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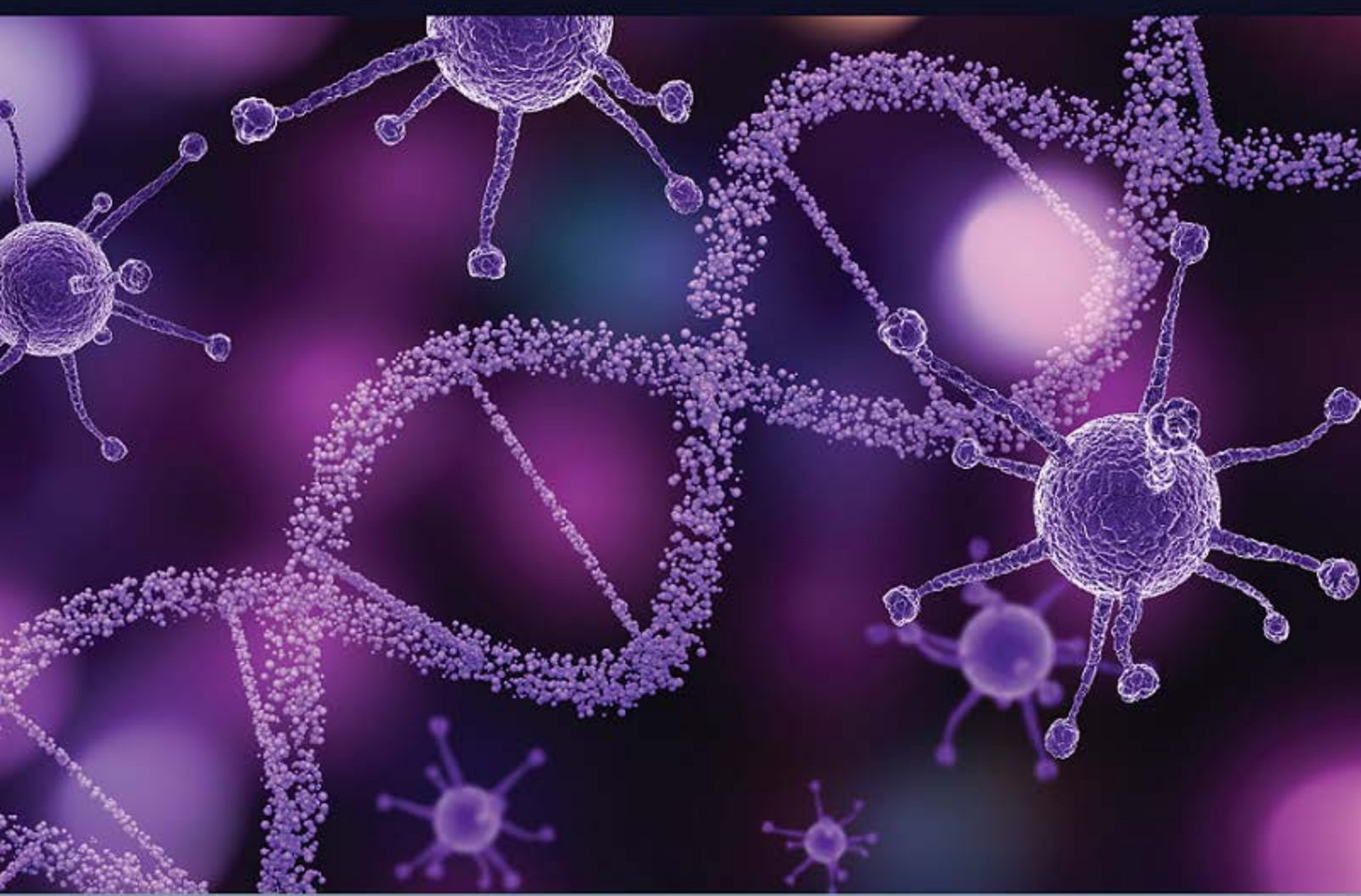
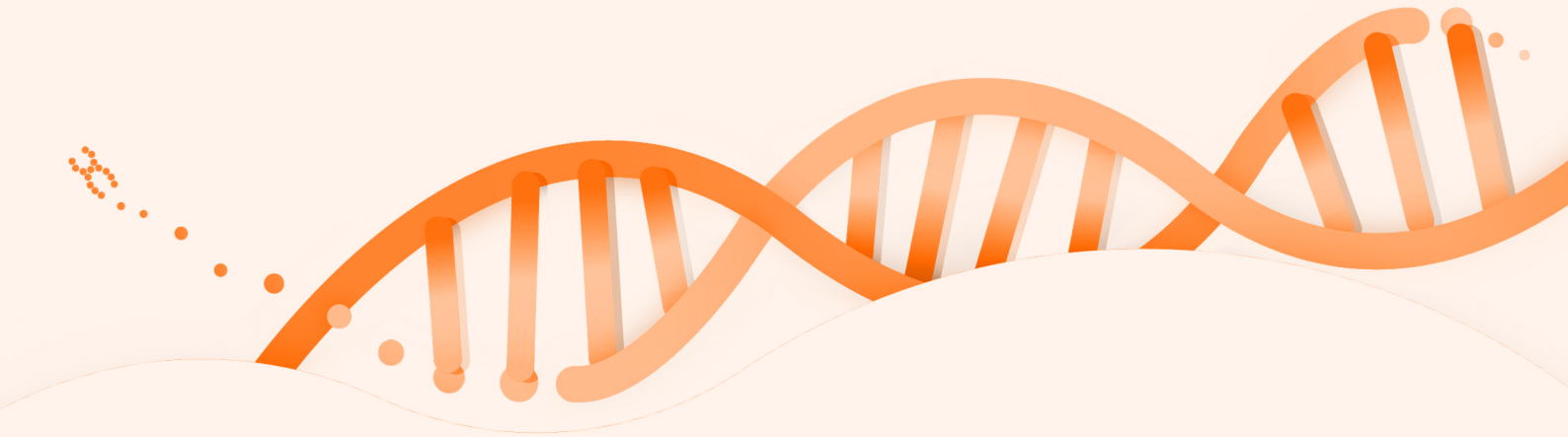


The Future of Medicine is Personalized



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The Role of Next Generation Sequencing Panels in Personalized medicine of Lung Cancer: A Review Study

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Abstract:
Lung cancer remains a leading cause of cancer-related deaths globally, with high mortality rates due to late-stage diagnosis. Early detection is crucial but challenging due to the asymptomatic nature of early-stage disease. Next-generation sequencing (NGS) has revolutionized oncology by enabling comprehensive genomic profiling, which can identify various genetic alterations from small samples. This review highlights the role of NGS panels in early lung cancer detection within personalized medicine. NGS allows the identification of actionable biomarkers, facilitating precision therapy and improving patient outcomes. Its efficiency in analyzing multiple genes simultaneously makes it a valuable tool for identifying therapeutic targets and resistance mechanisms. NGS is also cost-effective, reducing the need for multiple diagnostic tests, and its rapid data processing capabilities have led to increased adoption in clinical practice. As personalized approaches to cancer treatment gain traction, NGS is expected to play a key role in early diagnosis, prognosis, and monitoring of lung cancer. The ongoing development of advanced NGS panels and bioinformatics tools will enhance its clinical utility, positioning NGS as a cornerstone technology in lung cancer management.

INTRODUCTION

Lung cancer (LC) is a prevalent form of cancer on a global scale, with the distinction of being the primary cause of cancer-related mortality among males and the second most significant cause among females (1). Individuals diagnosed with LC generally have a poor prognosis, as evidenced by a 5-year survival rate of 19%. It is estimated that LC is responsible for 13% of

cancer cases and 24% of cancer deaths (2). LC is the second most prevalent cancer in both men and women in the United States. In Europe, this particular cancer ranks as the third most prevalent in both genders, with the greatest mortality rate (3). The incidence of LC is highly influenced by the geographic region, with greater rates observed in developed nations compared to undeveloped ones. Additionally, the prevalence is increasing in Asian countries (4). Central Africa exhibited the lowest prevalence of LC, whereas North America demonstrated the greatest occurrence. By 2030, it is estimated that LC would rank as the seventh leading cause of mortality, accounting for 3% of all deaths (5). The incidence rate of LC, the fifth most common cancer in Iran, ranges from 4.7 to 9.2 per 100,000 people. According to another study, the age-standardized rate (ASR) of this particular cancer was reported to be 9.7 in 2014 and 27 in 2030 (6).

The prevalence of LC in Iran is increasing, with the North West and West provinces of Iran having a higher incidence compared to other regions, particularly among males (7, 8). The rising prevalence of lung cancer in Iran can be attributed to the escalating rates of urbanization, the prevalence of smoking, and environmental pollution in the country (7). Small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) are the two primary classifications of lung cancer. SCLC makes up around 15% of bronchogenic carcinomas, while NSCLC makes up the remaining 85%. These carcinomas are categorized into squamous cell carcinoma (SCC), adenocarcinoma (ADC), and large cell carcinoma (9, 10). Smoking and tobacco use are significant risk factors for the development of LC. Furthermore, exposure to second-hand smoking can elevate the likelihood of developing LC by up to 26%. Additional risk factors for LC encompass dietary choices, occupational asbestos exposure, a familial predisposition to LC, gender, air pollution, and exposure to hazardous substances such as polycyclic aromatic hydrocarbons, heavy metals, and radon gas (11-13). The elevated mortality rate associated with LC can be related to multiple factors. Initially, the majority of individuals diagnosed with LC are often in an advanced stage of the disease. Furthermore, even in cases of early detection, the efficacy of treatments for LC is comparatively lower than that of other forms of cancer. The mutation burden of LC patients with a smoking history is shown to be significantly higher compared to people with malignancies that are considered “age-related” (14). Due to the elevated death rate associated with LC, researchers are increasingly focusing on its diagnosis and prognosis. Early detection and treatment are crucial in effectively reducing the mortality rate among LC patients. Tumor markers are crucial in the timely identification of lung cancer and hold significant importance in the realms of early detection, personalized treatment, and clinical prognosis.

Next-generation sequencing (NGS) is a valuable method for the identification of tumor markers and plays a significant role in the early detection of lung cancer. The purpose of this study was to examine the significance of NGS panels in early detection of lung cancer through the use of personalized medicine strategies.

Genetic of Lung Cancer

The etiology of LC involves the accumulation of genetic alterations within the cellular composition of lung tissue. The primary focus of the LC genetic analysis consists of the discovery of mutations (15). The presence of genetic modifications in the LC has been widely acknowledged in over 60% of the situations (16). LC is distinguished by a wide range of genetic abnormalities, including mutations, chromosomal area gains and losses, gene rearrangements, and copy number gain or amplifications in oncogenes and tumor suppressor genes (17).

The proto-oncogenes that undergo mutation most frequently in LC belong to the MYC, RAS, and HER families. Additionally, the most commonly observed changes in tumor suppressor genes include mutations in TP53, RB, and p16 (15,18-20). The initial genetic alterations observed in LC were identified in the KRAS and TP53 genes. The identification of KRAS and EGFR mutations is predominantly observed in LC, similar to the rearrangements involving ALK and ROS1, as reported in 2007. Several significant oncogenic alterations have been found in LC, such as B-Raf proto-oncogene (*BRAF*), Erb-B2 Receptor Tyrosine Kinase 2 (*ERBB2*), mesenchyme-epithelial transition factor (MET), and rearranged during transfection (RET). These modifications have been utilized as tumor markers for diagnostic purposes (21-27). Other studies have uncovered a variety of recurrent alterations in lung cancer, including gene amplifications (such as CCND1-3, CDK4, FGFR1-3, MET, PDGFRA, PIK3CA, and SOX2), gene fusions (like FGFR3-TACC3), tumor suppressor mutations (such as PTEN and TP53), and point mutations (including EPHA2, AKT1, and DDR2). Some research indicates that certain driver gene variants—such as mutations in EGFR, KRAS, and BRAF; mutations or amplifications of HER2; rearrangements in ALK, ROS1, and RET; and MET copy number amplifications or splice variants in MET exon 14—form a “core gene list” crucial for lung cancer. Table 1 presents genes involved in the carcinogenesis of lung tissue. The advent of next-generation sequencing has enabled a more detailed understanding of the specific genomic alterations relevant to lung cancer diagnosis.

