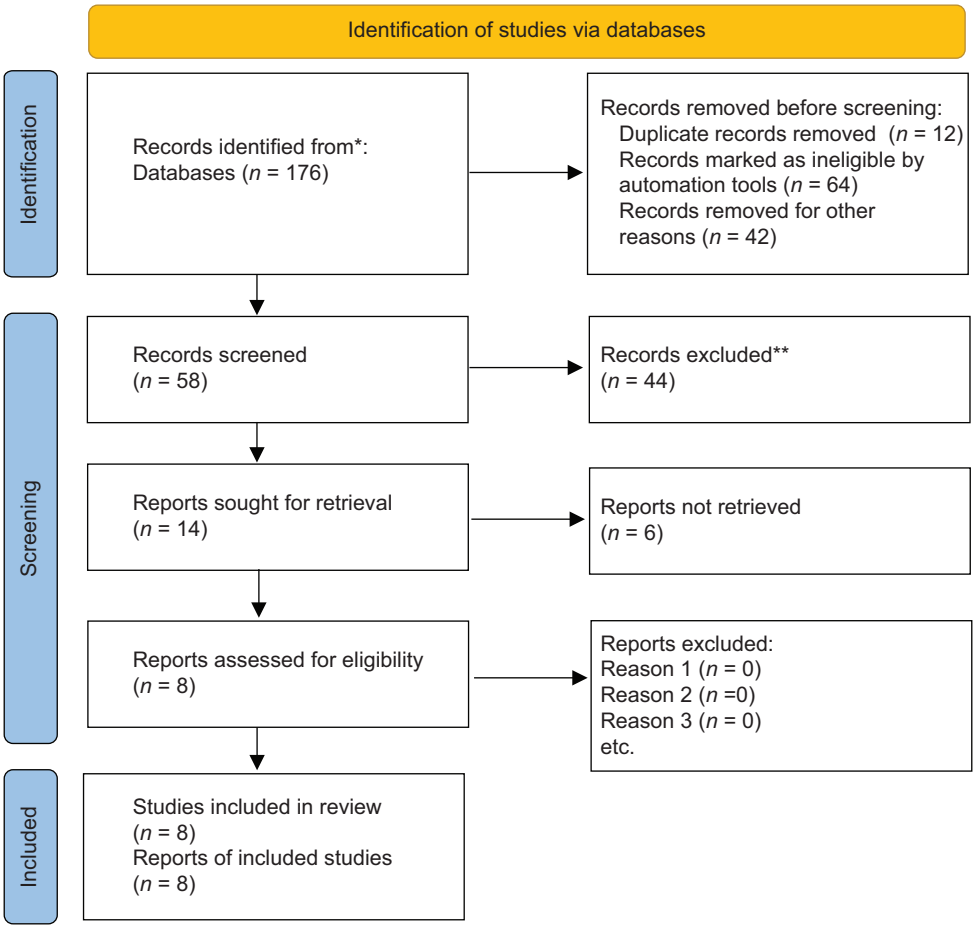

Figure 1: Study flowchart

Xiang Z *et al*. (2023) discussed the role of exDNA in revealing genetic heterogeneity within tumors, advocating for its analysis to enhance mutation detection and treatment personalization.[25]

Efficacy of exosomal DNA

Collectively, the reviewed studies demonstrated that exDNA is a viable alternative method for detecting EGFR mutations in NSCLC. The sensitivity of exDNA techniques (including nested PCR and assays targeting dsDNA from pleural effusions) showed promise in early detection and real-time monitoring of genetic alterations.

Clinical implications

The evidence suggests that integrating exDNA analysis into clinical practice may lead to improved patient outcomes through timely and accurate detection of mutations, facilitating personalized treatment strategies [Figure 2]. Given the non-invasive nature of these techniques, they could significantly enhance patient comfort and reduce the risks associated with traditional biopsy procedures.

Summary of findings

This review analyzed several studies exploring EGFR mutation detection in NSCLC, employing diverse methodologies and sample types. Findings reveal a significant range in EGFR mutation prevalence and highlight the evolving landscape of diagnostic approaches.