Next Generation Sequencing

Sanger sequencing, a pioneering technology, was

Table 1. The known genes involved in the development of lung cancer.
LC: Lung Cancer, ADC: Adenocarcinomas, SCC: Squamous Cell Carcinoma.

Gene	Type of LC	Genomic Aberrations	Frequency [%]
EGFR	ADC	*EGFR* exon 21, *EGFR* exon 19, *G719X*, *L861Q* point mutations and copy number variations	**30-40**
KRAS	ADC	G12C mutation in *KRAS* gene	**20–30**
MET	ADC	MET exon 14 mutation (MET ex14), skipping mutations, overexpression, amplifications	**2–5**
ALK	ADC	ALK fusions	**3–7**
BRAF	ADC	V600E mutation in *BRAF* gene	**0.5–5**
ROS1	ADC	ROS fusions	**2-3**
RET	ADC	RET rearrangements, gene fusion of KIF5B-RET; point mutations	**1-2**
NTRK	ADC	NTRK rearrangements, gene fusions of *NTRK1 (NTRKA), NTRK2 (NTRKB), NTRK3 (NTRKC)*	**1-2**
HER2	ADC	mutations in the kinase domain (exon 20), the most frequent is p.A775_G776insYVMA insertion amplifications	**1-5**
PTEN	ADC	mutations copy number variations	**1.7**
PDGFRA	ADC	mutations copy number variations	**6-7**
PIK3CA	ADC	mutations copy number variations	**5**
TP53	ADC	mutations copy number variations	**52**
ERBB2	ADC	mutations copy number variations	**2-5**
TERT	ADC	mutations copy number variations	**75**
CDKN2A	ADC	mutations copy number variations	**7**
FGFR	SCC	gene fusion of FGFR3-TACC3, mutations of *FGFR1*, *FGFR2*	**23**
TP53	SCC	tumor suppressor mutations	**79**
NF1	SCC	mutations of *NF1*	**10**
DDR2	SCC	point mutations of DDR2	**2-3**
PDGFRA	SCC	amplification	**4**
PIK3CA	SCC	amplification	**15**
PTEN	SCC	tumor suppressor mutations	**10**
SOX2	SCC	copy number variations	**8**
CDKN2A	SCC	amplification and copy number variation	**15**

pioneered by Fred Sanger in 1977 and has since been extensively employed in the field of clinical genetics for several decades. After a span of thirty years, NGS technologies have seen significant advancements, resulting in the development of second, third, and fourth-generation sequencing technologies (34). In Figure 1, types of sequencing generation technologies are introduced. There are two distinct methods in first-generation sequencing, specifically the Sanger method and the Maxam-Gilbert approach. The second

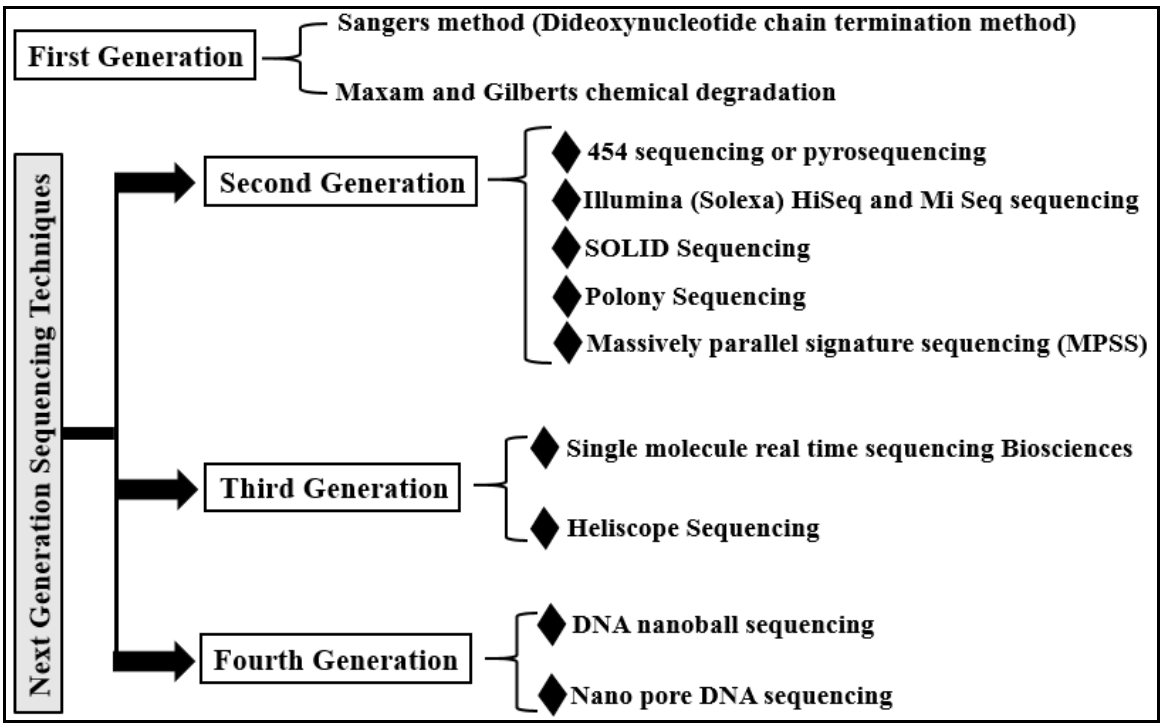

Fig 1. Classification of types of sequencing generation technologies.

generation of sequencing techniques encompasses many methods including pyrosequencing, Illumina, SOLID, polony, and massively parallel signature sequencing (MPSS). third-generation sequencing methods, such as Heliscope and single-molecule real-time sequencing by Biosciences, have advanced DNA analysis capabilities. High-throughput technologies, including DNA nano-ball sequencing and nanopore DNA sequencing, are regarded as fourth-generation sequencing (35). Next-generation sequencing generally encompasses second-, third-, and fourth-generation sequencing technologies, all of which are significantly more efficient than first-generation sequencing (36).

The Sanger sequencing method is the gold standard for detecting single nucleotide variants and modest insertions/deletions. However, its efficacy is limited when it comes to detecting gross insertions/deletions and major rearrangements. NGS is capable of detecting all types of mutations in target genes and abnormalities in chromosomes (37, 38). In essence, NGS is a DNA sequencing methodology that employs parallel sequencing of several small DNA fragments to identify particular sequences (39). The NGS technology can simultaneously identify single nucleotide variants, small insertions, deletions, copy number alteration, structural variations, gene fusions, and chromosomal rearrangements, and has significantly transformed the fields of personal medicine and genomic research (35). The NGS methodologies encompass a range of techniques that focus on the analysis of tumoral DNA and RNA (41). NGS involves several types of sequencing techniques, such as whole genome sequencing (WGS), whole-exome sequencing (WES), whole-transcriptome sequencing (RNA-seq), and targeted sequencing (both DNA and RNA). These techniques are mostly used to detect genetic alterations (42). WGS enables the sequencing of the entire genome, while whole exome sequencing (WES) specifically targets the coding portions of a genome to identify both rare and common mutations that are linked to a disease or phenotype. The utilization of RNA sequencing provides the potential to identify alternative gene-spliced transcripts, posttranscriptional modifications, gene fusion, mutations, single-nucleotide polymorphisms, and alterations in gene expression. The RNA that has been isolated is initially concentrated and then converted into complementary DNA (cDNA) using reverse transcription. Furthermore, the use of the. NGS methodology enables the examination of epigenetic modifications, including promoter methylation, microRNAs, and the expression of additional small RNAs (41).

The flexibility of NGS technology allows for the simultaneous analysis of mutational hotspots in many gene targets across different cancer patients (43). Various methodologies are employed across diverse platforms. HiSeq 2000 Illumina-Solexa is primarily utilized for whole-genome sequencing (WGS), whole-exome sequencing (WES), and RNA sequencing. On the other hand, Miseq Illumina-Solexa is primarily employed for WES and targeted sequencing (44). The two comprehensive molecular profiling NGS tests

are the FoundationOne CDx (F1CDx) and the MSK-IMPACT, which were approved by the Food and Drug Administration (FDA) in 2017. These tests examine a larger number of genes simultaneously. Several NGS tests have received approval from the FDA. These include the Oncomine Dx Target Test for lung cancer, the Illumina Extended RAS Panel for colon cancer, and the Foundation Focus CDx BRCA LOH in 2018. Additionally, there are various NGS diagnostics now in development. One such test is the Caris MI Transcriptome CDx, which is an in vitro diagnostic test based on next-generation sequencing. This test utilizes RNA extracted from formalin-fixed paraffin-embedded (FFPE) tumor tissue to identify structural rearrangements. The Breakthrough Device Designation for Illumina's pan-cancer assay, TruSight Oncology 500, was granted by the FDA in 2019. This assay uses DNA and RNA extracted from tumor samples to detect small DNA variants, fusions, and splice variants. NGS technologies have significantly contributed to the comprehension of the modified genomic pathways implicated in human cancer. Utilizing various panels of NGS technologies in both research and clinical settings can play a crucial role in the early detection and treatment of LC (41).

NGS technologies have facilitated the precise and effective identification of somatic mutations (46). NGS exhibits several advantages in comparison to genome-sequencing approaches. Firstly, this technology is classified as high-throughput since it enables the extensive sequencing of several targeted genomic areas in numerous samples simultaneously. This allows for the detection of contemporaneous mutations in the same run. Another significant benefit of routine tumor sequencing is the decreased time required for analysis, resulting in a shorter duration for clinical reporting. Furthermore, NGS analysis necessitates a minimal amount of DNA/RNA input, as opposed to previous sequencing techniques. It is possible to concurrently identify a range of genetic alterations with a high level of accuracy and sensitivity. NGS exhibits greater sensitivity compared to Sanger sequencing, with the ability to identify allele frequencies ranging from 2% to 10% and 15% to 25% respectively. Additionally, NGS enables quantitative assessment of the mutant allele (41). Furthermore, NGS enables the identification of simultaneous genetic changes in a particular subset of patients with molecular classifications, so providing a more comprehensive understanding of the genomic intricacy and prognosis of patients with LC. NGS also decreases the cost and duration of a comprehensive genetic evaluation (47-49). One of the limitations of NGS is the requirement for robust bioinformatics tools and skilled individuals to do both experimental and data analysis (50). NGS will generate hundreds of megabytes of data. Filtering redundant and large volumes of data is a challenging and intricate undertaking for bioinformatics personnel. Most NGS initiatives require the use of specialized applications. Furthermore, the storage, processing, and analysis of NGS data need the utilization of a high-performance computer (51, 52). Additional benefits and constraints of Next-Generation Sequencing (NGS) are illustrated in Figure 2.

The role of NGS in lung cancer diagnosis

Diagnosing LC in its early stages might be challenging due to the absence of symptoms in patients. Furthermore, prior methodologies, such as first-generation sequencing, have yielded numerous false-negative outcomes in the detection of lung cancer due to various factors, including the quality and amount of the samples, as well as the sensitivity of the test (53). NGS technologies have facilitated the precise and effective identification of somatic mutations. NGS has proven to be an effective method for identifying new mutations in LC (46), as it enables the sequencing of several genomic areas within a single test and platform. A technique based on NGS has the potential to offer a more thorough genetic analysis of LC, which could have an impact on the available diagnostic choices and the prognosis of patients (27). Furthermore, NGS has emerged as a prominent methodology employed in clinical practice to acquire thorough genetic profiling in individuals diagnosed with LC (54). NGS offers superior performance in terms of throughput, sensitivity, and specificity compared to traditional PCR testing. This enables the simultaneous amplification of a predetermined set of genes in a single reaction, allowing for multiplex PCR (27, 55, 56). Numerous studies have indicated that NGS might be advantageously utilized to identify particular LC mutations in circulating tumor DNA inside a liquid biopsy sample (57-59).

Several studies have shown that the utilization of NGS in plasma analysis yields a genomic profile of LC patients that is comparable to tissue testing. Furthermore, the incorporation of plasma NGS assays into regular management practices has been found to enhance the identification of clinically relevant mutations (60-62). Additional research has demonstrated that NGS is employed in LC to uncover potential biomarkers for early detection and identify mutations in clinical situations (36).

The advantages of NGS in examining changes linked to lung cancer diagnosis have opened up new possibilities for developing targeted commercial kits aimed at early LC detection. Most genomic profiling data from lung cancer patients who have had cfDNA NGS testing has been gathered using the Guardant360® panel, created by Guardant Health. The panel is equipped to

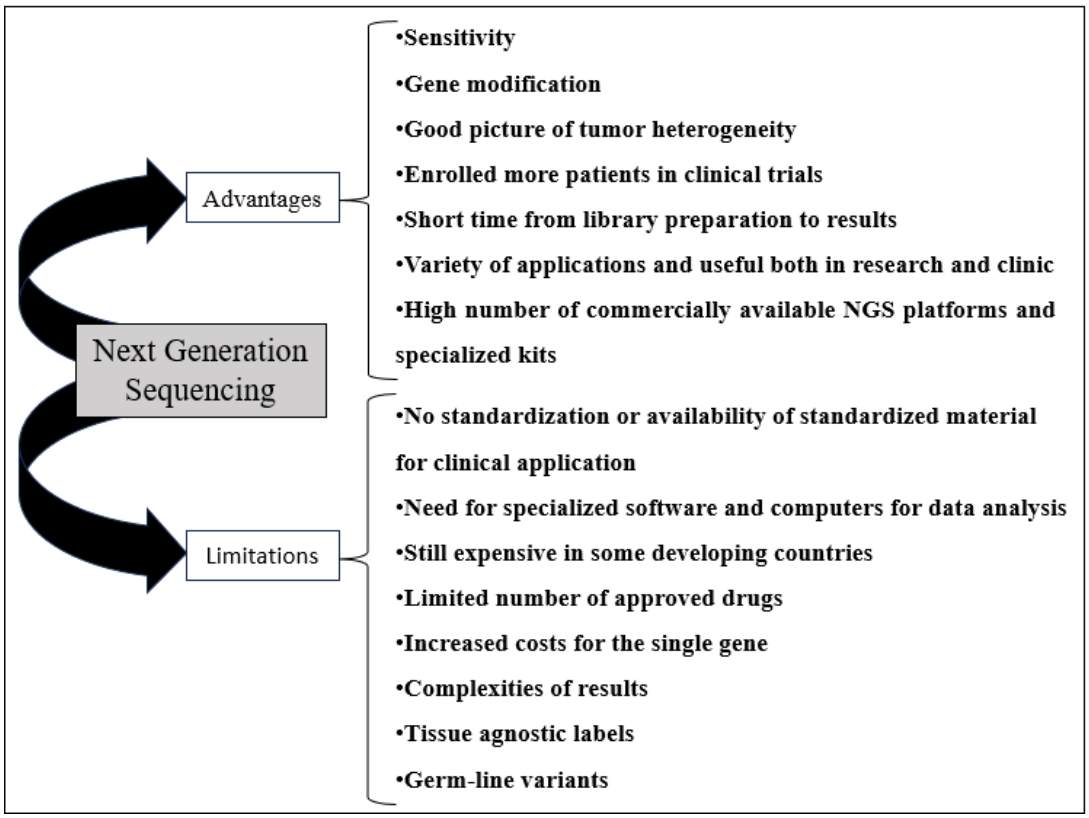

Fig 2. Advantages and limitation of Next Generation Sequencing
Also, NGS offers many benefits in comparison with traditional sequencing methods.

detect genetic alterations, including single nucleotide variants (SNVs), insertions/deletions (indels), copy number variations (CNVs), and gene fusions, across 73 genes. Recently published findings highlight the analysis of cfDNA using the Guardant360® panel, which encompasses over 8,000 plasma samples from lung cancer patients. Additionally, the Department of Public Health at the University of Naples Federico II in Italy has developed the SiRe® NGS panel, designed to assess 568 mutations in six specific genes—EGFR, KRAS, NRAS, BRAF, cKIT, and PDGFR³—in lung cancer tissue samples. Numerous studies have validated the high clinical performance of NGS-based cfDNA analysis, especially regarding success rates and mutation detection accuracy. Consequently, the SiRe® NGS panel is recognized as a valuable diagnostic tool for cfDNA analysis. (43, 65).

In 2013, Scarpa et al. highlighted the diagnostic value of the Ion AmpliSeq Colon and Lung Cancer Panel for lung adenocarcinoma samples (66). The initial version of this panel targeted 504 regions with high mutation rates across 22 cancer-related genes and was capable of detecting variants with an allele frequency as low as 1%, representing approximately 2% of cancer cells in a sample (67). Using the Ion AmpliSeq Colon and Lung Cancer Panel is essential for identifying the EGFR deletion type, a detail not provided by in vitro diagnostic molecular testing on Rotor-Gene real-time PCR. (41).

The TruSeq Amplicon-Cancer Panel, developed by Illumina, Inc. in the United States, is a targeted resequencing assay that exhibits a high level of multiplexing. It is designed to detect somatic mutations in cancer genomes by identifying numerous mutational hotspots. The presented panel offers an efficient workflow, encompassing a quality control assay designed for DNA extracted from FFPE samples. Additionally, this panel facilitates the detection of very sensitive mutations in significant genes, including *BRAF*, *KRAS*, and *EGFR*. LC is associated with mutations in these genes. The assay possesses a distinctive capability to evaluate significant FFPE samples for these crucial variations, hence enabling the extraction of a substantial amount of genomic data from LC tumors (68).

The mutations of *EGFR* exon 18, 19, 20, 21, *KRAS* exon 2, 3, *PIK3CA* exon 9, 20, and *BRAF* exon 11, 15 were detected using the NextDaySeqLung panel, developed by Beijing ACCB Biotech in Beijing, China. According to previous studies (69), the NextDaySeq-Lung panel has exhibited superior outcomes compared to Sanger sequencing or qRT-PCR.

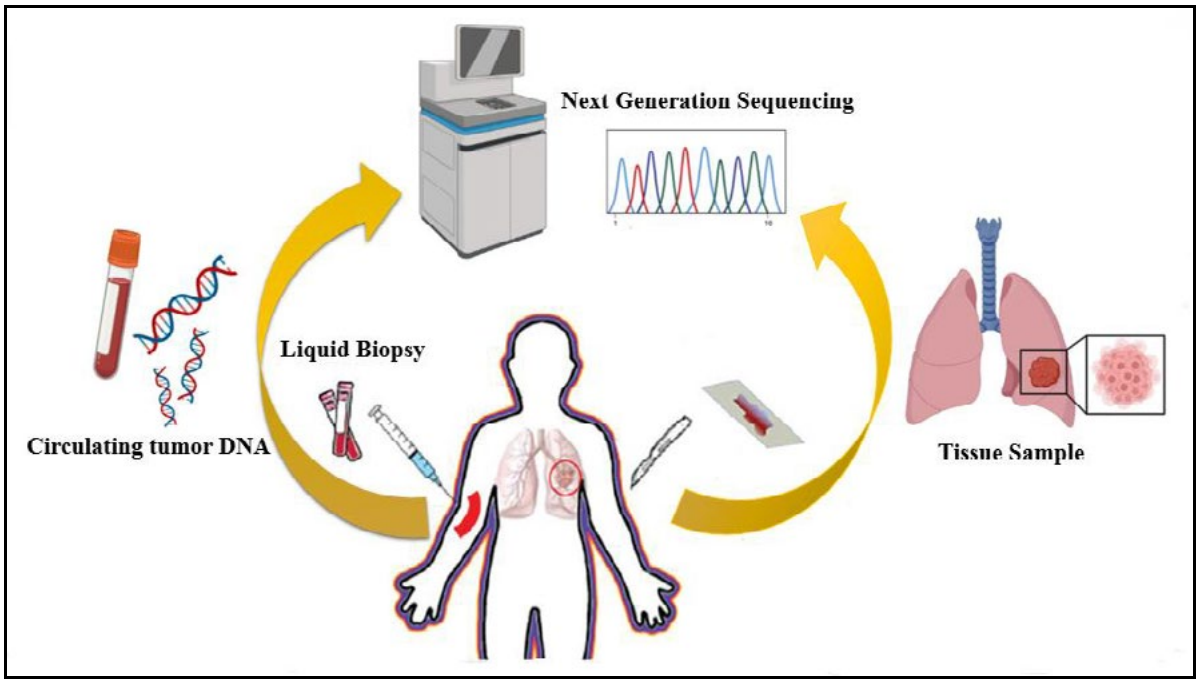

Fig 3. NGS analysis of liquid biopsy and tissue sample for detect of specific mutations.

Furthermore, several studies have demonstrated that the NextDaySeq-Lung panel exhibits notable technical benefits compared to Sanger and qPCR tests, indicating its promising potential as a molecular diagnostic panel for lung cancer (70, 71). LC gene panels utilizing RNA sequencing have been developed to examine gene fusion, translocations, chromosomal inversions, and interstitial deletions. The Ion Ampliseq RNA fusion lung cancer panel, developed by ThermoFisher Scientific in Waltham, USA, is an example of such a panel. This panel specifically targets 70 well-known fusion transcripts of *ALK, RET, ROS1,* and *NTRK*. This panel has exhibited a high level of sensitivity and a strong agreement with the conventional techniques employed for fusion testing (72). Displays the NGS panels that are currently accessible to diagnose LC. As previously said, cancer is known as a genetic disorder (16). LC is a multifaceted illness characterized by a wide range of somatic mutations (73). It arises from a process of polyphase carcinogenesis, wherein genetic alterations gradually accumulate over time. The utilization of screening for the distinctive genetic mutation as a biomarker has the potential to facilitate the early detection of LC (15). NGS technologies have significantly contributed to the identification of modified genomic pathways implicated in cancer. Consequently, the utilization of diverse panels of NGS technology has proven valuable in both research and clinical settings (41).

NGS is primarily used for the early detection of lung cancer by identifying mutations in genes such as *EGFR, BRAF, KRAS, TP53, HER2, ROS, ALK, PIK3CA, NTRK, RET*, and *MET*. These genes are considered potential candidates in the development of lung cancer (74, 75). NGS techniques are extensively employed in the field of oncology, particularly for the early detection of biomarkers and the identification of driving mutations in LC. Numerous studies have been conducted to examine the significance of NGS in the diagnosis of lung cancer and to compare it with conventional testing methods. The study conducted by Gabriela et al. (2019) aimed to compare the efficacy of a NGS method with a Sanger sequencing and Fluorescence In Situ Hybridization (FISH) sequential strategy in detecting actionable genomic alterations in a cohort of 117 patients diagnosed with advanced lung cancer. Patients were categorized as EGFR-mutated (n = 22, 18.8%), ALK-mutated (n = 9, 7.7%), and unclassifiable (n = 86, 73.5%) using Sanger and FISH techniques. On the other hand, the study using NGS resulted in the detection of at least one genetic variation in 56 patients (47.9%), resulting in a total of 68 variants across all samples. The study's findings indicate that the NGS-assay is a viable approach for conducting genomic profiling in individuals diagnosed with advanced lung adenocarcinoma (27). Gao et al. (2015) conducted a study to assess the practicality of using NGS-based assays to analyze mutations in important driver genes of lung cancer in a clinical environment. A total of 138 FFPE samples of lung cancer were analyzed simultaneously with NGS assays, quantitative PCR (qPCR), and Sanger sequencing platforms to detect the mentioned somatic mutations. The findings revealed that the NGS assays exhibited significantly greater

sensitivity compared to Sanger. Additionally, the NGS test demonstrated additional benefits compared to qPCR in terms of delivering precise data on allele sequence and mutation frequency, as well as identifying non-hotspot alterations. The findings indicate that the NGS assay possesses notable technical benefits compared to Sanger and qPCR assays, making it a promising molecular diagnostic panel for lung cancer (70, 71). In Lim et al.'s (2016) investigation, a NGS-based assay was employed to detect 51 FFPE samples of lung cancer. The efficiency of this assay was compared to that of a traditional technique. The study found that 58% of wild-type patients exhibited mutations in one of these genes when the NGS approach was used (53). In previous studies, NGS has been utilized to diagnose lung cancer, as conventional approaches are not adequate for the limited availability of tissue samples (55). Another study has demonstrated that existing tools are limited to identifying a single hot spot at a time. However, NGS offers various advantages over traditional sequencing methods, including increased throughput and reduced testing time. NGS allows for the simultaneous sequencing of multiple hot spots within a short timeframe. Therefore, NGS decreases expenses and produces valuable genetic data on lung cancer, helping clinicians in the diagnostic process (36). Moreover, another study compared an NGS panel, Sanger sequencing, and qRT-PCR in the detection of mutation in 138 lung cancer FFPE samples showed that the NGS and qRT-PCR have a higher sensitivity than Sanger sequencing. Also, NGS is better than qRT-PCR and identifies mutations that are not in the hotspot area (70). In the study by Park et al. (2019) the single-gene assays, such as real-time PCR, IHC, and FISH were compared with the NGS method.