One large-scale study[20] utilizing both biopsy and cfDNA samples, reported an overall EGFR mutation prevalence of 26.8%, with the majority of mutations located in exons 19 and 21. This aligns with established literature on the prevalence of these common mutations. However, a considerable portion of samples (70.2%) showed no detectable EGFR mutations.

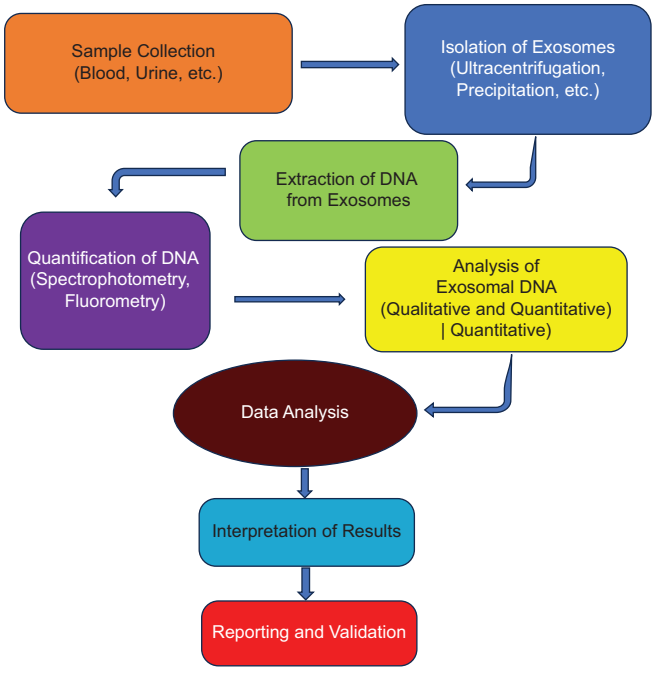

Figure 2: Workflow of exosomal DNA analysis

A significant multicenter study[26] focusing on rare exon 18 and 20 mutations across 10,117 NSCLC samples identified EGFR mutations in 10% (1047 samples). Among these, 10% (102 samples) harbored rare mutations: 4% in exon 18, 5% in exon 20, and 1% in other locations. This study underscored the clinical heterogeneity of these rare mutations, highlighting differing sensitivities to EGFR–TKIs and the need for individualized treatment approaches. Specifically, distal exon 20 insertions demonstrated resistance, while exon 18 and complex mutations showed improved sensitivity to EGFR–TKIs.

Several studies explored the use of liquid biopsy methods for EGFR mutation detection. A meta-analysis[21] examining CTCs found no significant difference in sensitivity across subgroups but noted considerable heterogeneity in specificity, dependent on factors such as blood volume, histological type, prior EGFR–TKI therapy, and methodologic differences in CTC/tissue testing. Other studies investigated the use of exDNA,[22-25] demonstrating its potential as a source for EGFR mutation detection and monitoring tumor heterogeneity and treatment response. These findings suggest that liquid biopsy techniques offer valuable non-invasive alternatives to traditional biopsy methods, although standardization and further validation are necessary. A study from India investigates the range of unusual and combined epidermal growth factor receptor (EGFR) mutations in non-small-cell lung cancer (NSCLC). This research correlates these specific mutations with how patients respond to treatment and their overall outcomes. The goal is to understand how these less common EGFR mutations impact treatment efficacy and patient survival in an Indian patient population. Further research is needed to refine targeted therapies for these specific mutations.[27]

Discussion

The results section succinctly summarizes the key findings of the systematic review and presents how exDNA testing could change the landscape of EGFR mutation detection in NSCLC.

The prevalence of NSCLC is steadily increasing. The pathogenesis is diverse, and many hereditary and acquired factors have been identified in connection with it.[28,29] However, studies have shown that EGFR mutations are one of the main factors in the pathogenesis of NSCLC, which has a high prevalence. It is often considered that the tumor tissue is the gold standard sample for detecting EGFR mutations. Nonetheless, even in prospective, well-designed clinical trials, it is still impossible to obtain enough tumor tissue samples for molecular detection from one in three patients.[30] This has become one of the major limitations of precision treatment for NSCLC.