The NGS data indicate that EGFR PCR had a sensitivity of 80.3% and specificity of 99.4%, ALK FISH had a sensitivity of 71.4% and specificity of 100%, and ROS1 FISH had a sensitivity of 100% and specificity of 99.5%. The observed outcomes are associated with the diminished sensitivity of single-gene assays when compared to deep-targeted NGS (76). Numerous studies have demonstrated various transformations resulting from the integration of NGS into clinical practice for the diagnosis of lung cancer. These studies have reported a notable level of sensitivity in detecting alterations through the utilization of a gene panel-based NGS approach on lung cancer specimens (77-79). For instance, Lim et al. (2016) found that 58% of patients who tested for EGFR/KRAS/ALK using standard methods exhibited alterations that were identified by NGS (53). The research conducted by de Leng et al. (2016) and Jing et al. (2018) demonstrates a strong concurrence between NGS and single gene assays in identifying driver-gene mutations in individuals with lung cancer. These studies exhibit notable levels of sensitivity and specificity, indicating the potential for the widespread adoption of NGS assays in informing clinical decision-making (80, 81). The examination of fusion alterations to lung cancer revealed that NGS has greater sensitivity and specificity compared to FISH or IHC. Lin et al. (2019) reported a positive rate of 92.7% for ALK rearrangement when employing NGS, 82.4% for FISH, and 94.5% for Immunohistochemistry (IHC). Additionally, they observed a concordance of 87.3% between NGS and IHC results (82). The NGS technique has facilitated the development of liquid biopsy testing for the diagnosis of lung cancer. Leighl et al. (2019) reported a significant level of agreement between the NGS results obtained from cfDNA and tissue DNA in lung cancer cases. The benefits of NGS have facilitated the creation of various assays utilizing liquid biopsy samples for the timely detection of lung cancer (83, 84). A study has demonstrated that NGS-based methodologies are very suitable options for achieving optimal characterization of circulating tumor DNA in individuals diagnosed with lung cancer. Various NGS techniques have been created and verified for identifying anomalies in lung cancer ctDNA. These methods have the benefit of being able to discover uncommon mutations that were previously undetectable using conventional methods (85). The meta-analysis of 1639 lung cancer patients from 21 studies found that the combined sensitivity and specificity of NGS-based ctDNA T790M testing were 0.87 (95% CI 0.76–0.95) and 0.89 (95% CI 0.82–0.94), respectively. These values were higher than those of other detection methods such as Real-Time PCR and ddPCR, indicating that NGS is more efficient (86). In addition, a tag-based NGS panel was utilized to detect T790M in plasma samples obtained from patients with lung cancer. This approach yielded a notably higher detection rate of NGS in comparison to Real-Time PCR, with rates of 42.85% and 21.4% respectively. In addition, NGS demonstrated the capability to detect mutations at extremely low AFs (up to 0.07%), resulting in a drop in false negative instances necessitating repeated tumor biopsies and a reduction in turnaround times compared to alternative approaches (87). In a recent study, the effectiveness of an 11-gene NGS panel in detecting target mutations in two separate groups of lung cancer patients was compared to conventional approaches and focused NGS. NGS has demonstrated the ability to accurately detect the EGFR T790M mutation in liquid samples of lung cancer patients with a high level of sensitivity and specificity. Reckamp et al. (2016) conducted a study. NGS assays were employed to investigate the presence of EGFR activating mutations and the T790M resistance mutation in urine or plasma samples obtained from patients diagnosed with advanced NSCLC who tested positive for EGFR mutants. The NGS assay conducted on urine and

plasma samples revealed a higher prevalence of EGFR mutated positive results compared to tissue samples subjected to RT-PCR testing. The urine specificity is 94% and the plasma specificity is 96-100%. The urine and plasma sensitivity rates are 80-93% and 87-100%, respectively (89). In their study, Liu et al. (2021) offer a collection of three cases wherein the utilization of NGS enabled the identification of genomic alteration patterns, hence aiding in the differentiation between various original lung malignancies and intrapulmonary metastases. NGS exhibits unique molecular attributes that distinguish it from conventional disease detection methods. This finding supports the potential of NGS in aiding the diagnosis of lung cancer (90). Jiang et al. (2021) conducted a retrospective investigation. Both IHC and NGS were utilized to identify changes in the *ALK, EGFR, KRAS, BRAF, RET, ROS1, V-Erb-B2, CerbB-2*, and *MET* genes in 19 cases of FFPE lung adenocarcinoma. The findings of this investigation indicate a strong agreement between the NGS assay and the IHC technique (91). In their study, Pekar-zlotin et al. (2014) conducted a thorough analysis of FISH and IHC to detect *EML4-ALK* rearrangement in 51 patients with lung cancer. If there was any disagreement, they resorted to NGS. The findings of this study indicate that the FISH-based approach for identifying *EML4-ALK* rearrangement in lung cancer may overlook a considerable number of patients who could potentially benefit from targeted ALK therapy. Therefore, it is highly recommended to strongly consider screening for EML4-ALK rearrangement through IHC and to consider NGS in cases that are on the borderline (92). The study conducted by Clavé et al. (2019) evaluated the identification of ALK and ROS1 rearrangements in a retrospective cohort of forty patients with non-small cell lung cancer who had known FISH data. This assessment was performed using NGS and immunohistochemical techniques. The findings indicate that the presence of a 3 isolated signal FISH pattern in cases of ALK and ROS1 may indicate a false positive outcome.NGS appears to be a reliable technique for evaluating ALK and ROS1 rearrangements, with the benefit of discovering additional molecular changes that may have therapeutic significance, in comparison to immunohistochemistry (93). In their study, Dacic et al. (2016) conducted a comparative analysis between different ALK-FISH patterns and NGS for gene fusion detection and ALK immunohistochemistry. The authors propose that relying solely on ALK FISH may not be the most dependable method for detecting ALK gene rearrangements. Instead, they suggest that a combination of ALK IHC and NGS should be employed to detect gene fusions and mutations in lung cancer (94). The studies conducted by Moskalev et al. (2014) and Beadling et al. (2016) showed a strong agreement between the NGS assay and conventional methods (95, 96). Drilon et al. (2015) showed that employing NGS assay is a superior and more effective approach in identifying actionable mutations in lung adenocarcinomas, as compared to non-NGS testing (97). A multitude of clinical trials have been conducted to investigate the utilization of NGS technology in the diagnosis and treatment of LC. A search conducted on clinicaltrial.gov on 24 March 2022 revealed a total of 117 trials focused on lung cancer that employ NGS.

Certain of these trials are formerly complete and some others are recruiting or enrolling.

CONCLUSIONS

In the new era of precision oncology, detecting genomic alterations is the priority and guiding principle for personalized care. The next-generation sequencing method is an ideal technique than other standard techniques for investigating different types of lung cancer in a short period and at a low price. NGS has allowed the obtainment of a wide spectrum of genomic alterations occurring within lung cancer. NGS is widely used in the clinic for the detection of a greater number of driver genes and leading to early diagnosis of lung cancer. NGS maximises the identification of clinically related genomic alterations using a limited amount of tissue, thus reducing time and costs. Molecular biomarkers can be useful in diagnosis at an early and non-invasive stage of lung cancer. It is essential to develop innovative approaches using NGS panels to generation biomarkers in lung cancer screening programs for early diagnosis. The application of a standard NGS panel for lung cancer diagnosis is more efficient at a lower cost point than other methods such as Sanger-Sequencing and provides a much better resolution compared to microarrays. NGS has been used to identify new biomarker candidates for the early diagnosis of lung cancer. The different clinical studies were developed to obtain specific NGS panels for lung cancer diagnosis. Different NGS panels should be used to develop clinical tests in personalized medicine for early diagnosis of lung cancer. The apply of NGS-specific panels in the early diagnosis of lung cancer can play an important role in reducing mortality, increasing response to treatment and reducing treatment costs from this cancer. All the mention advantages, make NGS the preferred method to extend clinical tests for personalized medicine and early diagnosis of lung cancer.

Abbreviations

LC: Lung cancer, ASR: Age-standard rate, NSCLC: Non-small cell lung cancer, SCLC: Small cell lung cancer, SCC: Squamous cell carcinoma, ADC: Adenocarcinoma, NGS: Next generation sequencing, *BRAF*: *B-Raf* proto-oncogene, *ERBB2*: Erb-B2 receptor tyrosine kinase2, MET: Mesenchyme-epithelial transition factor, RET: Rearranged during transfection,

HER2: Human epidermal growth factor receptor2, MPSS: Massively parallel signature sequencing, WES: Whole-exome sequencing, WGS: Whole-genome sequencing, RNA-seq: RNA sequencing, DNA-seq: DNA sequencing, FFPE: Formalin-fixed paraffin-embedded, MSI: Microsatellite instability, TMB: Total number of mutations, PDL-1: Programmed death-ligand 1, SNVs: Single-nucleotide variants, Indels: Insertion–deletion mutations, cfDNA: Cell-free DNA, FISH: Fluorescence in situ hybridization, IHC: Immunohistochemistry, ctDNA: Circulating free DNA.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

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A Review Study for the Treatment of Diabetes Using New Biotechnological Methods

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Abstract:

A metabolic disease known as diabetes mellitus (DM) is brought on by a reduction in insulin production and activity. Nephropathy, retinopathy, and cardiovascular problems are among the pathological alterations that the body will unavoidably experience as the condition progresses. Type I DM and Type II DM are the two basic subtypes of DM. Oral hypoglycemics are used to treat type II diabetes, while insulin replacement treatment is often used to treat type I diabetes. Insulin secretagogues, biguanides, insulin sensitizers, alpha-glucosidase inhibitors, incretin mimetics, amylin antagonists, and sodium-glucose co-transporter-2 (SGLT2) inhibitors are the main medications used to treat type II diabetes. When first-line oral hypoglycemic medications are not as effective as monotherapy, dual-drug treatments are often advised for patients. Despite the significant therapeutic advantages, traditional dosage forms have a short half-life and varied bioavailability, which require frequent dosing and increased side effects. This may render treatment ineffective and result in patient non-compliance. With the extra benefit of site-specific medication delivery with increased bioavailability and a lower dose regimen, nanotechnology-based techniques are more alluring, given the pathological intricacy of the condition above. In this review study, we have attempted to examine the biology of type II diabetes, traditional treatment modalities (mono and combination therapy), and drug delivery methods based on nanotechnology.

INTRODUCTION

Diabetes is a prevalent chronic illness. The International Diabetes Federation reports that 463 million people worldwide had diabetes in 2019; by 2030, that number is expected to rise to 578 million individuals (1). Diabetes is a condition that is characterized by high blood glucose amounts; it is brought on by inadequate production of insulin or impaired insulin action (2). Type 2 diabetes is the most common type of diabetes, accounting for over 90% of cases (3). Persistently high blood glucose levels may lead to damage to the kidneys, eyes, cardiovascular system, nervous system, and other organs. Diabetes frequently leads to numerous complications, including diabetic nephropathy, diabetic neuropathy, and diabetic foot problems (4). These problems put human health in grave peril since they often result in blindness, incapacity, and even death (4).

A review of new diabetes treatments

Diabetes management has changed dramatically over the past few thousand years. The option preferred by the "experts" of the Egyptian pharaoh 3500 years ago was a mixture of "bird pond water," elderberry, ascites plant fiber, milk, beer, cucumber flowers, and green dates. Although our treatment options are significantly more effective today, if the current path of treatment development continues, they will likely be considered secret by our successors in the next 100 years. However, the current drug arsenal used to manage diabetes has resulted in a significant reduction in mortality (5).