Several studies have been conducted to identify EGFR gene mutations in the serum and plasma of NSCLC patients as a less invasive approach. The findings indicate that the accuracy of the test varies depending on the methods employed. In

Table 2: Specifications of the studies included in the study

Study	Authors/year	Method	Sample type	Key findings	Clinical relevance
Ten Years Investigation of EGFR Mutation Screening in NSCLC	Maryam Safa *et al*. 2024[20]	Reverse Strip Assay (RSA) and Real-time PCR (RT-PCR)	Biopsy samples and cell-free DNA (cfDNA)	- 26.8% of patients exhibited EGFR mutations. Common mutations in exons 19 and 21; 70.2% of samples had no detectable mutations.	- cfDNA as a non-invasive alternative to biopsy. Comparable sensitivity of cfDNA methods to traditional biopsy. Potential for improved patient comfort and outcomes.
Meta-analysis of Circulating Tumor Cell (CTC) Performance in Detecting EGFR Mutations in Advanced NSCLC	Hu *et al*., 2018[21]	Meta-analysis and systematic review	Eight eligible publications with 255 advanced NSCLC patients were included	Subgroup heterogeneity, CTCs may reflect EGFR mutation status. Reliability of CTCs compared to traditional methods explored.	Significant differences in specificity were noted based on blood volume, histological type, EGFR–TKI therapy, and CTC/tissue testing methods. No significant difference in sensitivity across subgroups. Supports the use of CTCs in mutation screening, particularly when biopsy is not feasible.
dsDNA in Exosomes from Malignant Pleural Effusions	Qu *et al*. 2019[22]	Analysis of exosomal dsDNA	Exosomes from pleural effusions	- dsDNA in exosomes can detect EGFR mutations. Potential for new diagnostic approaches in lung adenocarcinoma.	- Highlights the utility of exosomal DNA in genetic testing and monitoring.
Nested PCR for Exosomal DNA	Jahani *et al*. 2024[23]	Nested PCR followed by sequencing	Exosomal DNA	- Evaluates the sensitivity of nested PCR for EGFR detection.	- Suggests exosomal DNA as a viable source for mutation detection in NSCLC.
Monitoring EGFR Mutations in NSCLC	Fabrizio FP *et al*. 2023[24]	Literature review	Exosomes	- Investigates advantages of monitoring EGFR mutations via exosomes.	- Emphasizes potential benefits over traditional monitoring methods in treatment response assessment.
Exosomal DNA for Tumor Heterogeneity	Xiang Z *et al*. 2023[25]	Review of exosomal DNA	Exosomes	- Discusses the role of exosomal DNA in revealing tumor genetic heterogeneity and monitoring.	- Supports the integration of exosomal DNA analysis in clinical practice for better treatment outcomes.
Rare EGFR exon 18 and exon 20 mutations in non-small-cell lung cancer in 10, 117 patients: a multicentre observational study by the French ERMETIC-IFCT network	Beau-Faller M, *et al*. 2013[26]	Multicenter observational study using data from the French ERMETIC-IFCT network.	10,117 NSCLC samples	10% (1047 samples) had EGFR mutations. Of these, 10% (102 samples) harbored rare mutations.	Rare EGFR mutations in NSCLC are heterogeneous, with varying responses to EGFR-TKIs. Distal exon 20 insertions showed resistance, while exon 18 and complex mutations demonstrated better sensitivity. Individualized assessment is crucial.
Spectrum of uncommon and compound epidermal growth factor receptor mutations in non-small-cell lung carcinomas with treatment response and outcome analysis: A study from India	Varsha Singh(2020)[27]	combined retrospective (January 2010–December 2015) and prospective (January 2016–February 2020) design spanning 10 years.	1227 tumor samples were tested.	EGFR mutations were detected in 391 samples (31.8%), and included 79.5% (311/391) single common (exon19del/L858R), 6.6% (26/391) single uncommon (non-exon19del/L858R), and 13.8% (54/391) compound mutations. Exon 20 T790M mutations were most prevalent among uncommon/compound mutations (40/391, 10.2%).	Collectively, uncommon and compound EGFR mutations are detected in 6.5% of NSCLC patients. Single exon 20 T790M mutations were the third most frequent EGFR mutations. Uncommon/compound EGFR mutations showed poor treatment outcomes following EGFR TKI and chemotherapy.