Insulin

Before the 1920s, there were no effective drugs to manage diabetes; That is why type 1 diabetes is a disease. It was fatal. This changed dramatically with the work of Frederick Bunting. In addition to the formulation changes that later resulted in the structure

of insulin, countless improvements in the field of insulin delivery devices and Prescription ways have been done or are in progress, including better syringes, insulin Pulmonary, insulin pumps, and closed-loop insulin delivery systems. Insulin today Widely in patients with diabetes Type 1 or Type 2 is used and arguably the most effective and predictable (in most, but not all) cases of all current antihyperglycemic drugs (6).

Biguanids

French lilac or goat (officinalis Galega) was used as a folk medicine for diabetes in Eastern and Southern Europe in the Middle Ages. In the early 20th century, the antihyperglycemic part of this plant, guanidine, was isolated. Researchers Synthesized a guanidine compound called cynthalin in Germany and used it to treat diabetes in the 1920s. Hyguanidine homologs (e.g., cynthalin) were used for a short time but had hepatotoxicity, and the use of these compounds ended with the discovery and proliferation of insulin (7). However, in the following years, interest in biguanides increased again. In the 1960s and 1970s, phenformin was studied extensively in the United States, While Metformin was studied in France and Buforminder in Germany. Although phenformin and buformin were used clinically, their association with lactic acid led to their withdrawal from the market in most countries (8). Metformin was introduced as an antihyperglycemic agent in 1959 but was not approved in the United States until 1990. Today, metformin is the only clinically important biguanide and the most widely used antihyperglycemic agent in the world. The main mechanism of its effect is its ability to reduce hepatic glucose production, but also, through A slight increase in insulin-stimulated glucose uptake, lowers glucose. This drug is generally well tolerated and is usually accompanied by a significant reduction in A1C levels (~1.5%) (9).

Sulfonylureas

The history of sulfonylureas (Sus) began in 1937 with the observation of the hypoglycemic activity of synthetic sulfur compounds. Five years later, Marcel Janbon and his colleagues treated patients with the antibiotic amino sulfonamide isopropyl thiadiazole and observed hypoglycemia. In August 1946, Lobatieres confirmed that Aryl compounds of SU stimulate the release of insulin and, therefore, induce effects on some β-cell functions. In 1950, the first SU, tolbutamide, was marketed in Germany. Following this, other first-generation agents were chlorpropamide, acetohexamide and tolazamide. Further advances in the treatment of SU in the United States only occurred once the release of the more potent second-generation agent's glipizide and glyburide in 1984. These agents had been used in Europe for several years before this. The next SU agent, glimepiride, which is sometimes known as a third-generation agent, was released in 1995 (10).

Thiazolidinediones

Thiazolidinediones (TZDs), also known simply as "glitazones," were first introduced to the US market in 1996. These receptor-activating agents Γ are activated by peroxisome proliferators whose mechanism of action is to increase skeletal muscle insulin sensitivity and decrease it. Hepatic glucose production, these agents do not increase the risk of hypoglycaemia, and it has a more lasting effect than troglitazone, the only agent in this category that the FDA approved. With increasing use of troglitazone, specific liver failure was reported. By March 2000, the FDA had received reports of 63 cases of fatal liver failure in patients treated with troglitazone, and shortly after that, the drug was withdrawn from the market. Two other TZDs, pioglitazone and rosiglitazone, are currently on the market. Are they associated with non-hypoglycaemic problems? Both factors are associated with fluid retention and should be used in patients with congestive heart failure (11).

The heart should be used with caution. Pioglitazone has been shown to have a potentially beneficial effect on cardiovascular disease, but it is also associated with a possible increase in the incidence of bladder cancer. It was not α-glucosidase inhibitors A-glucosidases (AGIs) that have a local effect on Ruymerz applying the brush of the small intestine and They inhibit α-glucosidase enzymes (12).

They are saccharides and disaccharides. These enzymes include maltase, isomaltose, and glucoamylase, and they are sucrose. Control these systems. Enzyme effectively speeds up absorption. Carbohydrates reduce, but absorption does not change the absolute. Reduction results in Postprandial glucose level, with effect, it is moderate on fasting glucose. The FDA, which previously imposed restrictions on Rosiglitazone, began easing those restrictions in November 2013. Their change of position was based on the findings of the large Rosiglitazone Evaluated for Cardiovascular Outcomes and Blood Glucose Regulation in Diabetes (RECORD) study, which concluded that subjects treated with Rosiglitazone compared with patients treated with other antidiabetic drugs. There is no significance in A1C reduction between pioglitazone and rosiglitazone (13).

α-glucosidase inhibitors

α-glucosidases (AGIs) have a local effect on Ruymerz applying the brush of the small intestine, and they inhibit α-glucosidase enzymes. Responsible for the breakdown of oligosaccharides, they are saccharides and disaccharides. These enzymes include maltase,

isomaltase, glucoamylase, and sucrose (14).

Meglitinides

Meg Lytin Ida (the so-called "Glinides" are called) has the same mechanism of action as they have SU. This class of drugs irritates Insulin secretion from the pancreas and glucose levels. They reduce blood. The second generation has a faster onset and a shorter duration of action that requires multiple doses of glinides and can cause hypoglycemia. However, they do it at a slower rate than SUs. Decrease the A1C of glinides, which is generally between 1 and 5.1% sucrose (15).

Glucagon-like peptide-1

The idea of the "incretin effect" has been around for a long time. Budo, based on experimental data, shows that there is more insulin response with the administration of glucose ingestion than with an intravenous injection. Incretin pathway overview Insulin was identified in the 1980s. A key study evaluated the effect of native GLP-1 receptor agonists, all of which are administered subcutaneously. One of the benefits of treatment with incretin-based drugs is a 5.0% to 1% reduction in Treatments and 11.7 weight loss. However, these compounds can cause complications and create significant side effects in the digestive system, especially in early treatment (15).

4-DPP inhibitors

As mentioned above, researchers can develop 4-DPP inhibitors by turning on the incretin-insulin pathway. These factors can be taken orally, and the circulating half-life of incretin is too long. The first of these agents is not available in the United States. Sitagliptin was included in 2006, and after that, Saxagliptin and linagliptin were produced (16).

Pathophysiology of diabetes

Many hormones are responsible for maintaining the body's glucose homeostasis. However, glucose homeostasis is primarily regulated by two hormones, insulin and glucagon (16). β cells release insulin as the glucose concentration increases. Insulin lowers blood glucose levels either a) by preventing the liver from producing glucose via gluconeogenesis and glycogenolysis (17) or b) by promoting the liver, muscle, and adipose tissue to absorb glucose more readily.

When the amount of glucose is low, the pancreatic α cells produce glucagon. Glucagon works by: a) Counteracting the effects of insulin by promoting the liver's gluconeogenesis and glycogenolysis (18).

b) Plasma glucose levels are likewise raised by catecholamines, cortisol, and glucagon (18).

Amylin, a 37 amino acid peptide, glucagon-like peptide-1 (GLP-1), a 30 amino acid peptide, and glucose-dependent insulinotropic polypeptide (GIP), a 42 amino acid peptide, are additional hormones that contribute to the maintenance of normal glucose levels (18). Insulin and amylin are secreted together. It improves the absorption of glucose after a meal by reducing stomach emptying. The gut produces the peptides or incretins GLP and GIP. These incretins help the pancreatic β cells synthesize and secrete insulin (19). Neither the intestinal tract nor cells in need of energy can readily absorb glucose. Therefore, glucose transporters are responsible for distributing glucose to the cells. There are two categories for the family of membrane-bound glycoproteins known as glucose transporters.

- Sodium-glucose co-transporter (SGLT)
- Facilitative glucose transporter (GLUT)

There are two basic subtypes of diabetes mellitus, and the underlying causes of each are distinct (20).

•Type I diabetes (T1DM): In this condition, the immune system unintentionally targets the pancreatic β cells, where genes are essential.

•Type-II DM (T2DM): Genetics and lifestyle factors interact to cause this condition. Being overweight or obese raises the hazards involved.

Advanced treatment now includes new medication classes

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•Type I diabetes (T1DM): In this condition, the immune system unintentionally targets the pancreatic β cells, where genes are essential.

• Type-II DM (T2DM): Genetics and lifestyle factors interact to cause this condition. Being overweight or obese raises the hazards involved.

Alpha-glucosidase inhibitors (AGIs)

The two main enzymes in charge of the metabolism of carbohydrates are alpha-amylase and alpha-glucosidase (22). The alpha-glucosidase Oral anti-diabetic medications called AGIs are mostly used to treat type 2 diabetes. By relocating the undigested carbohydrates to the distal portion of the small intestine and colon, AGIs slow down the absorption of carbohydrates in the gastrointestinal system. Postprandial hyperglycaemia is reduced by this family of medications (23). AGIs are saccharides that work as competitive inhibitors of the small intestine's enzymes to slow down the breakdown of starch and other carbs. This causes glucose from meals to reach the circulation more slowly, which lowers postprandial hyperglycaemia. When used in conjunction with other diabetes medications, these medications help reduce post-meal blood sugar levels, which in turn lowers HbA1c (23).

They aid in increasing GLP-1 levels after meals, which postpones digestion and reduces appetite (60).

Bloating, flatulence, and gastrointestinal discomfort are common AGI side effects that may go away in a few weeks (24).

Alpha-glucosidase inhibitors are not advised for those with inflammatory bowel diseases such as Crohn's disease or ulcerative colitis, intestinal obstruction, intestinal digesting disorders, or diabetic ketoacidosis, a condition in which the body burns fat for energy rather than carbs (25). Acarbose is not advised for pregnant women, those with large intestinal ulcers, or those with liver cirrhosis.

Amylin analogues

The hormone amylin is made up of a single chain of 37 amino acids. It is co-secreted by the pancreatic β cells with insulin (25). It keeps blood glucose levels stable during fasting and after meals by delaying stomach emptying and inhibiting glucagon secretion. Adjusting the brain's appetite center controls how much food is consumed (25). Since both T1DM and T2DM lack amylin, research, and development of amylin analogs that preserve glucose homeostasis by any one of the following pathways were conducted.

i) Postponing stomach emptying ii) Preventing glucagon release after meals iii) Restricting food intake and weight gain by regulating appetite

Biochemical analogs that mimic amylin's effect were created since amylin aggregates and is insoluble in solution, making it unsuitable as a medication Amylin analogs may be administered parenterally to treat both type 1 and type 2 diabetes (25). This sort of molecule is given before meals and functions similarly to amylin. The available medication in this class is pramlintide acetate, which is marketed under the Symlin® brand and is given subcutaneously.

Amylin analogs most often cause headaches, nausea, vomiting, and hypoglycemia when used with insulin. Once the patient gets used to the drug, these adverse effects disappear (25).

Incretin mimetics (GLP-1 agonists and DPP-IV inhibitors)

The gut produces the incretins, or peptides, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide (GLP). A class of naturally occurring metabolic hormones known as incretins promotes a drop in blood glucose levels (26). After a meal, several hormones are released. After a meal is introduced, the gut's L cells release a peptide called GLP-1, which is composed of 36 amino acids. GLP-1 secretion is comparable to insulin secretion from the pancreatic β cells (27). GLP-1 causes the pancreatic β cells to synthesize and secrete insulin. In intestinal L cells, the metabolism of carbohydrates results in the depolarization of the membrane and the closure of the ATP-sensitive potassium channel, which allows calcium ions (Ca2+) to enter. This results in GLP secretion. Dipeptidyl peptide-IV (DPP-IV) enzymes rapidly metabolize GLP-1, resulting in a half-life of around 1-2 minutes (28, 29). Therefore, one method for treating T1DM and T2DM may be the creation of GLP-1 analogs with longer half-lives. Once again, DPP-IV inhibitors exhibit incretin mimic behavior.

Sodium-glucose co-transporter 2 antagonists/ inhibitors

Facilitative glucose transporter (GLUT), a passive transporter, and sodium-glucose co-transporter (SGLT), an active co-transporter, are responsible for the reabsorption of glucose in the proximal convoluted tubule (PCT) (30).

By blocking the SGLT2 found in PCT, SGLT2 inhibitors stop glucose from being reabsorbed and increase its excretion in urine. The blood glucose level and other glycaemic indicators are maintained. In contrast, glucose is eliminated in the urine (31). Canagliflozin, Dapagliflozin, Empagliflozin, Ipragliflozin, Luseogliflozin, and Tofogliflozin are the compounds that currently fall within this group (31).