2013, Kim *et al*.[31] attempted to evaluate the sensitivity of peptide nucleic acid-mediated PCR clamping to identify EGFR mutation on ctDNA of patients with NSCLC. They detected EGFR in 17% of plasma samples, with 70% showing exon 19 deletions and 30% showing exon 21 point mutations. Zaini *et al.* conducted a study using high resolution melt analysis, restriction fragment length polymorphism, and direct sequencing techniques to identify the common *EGFR* gene mutations in the tumoral tissue and plasma of patients with NSCLC. Their study showed that the detection sensitivity was different and varied from 9.1% to 39% depending on the technique employed.[32]

In a recent study, pyrosequencing was used to detect EGFR mutations in tissue biopsy and exosome samples from 28 patients with NSCLC. The findings indicated that exosomes are valuable tools for monitoring EGFR mutation status and could serve as a noninvasive method for assessing tumor mutation status, thereby facilitating personalized treatment for patients with NSCLC.[33]

This review analyzed eight studies exploring the utility of plasma exDNA and related methodologies for detecting EGFR mutations in NSCLC. The studies employed diverse approaches, including RSA, RT-PCR, nested PCR, and NGS, analyzing various sample types such as pleural effusions, plasma-derived exosomes, and cfDNA for comparative purposes. While one study using cfDNA demonstrated sensitivity comparable to tissue biopsy in detecting EGFR mutations (26.8% prevalence, primarily exons 19 and 21),[20] the majority focused on exosomes or CTCs.

A meta-analysis of CTC studies[21] reported high pooled sensitivity (0.82, 95% CI: 0.50–0.95) and specificity (0.95, 95% CI: 0.24–1.00) for detecting EGFR mutations, although substantial heterogeneity existed across subgroups owing to factors such as blood volume and methodology. Studies directly examining exDNA[22,24] demonstrated the feasibility of this minimally invasive approach but often lacked substantial sample sizes and comprehensive comparisons with established techniques.

Review articles[24,25] highlighted the potential advantages of exosome-based EGFR mutation monitoring over traditional methods, particularly for assessing treatment response and characterizing tumor heterogeneity. However, a significant limitation across the reviewed studies was the lack of standardization in methodologies and the absence of large-scale, prospective studies directly comparing exDNA approaches with current gold standard methods. This necessitates further research to establish robust sensitivity and specificity metrics against established benchmarks, ultimately determining the clinical utility of plasma exDNA for EGFR mutation detection and personalized therapy in NSCLC. Future studies should prioritize standardized protocols, larger cohorts, and direct comparisons with traditional diagnostic methods to validate the potential of this approach.

Exosome isolation methods and mutation detection techniques vary, leading to heterogeneous results. This variability can impact the accuracy of diagnoses and effectiveness of treatment plans. Differences in exosome isolation yields and purity can affect the reliability of the analysis, potentially leading to misinterpretations. Similarly, variations in mutation detection methods can lead to inconsistent results, which might affect clinical decisions about treatment strategies.[34]

In summary, while conventional methods reveal a significant prevalence of common EGFR mutations, this review emphasizes the rising importance of detecting rare mutations and utilizing liquid biopsy techniques to improve the accuracy, accessibility, and efficiency of EGFR mutation screening in NSCLC. Further research is required to optimize liquid biopsy methodologies and fully characterize the clinical significance of rare EGFR mutations.

While the emerging body of research on exDNA has provided valuable insights into its potential applications in diagnostics, several limitations must be addressed to enhance the reliability and generalizability of these findings.

Small sample sizes

A significant limitation observed across many studies is the small sample size employed in the analysis. Limited participant numbers can result in insufficient statistical power, which hinders the ability to draw definitive conclusions about the effectiveness or applicability of exDNA in various contexts. Small sample sizes also increase the risk of type I and type II errors, potentially leading to an overestimation or underestimation of the relevance of exDNA in disease processes.

Methodological variability

Another challenge is the variability in methodologies used for the isolation, extraction, and analysis of exosomal DNA. Different techniques, such as ultracentrifugation, commercial kits, or precipitation methods, can yield significantly different results regarding yield and purity of extracted DNA. This variability complicates comparisons across studies and may affect the reproducibility of findings. Furthermore, the lack of standardized protocols for the characterization and quantification of exosomes adds another layer of complexity, making it difficult to assess the validity of results across different research groups.

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Conflicts of interest

There are no conflicts of interest.

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