SGLT2 inhibitors may be administered alone, in conjunction with insulin, sulfonylureas, metformin, or thiazolidinediones, or as an adjuvant.

An innovative method of delivering antidiabetic medications for type 2 diabetes

Some drawbacks of traditional drug delivery methods include reduced potency or altered effects as a result of drug metabolism, lack of target specificity, and ineffectiveness owing to incorrect or inefficient doses (33). Because of its advantages in lowering the frequency of doses, improving bioavailability, preventing degradation in an acidic stomach environment, and providing tailored therapeutic effectiveness with fewer adverse effects, novel drug delivery systems, or NDDSs, have been a rapidly developing topic in recent years (34). Few NDDSs have been documented to treat type 2 diabetes, even though several are being studied for the treatment of other illnesses. They may be categorized as:

The first kind of particle system is the microparticulate system, followed by the nanoparticulate system.

Niosomes and Liposomes in the Vesicular System

Other: i) Drug delivery system that self-emulsifies (SNEDDS), ii) Transdermal medication administration

The particulate system is made up of tiny particles that may carry drugs into cells and are identified by certain receptors via ligand coupling. As a result, these systems are seen to be the best options for delivering medications that prevent diabetes (35).

Microparticle-based treatment allows for the controlled release of entrapped medications at the

Table 1. novel drug delivery of antidiabetic drug delivery for T2DM.

Type of delivery system	Class of drug	Drug	Polymer used	References
Niosome	insulin Secretagogues	Repaglinide	Span 60, cholesterol	40
Liposome	Incretin Mimetics	Glucagon-like peptide-1 (GLP-1)	Anionic liposomes Containing DSPEPG8G (10%), DPPC (27%), Cholesterol (36%) and DPPG (27%)	41
Polymeric Nanoparticles	Biguanides	Metformin	Chitosan-PLGA	42
Carbon Nanotubes	Biguanides	Metformin	---	43

desired location. Mechanisms control the drug's release rate to maintain its concentration in plasma. Because of their greater surface-to-volume ratio and smaller size, microparticles are used to improve the dissolving of insoluble medications. Receptor-mediated endocytosis is the transcellular transport mechanism for microparticulate systems. Because of their size, microparticles are unable to pass through the mucosal membrane's tight junctions and enter cells by paracellular transport; in contrast, nanoparticulate systems have greater intracellular absorption than microparticulate systems (36). The nanoparticles are divided into four categories: metallic, lipid-based, polymeric, and biological nanoparticles (NPs) (36). Drugs that are encapsulated in nanoparticles are delivered by both transcellular and paracellular pathways during cellular absorption (37). In addition, since the positively charged NPs interact electrostatically with the negatively charged mucus and endothelium layer to stay in the gastrointestinal system, they exhibit enhanced mucoadhesion. Sometimes, the mucous physically captures the NPs.

The transdermal delivery system (TDS) is an alternate method of medication administration to oral and parenteral routes. It is administrable, noninvasive, affordable, and patient-compliant. TDS may solve the issue of first-pass metabolism's premature drug metabolism (38). Using penetration enhancers, TDS may be a viable method for delivering hydrophilic medications, macromolecules, and vaccinations (39, 44). Table 1 shows several reports on innovative antidiabetic medication administration.

CONCLUSION

An ever-increasing number of people have diabetes as a result of the increasing incidence of obesity and sedentary lifestyles. This has led to a huge demand for anti-diabetic medications and increased investment by companies in research and development to create targeted formulations. Nanotechnology promises to introduce several real, revolutionary medicinal breakthroughs into our everyday lives. The development of nanoparticulate drug delivery systems for anti-diabetic medications has advanced significantly as a result of years of thorough nanoformulation research. The implementation of the most recent FDA rules for the regulation of these goods, together with long-term safety and ethical problems about nanoformulations, is necessary to facilitate the safety of said products and improve their effectiveness.

Longer-term glucose regulation may be possible with active targeting techniques that functionalize appropriate ligands or with combinatorial medication treatment that combines two or more antidiabetic medications. Such ongoing developments in nanotechnology provide promising opportunities for the creation of an effective treatment modality that lowers blood sugar levels in the near future.

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Authors' contributions

Conceptualization and writing the draft of manuscript Parinaz Shaqaqi, Nastaran Shojaei-Barjoei; All authors reviewed the manuscript.

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Availability of data and materials

The datasets analysed during the current study are available from the corresponding author upon reasonable request.

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Not applicable.

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Developing a Multiplex PCR Technique for the Identification of Halal and Non-Halal Meats

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Abstract:

Food safety is a major concern in most Muslim countries (e.g., Iran), where halal and non-halal meat adulteration is believed to cause significant social and health risks. Thus, developing a valid and reliable detection method to distinguish between halal and non-halal meats in processed foods is crucial, particularly for small-scale laboratories. In this regard, this current work aimed at designing and optimizing species-specific primers to identify halal and non-halal meats in processed foods with the use of the NADH Dehydrogenase and ATP Synthase genes via simplex and multiplex PCR assays. The results depicted that the DNA extracted from processed beef, poultry, and pork could be effectively amplified using the NADH Dehydrogenase and ATP Synthase primers, producing amplicon bands that were visible and consistent with the expected size in both simplex and multiplex PCR experiments. Despite the fact that non-specific bands were observed in several primer sets, the primary target bands remained distinct and easily identifiable. The existence of such primers is expected to promote the efficiency of halal food authentication, particularly in small-scale laboratories in rural regions of Iran.

INTRODUCTION

Iran has a large Muslim population and is globally ranked among the countries with the highest Muslim community. As such, observance of halal dietary regulations is a fundamental obligation for Muslims, who label certain food products (e.g., porcine-derived foods) as non-halal (haram)—prohibited for consumption (1). As a consequence, the availability of halal food is a critical concern in countries with large Muslim populations, such as Iran (2).

Meat is considered the principal source of protein in Iran, with poultry and beef being the most popular varieties (3). As declared by the Iranian Ministry of Agriculture, in the year 2024, the average annual consumption of poultry meat and red meat per inhabitant was respectively 27 kg and 14 kg. With the availability of several processed poultry products such as sausages, nuggets, chicken and beef meatballs, and meat floss, there is always the possibility that the halal meat products may have non-halal meat mixed during processing (4). Notably, among the many available products, halal meats, such as beef and lamb, are more susceptible to adulteration with pork by dint of analogous color and texture profiles (5). The mixing of non-halal and halal meats in processed products can result in consumer dissatisfaction, social and religious concerns, and potential health-related risks. Therefore, developing detection technologies capable of identifying porcine adulteration in processed foods is fundamental to ensure the integrity of halal meat products (5, 6).

To date, a number of analytical techniques have been developed and proposed for detecting pork adulteration in food products, videlicet Enzyme-Linked Immunosorbent Assay (ELISA) (7, 8, 9), high-performance liquid Chromatography-Mass Spectrometry (HPLC-MS) (10), Loop-Mediated Isothermal Amplification (LAMP) (11, 12), conventional Polymerase Chain Reaction (PCR) (13), Real-Time PCR (RT-PCR) (14, 15, 16), and gold nanoparticle-based assays (17, 18). Among these methods, conventional PCR is found to be a relatively easy-to-use and accessible technique, and this has made it a good candidate for use in small-scale laboratories in Iran. Not to mention that the PCR-based detection method enjoys high sensitivity that enables

the identification of porcine DNA contamination at concentrations as low as nanogram levels.

In standard PCR-oriented detection methods, molecular markers have been developed to recognize pork adulteration in processed products by targeting specific gene sequences. Typically, mitochondrial genes such as 12S rRNA, 16S rRNA, cytochrome b (cyt b), and D-loop have so far been used to develop these markers (19, 20, 21). Nonetheless, alternative genes, viz NADH Dehydrogenase and ATP Synthase have marginally been recruited to date (22). These genes encode essential enzymes involved in the mitochondrial respiratory chain, with NADH Dehydrogenase facilitating electron transfer and ATP Synthase catalyzing the ATP synthesis from ADP (23). These housekeeping genes play a pivotal role in balancing cellular respiration and showing high sequence conservation across different animal species. This has made these genes promising candidates for molecular marker development (23, 24).

Previous research (24) demonstrated the efficacy of a multiplex PCR assay that used the NADH Dehydrogenase gene as a molecular marker to identify meat from four different animal species (pig, ruminant, bird, and rabbit). Nonetheless, no previous work has been reported to have applied the ATP Synthase gene in this context. Noteworthy, the aforementioned study (24) focused on raw meat samples, whereas the present investigation aimed at developing and validating species-specific primers for differentiating between halal and non-halal meats in processed food products using simplex and multiplex PCR approaches. For this purpose, the NADH Dehydrogenase and ATP Synthase genes were considered molecular markers. The development of these primers is expected to enhance the precision and efficiency of halal food authentication, more specifically in small-scale Iranian laboratories, where access to advanced molecular diagnostic tools might be limited.

MATERIALS AND METHODS

Food samples

A total of seven processed food products, namely sausage, nuggets, meatballs, rendang, dendeng, floss, and skin crackers, were purchased from local markets and online retailers (see Table 1). The primary ingredients of these products included pork, beef, and chicken. The samples were stored at -20 ℃ in a refrigerated environment to protect their integrity and prevent degradation until further analysis.

Genomic DNA Extraction

A modified protocol (25) was used to isolate genomic DNA from the processed food samples. In brief, a sterile mixer was used to homogenize 0.1 g of each sample in 500 µL of lysis buffer (250 µL of 10%

Table 1. List of processed foods used in this study

Sample code	Processed food sample	Processed food source
1	Beef rendang	Local market
2	Beef dendeng	Local market
3	Beef floss	Online shop
4	Beef sausage	Local market
5	Beef meatball	Local market
6	Chicken floss	Online shop
7	Chicken meatball	Local market
8	Chicken nugget	Local market
9	Chicken sausage	Local market
10	Pork sausage	Online shop
11	Pork floss	Online shop
12	Pork meatball	Online shop
13	Pork dendeng	Online shop
14	Pork rendang	Online shop
15	Pork skin cracker	Online shop

sodium dodecyl sulfate (SDS) and 250 µL of sodium chloride-tris-EDTA (STE)). The obtained mixture was transferred to a 2 mL microtube and incubated at 58°C for 1 hour with 20 µL of Proteinase K, with periodic mixing every 10 minutes ensuring uniform digestion. Afterwards, the mixture was subjected to two consecutive extractions with 800 µL and 600 µL of chloroform:isoamyl alcohol solution (24:1), respectively, with centrifugation at 12,000 rpm for 10 minutes at 20°C between each extraction. Next, the acquired supernatant was precipitated with 3 M sodium acetate (pH 5.2) and cold isopropanol, followed by incubation at -20 °C for 1 hour and centrifugation at 12,000 rpm for 10 minutes at 20°C. The DNA pellet was washed with 70% (v/v) ethanol, dried overnight at room temperature, and reconstituted in 100 µL of TE buffer (10 mM Tris, pH 8.0, and 1 mM EDTA). The DNA concentration was adjusted to 20 ng/µL for subsequent amplification. The quality and purity of the isolated DNA were then evaluated with the use of the NanoDrop 2000 Spectrophotometer (Thermo Scientific, USA), with high-quality DNA exhibiting an A260/A280 ratio between 1.8 and 2.0.

Primer Designs

Gene sequences of the NADH Dehydrogenase and ATP Synthase genes from bovine (Bos taurus), chicken (Gallus gallus), and pig (Sus scrofa) were retrieved from the National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/gene/). The retrieved sequences were subsequently used to design specific primers using the Primer3Plus software (https://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) (26). To ensure specificity, the designed primers were then evaluated against reference sequences (RefSeq mRNA) from cattle, chickens, and pigs utilizing the Primer-BLAST tool(https://www.ncbi.nlm.nih.gov/tools/primer-blast/) on the NCBI platform. Confirmed by BLAST analysis, the primer design was then optimized to ensure a single annealing site for each primer (see Table 2 for the acquired primer sequences).

Simplex PCR assay

To evaluate the specificity and efficacy of the newly designed primers, a simplex PCR assay was carried out using the primers that targeted the NADH Dehydrogenase and ATP Synthase genes. The PCR reaction mixture comprised 2 µL of 20 ng/µL DNA template, 5 µL of 2x MyTaq HS (Bioline, UK), 0.5 µL

Table 2. Primer pairs that were used in this study

Gene	NCBI Accessions Number	Species	Primer (5'-3')	Ta* (°C)	Product Size (bp)
NADH Dehydrogenase	NM_174565.3	Bovine	F: GGAGCTGGAGGAGCCTTATT R: TTTCCAACAGGCTTTCGATT	58	290
	XM_046907522.1	Chicken	F: CTGAAGACCCACAGCAGACA R: GAGGACGCTGTCCTTCACTC	58	405
	NM_001243796.1	Pig	F: CTGCCTGTGAGGAAGGAAAG R: AGGATTTCGGCCACATACTG	58	113
ATP Synthase	NM_175796.3	Bovine	F: AGCCCATGGTGGTTACTCTG R: TTGCCACAGCTTCTTCAATG	58	881
	NM_001031391.3	Chicken	F: AGGGCAACGATTTGTACCAC R: GGTCAGCTTGTCCTCCTCAG	58	635
	NM_004046.6	Pig	F: TCCAGAAATGCTTTGGGTTC R: ATTGGCACCAAGCTATCCAC	58	542

*Ta: temperature annealing

each of forward and reverse primers at a concentration of 10 µM, and sterile ddH2O, in a total reaction volume of 10 µL. The PCR amplification was implemented in a Thermocycler (Biorad, USA) with the use of a thermal cycling profile consisting of an initial denaturation at 95°C for 5 minutes, followed by 32 cycles of denaturation at 94 °C for 30 seconds, annealing at 58 °C for 1 minute, and extension at 72 °C for 1 minute. The final extension was executed at 72 °C for 5 minutes. The resulting PCR products were separated by 1% agarose gel electrophoresis in a 1x TBE buffer at 90 V for 60 minutes. Afterwards, the gel was stained with a 10 mg/ml solution and was visualized under ultraviolet light using a UV transilluminator (Biorad, USA). For comparison, a previously reported primer (27).

Multiplex PCR assay

To simulate the detection of adulteration between non-halal meat (pork) and halal meats (beef and poultry) in processed food products, a multiplex PCR assay was conducted by combining DNA templates from processed meat products containing pig with those from products containing beef or chicken DNA in a 1:1 ratio (see Table 3). Specifically, 1 µL of DNA template from a beef sausage sample was mixed with 1 µL of DNA template from a pork sausage sample, and subsequently amplified in a total reaction volume of 10 µL, comprising 5 µL of 2x MyTaq HS (Bioline, UK), 0.5 µL each of forward and reverse primers at a concentration of 10 µM, targeting the bovine, poultry, and pig NADH Dehydrogenase and ATP Synthase genes in a 1:1:1 ratio, and sterile ddH2O. The thermal cycling profile and electrophoresis procedure were identical to those employed in the simplex PCR experiment. Furthermore, a multiplex PCR experiment was also conducted using a previously reported primer (27) as a control, following the same PCR and electrophoresis protocols as the newly developed primer.

RESULT

DNA Concentrations and Purities

The DNA concentration and purity measurements obtained by the NanoDrop 2000 Spectrophotometer (ThermoScientific, USA) are shown in Table 4. While the values of DNA purity ranged from 1.38 (pork rendang) to 2.02 (chicken nugget), the DNA concentrations extracted from processed food samples varied from 25.9 ng/µL (chicken floss) to 669.1 ng/µL (beef dendeng). Notably, the average DNA concentration and purity values for the entire dataset were 230.93 ng/µL and 1.77, respectively. Although some samples exhibited DNA purity values below the optimal threshold of 1.80, the repeated extraction process that used chloroform:isoamyl alcohol (24:1) mixture significantly reduced contamination by proteins and other impurities, as evidenced by the median DNA concentrations. The combination of chloroform and isoamyl alcohol was found to be essential for the degradation of protein and prevention of emulsion formation, which resulted in the enhancement of DNA precipitation and improvement of the overall quality of the extracted DNA (28).

The simplex PCR results using NADH Dehydrogenase primers yielded distinct and well-defined amplicon bands (see Figure 1). The amplicon sizes obtained from each processed product derived from beef, poultry, and pork corresponded to the expected target sizes of 290, 405, and 113 base pairs, respectively. Nevertheless, the primer obtained from the bovine sequence showed non-specific amplicon bands that were larger than the intended size, which were difficult to eliminate. Yet, as the primary amplicon band for this primer was visible and distinct from the non-specific bands, this result is still considered acceptable.

Consistent with the results obtained using NADH Dehydrogenase primers, the simplex PCR results

Table 3. DNA combination from processed food for multiplex PCR assay in this study

Sample Code	Food Processed Combination
A	Beef and pork rendang
B	Beef and pork dendeng
C	Beef and pork floss
D	Beef and pork sausage
E	Beef and pork meatball
F	Chicken and pork floss
G	Chicken and pork meatball
H	Chicken and pork sausage

Table 4. DNA concentration and purity extracted from processed foods in this study.

Sample code	Processed food sample	DNA concentration (ng/µL)	DNA purity (A_{260}/A_{280})
1	Beef rendang	478.4	1.80
2	Beef dendeng	669.1	1.77
3	Beef floss	444.8	1.93
4	Beef sausage	138.4	1.94
5	Beef meatball	314.0	1.87
6	Chicken floss	25.9	1.57
7	Chicken meatball	416.4	1.67
8	Chicken nugget	121.5	2.02
9	Chicken sausage	41.6	1.88
10	Pork sausage	186.6	1.71
11	Pork floss	41.0	1.50
12	Pork meatball	227.0	1.92
13	Pork dendeng	47.2	1.96
14	Pork rendang	31.3	1.38
15	Pork skin cracker	280.8	1.59
	Average	**230.93**	**1.77**

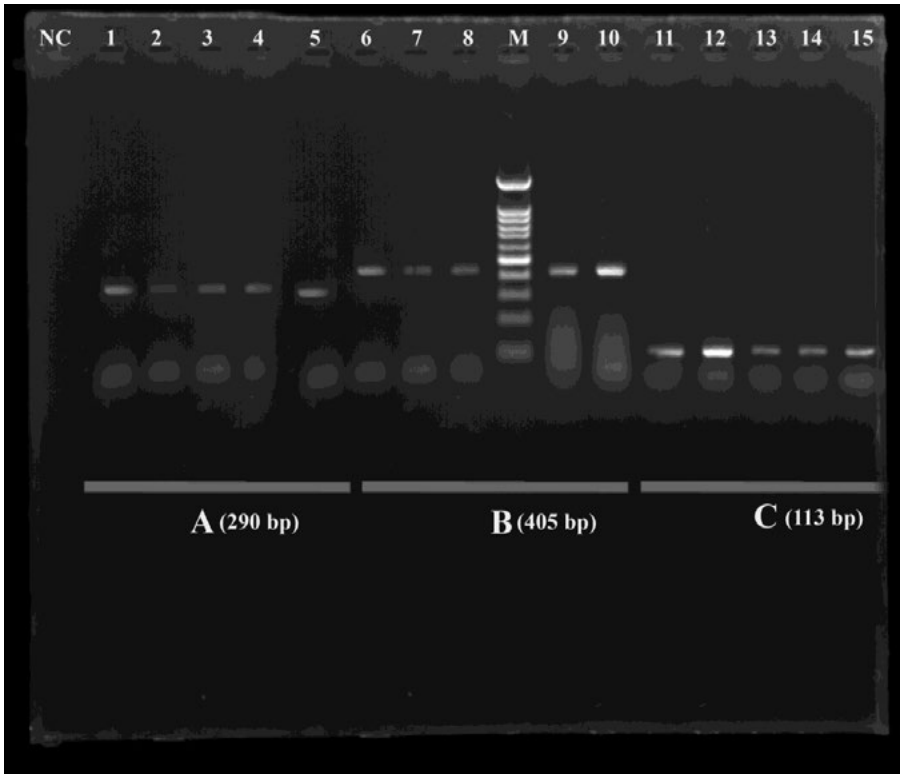

Fig1. Identification of simplex PCR results from: (A) 290 bp of beef, (B) 405bp of poultry, and (C) 113bp of pork, generated with NADH Dehydrogenase primers. M: 100 basis points DNA ladder, 1-15: enumeration of processed meals as delineated in Table 1.

employing ATP Synthase primers also produced visible amplicon bands (see Figure 2). The amplicon sizes achieved from each processed product derived from beef, poultry, and pork corresponded to the expected target sizes of 881, 635, and 542 base pairs, respectively. It should be noted that despite the presence of non-specific bands, the principal amplicon band for each primer remained distinct and well-defined, which is consistent with the results obtained using NADH Dehydrogenase primers for bovine, pig, and chicken.

In contrast to the results obtained with the NADH Dehydrogenase and ATP Synthase primers, the simplex PCR assay that used primers from earlier work (27) also yielded visible amplicons (see Figure 3). The amplicon sizes obtained from each processed product derived from beef, poultry, and pork corresponded to the expected target sizes of 274, 227, and 398 base pairs, respectively. Notably, the use of these primers led to a considerable reduction in non-specific amplicon bands, with all primers from bovine, chicken, and pig producing a single, distinct, and readily identifiable single band.

Multiplex PCR Assay

The results from multiplex PCR that used DNA mixtures from processed pork mixed with beef or chicken revealed the existence of clearly visible amplicon bands that matched the expected sizes for NADH Dehydrogenase, ATP Synthase, and Matsunaga et al. (1999) primers (see Figure 4). It is noteworthy that the genetic material from each component in the mixture was successfully identified and isolated, showing no evidence of interference from non-specific amplicons. The findings unraveled that the multiplex PCR assay is capable of simultaneously detecting and identifying the genetic material from diverse sources, thereby making the detection of any impurities in processed meat products easier.

This study employed a multiplex PCR approach to simulate real-world scenarios of halal detection in processed food products, including adulteration with non-halal components. A hypothetical example of a beef meatball from brand A suspected of containing pork was used to demonstrate the feasibility of the method. The approach involved isolating and amplifying DNA from the meatball using primer pairs targeting NADH dehydrogenase and ATP synthase genes in both bovine and porcine samples. To evaluate the efficacy of the primer mixture, DNA mixtures from bovine or chicken products were combined with DNA from pork products and amplified using a multiplex PCR assay with NADH dehydrogenase, ATP Synthase, and previously established primer pairs (27) for both bovine or chicken and pork. The results demonstrated that the primer mixture could successfully differentiate between halal (beef or chicken) and non-halal (pork)

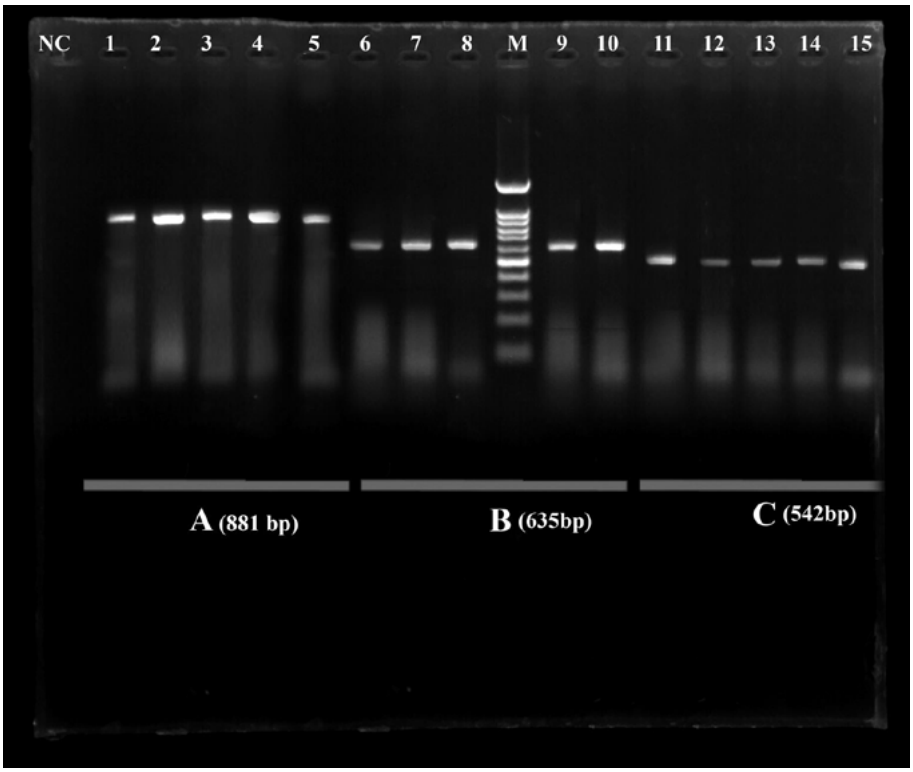

Fig 2. Characterization of simplex PCR results from: (A) beef, (B) chicken, and (C) pork, generated with ATP Synthase primers. M: 100 basis points DNA ladder, 1-15: enumeration of processed meals as delineated in Table 1.

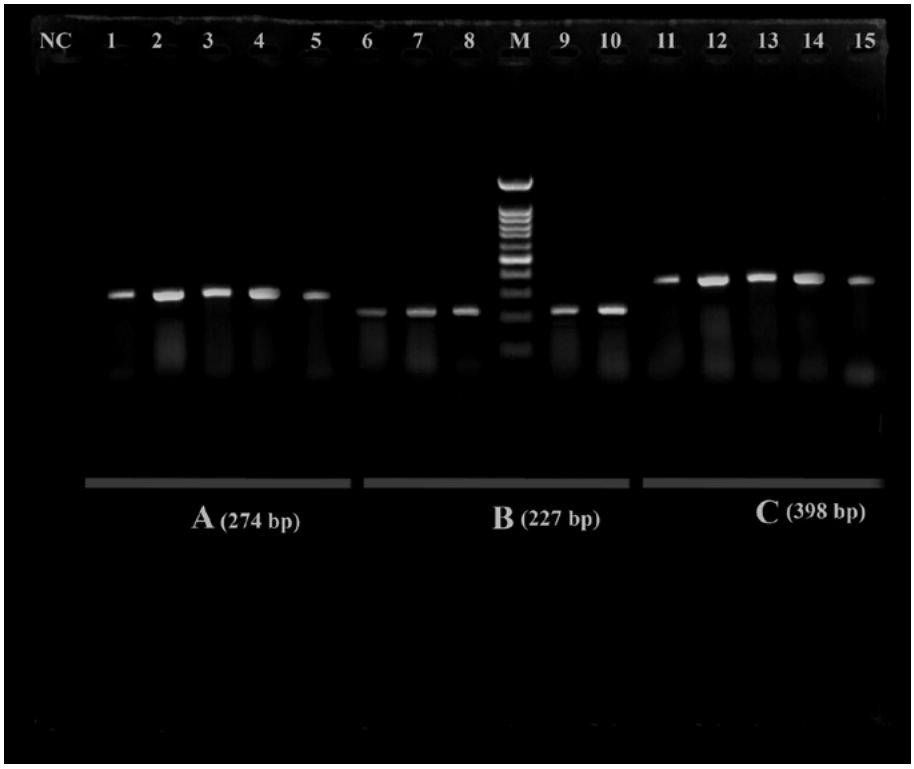

Fig3. Identification of simplex PCR results from (A) beef, (B) chicken, and (C) pork, generated with primers from prior research (27). M: 100 basis points DNA ladder, 1-15: enumeration of processed meals as delineated in Table 1.

DNA based on amplicon band sizes as separated in agarose gel, indicating the potential of this approach for detecting adulteration in processed food products.

DISCUSSION

Safeguarding food safety and security is a notable global concern, with food adulteration as a significant threat to public health. In Iran, food adulteration is still a persistent issue despite the establishment of the National Agency for Drugs and Foods Control (BPOM), which is responsible to regulate the food industry and oversee halal product certification (29). It is worth mentioning that small and medium-sized enterprises (SMEs) and traditional market sellers are more inclined to be engaged in meat adulteration (30). As such, developing a reliable detection technology that can recognize halal from non-halal meat in processed foods is essential to reduce such risks.

As an Islamic nation, the establishment of small-scale labs capable of conducting halal food detection in rural areas is essential to bolster the food safety program in Iran. Conventional PCR is a technology appropriate for use in small-scale labs in Iran due to its speed, sensitivity, accuracy, affordability, and ability to identify DNA contamination in minimal quantities, down to nanogram levels (31, 32).

In this study, the novel primers derived from the NADH dehydrogenase and ATP synthase genes effectively amplified all samples, producing clear and distinct amplicons that matched the target size in both simplex and multiplex assays. The consistency of amplicon size between the two PCR procedures was also observed. Furthermore, the previously developed primers (27, 32) targeting the cytochrome b gene also showed high amplification efficiency, yielding clear and visible bands of the predicted size.

The original primer set that was developed in this study could successfully amplify the target genes but also yielded non-specific amplicon bands. Multiplex PCR is a simultaneous amplification procedure of several DNA targets inside a single reaction, and primer design is a vital and fundamental phase in the construction of a multiplex PCR test (17, 31, 32). In the design process, several parameters, namely primer length, GC content, melting temperature, and potential secondary structures (e.g., such as dimers and hairpin loops) must be computed meticulously to ensure specific and efficient amplification of the target genes. Furthermore, other factors, such as PCR conditions, DNA template concentration, and PCR cocktail composition, that can influence multiplex PCR performance, must be thoroughly evaluated and optimized during the development process.

The annealing temperatures for the NADH dehydrogenase and ATP synthase primers, specifically 58 °C, were determined through prior

optimization studies. Yet, increasing the annealing temperature for the bovine NADH Dehydrogenase and pig ATP synthase primers to 58 °C or higher may help eliminate non-specific amplicon bands. Nevertheless, this approach may compromise the amplification of other primers, resulting in reduced or absent amplicon bands due to suboptimal annealing conditions. Therefore, the annealing temperature was carefully selected to make sure that all primers were able to produce distinct and visible amplicon bands. This optimization procedure is a critical constraint in multiplex PCR assays, as it is challenging to simultaneously optimize the melting, annealing, and elongation temperatures for multiple primers while minimizing the formation of secondary structures, primer-dimers, and non-specific amplicon bands (34). Notably, as shown in the current work, if the primary target band remains detectable and visible, the existence of non-specific bands may be tolerated.

The novel primers developed in this study have the potential to improve the halal detection technique using traditional PCR, which has been previously employed with various genes, including 12S rRNA, 16S rRNA, cyt b, and D-loop. The ability of these primers to amplify small amounts of DNA extracted from processed foods makes them a valuable tool for halal identification, thereby enhancing food safety programs in Iran. Furthermore, the newly designed primers may be adapted for use in a multiplex end-point PCR approach, as demonstrated in previous studies (27, 34), by utilizing a single universal forward primer in combination with multiple species-specific reverse primers, which could help minimize production costs.

CONCLUSION

The novel primer sets developed in this study, targeting the ATP synthase and NADH Dehydrogenase genes, demonstrated high efficacy in amplifying DNA from all processed food samples in both simplex and multiplex PCR assays. The generated amplicon bands were visible and corresponded to the expected size, comparable to primers from a previous investigation. However, a limitation of this study was the observation of non-specific bands in the bovine NADH Dehydrogenase and pig ATP Synthase primers. Nevertheless, the presence of these non-specific bands was deemed acceptable, as the primary target band remained distinct and visible. The newly developed primers in this study have the potential to improve the halal detection technique using traditional PCR, which has been established to date, and could serve as a valuable tool for halal detection, thereby supporting food safety programs in Iran.

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Authors' contributions

Conceptualization: Romina HosseinZadeh, Methodology; Romina HosseinZadeh and Zahra Sorkhou, Investigation: Romina HosseinZadeh and Zahra Sorkhou, Writing-review and editing: Romina HosseinZadeh and Zahra Sorkhou. All authors reviewed the manuscript.

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Availability of data and materials

The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

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A Review of Exosomes: Isolation Methods and Applications

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Abstract:

Exosomes are becoming recognized as crucial facilitators of interaction between cells. They deliver biological agents to target cells, play essential roles throughout multiple biological and pathological events, and have significant potential as innovative alternative therapies for illnesses. Exosomal communication between cells appears to have a role in the development of several illnesses, involving cancer, neurodegenerative conditions, and inflammatory disorders. Exosomes are diminutive (20–150 nm) entities characterized by a unique bilipid protein architecture. They transport and switch diverse cargos across cells and serve as noninvasive indicators for multiple disorders. Exosomes are regarded as the most effective indicators for cancer detection due to their distinctive properties. This document will examine the current uses of exosomes, their origins, and diverse isolation techniques. Furthermore, the function of exosomes and their use in biomedical studies and preclinical experiments are succinctly addressed.

Summary of extracellular vesicles (EVs)

Synthetic delivery of drug technologies, such as polymeric nanoparticles, dendrimers, micelles, and liposomes, have been utilized to improve pharmaceutical efficacy and curative usefulness in clinical contexts (1). Notwithstanding the considerable benefits of liposomes, the most established and extensively researched drug delivery system, their utilization is constrained by their low stability, long-term safety concerns, and the potential to trigger severe hypersensitivity responses (2). EVs, considered a natural transport system, may surmount the limitations associated with liposomes as well as additional artificial drug transport methods. EVs are categorized based on their size, source, appearance, and operations, including numerous kinds of microvesicles generated from the plasma membrane (3). Exosomes, as a subgroup of EVs, are now garnering significant interest from the scientific community. Exosome creation transpires in three phases: budding, multivesicular body (MVB) development, fusion of the plasma membrane with MVBs, and the dissolution of vesicular components as exosomes (4). In contrast to apoptotic bodies (1000–5000 nm) and microvesicles (50–1000 nm), which are produced by dead cells and the external budding of the plasma membrane respectively, exosome biosynthesis commences with the inward budding of the plasma membrane, typically initiating the formation of intraluminal vesicles (ILVs) within early endosomes (5). Endocytosis results in the formation of early endosomes that sequester cellular proteins and genetic materials from the cytoplasm, subsequently transforming to late endosomes, which give rise to multivesicular bodies (MVBs). MVBs are subsequently destroyed by lysosomes or merged with the plasma membrane to release ILVs as exosomes (6).

The therapeutic uses of exosomes are many and diverse. A very interesting study domain is reconstructive medicine, whereby exosomes produced from stem cells have demonstrated the capacity to facilitate regeneration and repair of tissues (7). Exosomes have been employed for the treatment of heart disorders, including myocardial infarction,

by facilitating angiogenesis and enhancing cardiac function. Exosomes have also been investigated as a therapeutic modality for neurological illnesses, such as Parkinson's disease and Alzheimer's disorder (8).

Besides the possibility of treatment, exosomes have been examined for their monitoring qualities. Exosomal indicators have been discovered for several illnesses, such as cancer, facilitating the creation of non-invasive diagnostic assays (9). Moreover, exosomes have been investigated as a strategy for delivering tailored medicines to certain cells or tissues, overcoming the constraints of conventional delivery techniques. Notwithstanding the considerable advancements in exosome research, several obstacles and ambiguities remain to be resolved (10). The best techniques for the separation and purification of exosomes are still under discussion, and both the stability and the bioavailability of exosomal cargo continue to be problematic. The regulatory framework governing the application of exosomes as medicinal products is in flux (10).

This critical study seeks to offer a thorough summary of the existing information about the therapeutic uses of exosomes. We will conduct a critical study of the current literature to examine the advantages and drawbacks of exosomes as a means of treatment, discuss the obstacles and difficulties that require resolution, and provide suggestions for additional study avenues.

Composition of exosomes

Exosomes are endowed with lipids, proteins, and DNA or RNA throughout biosynthesis. Lipids such as sphingomyelin, cholesterol, ganglioside GM3, desaturated lipids, phosphatidylserine, and ceramide constitute the bilayer framework of the exosomal membrane and enable exosomes to serve as cargo transporters (11). Proteins found in the outer layer or cytoplasm of exosomes involve fusion proteins, enzymes, chaperones, and proteins associated with MVB production (e.g., CD9, Annexin A2, Meosin, ICAM1, MHC) (12).

Nucleic acids present in exosomes including mRNAs, miRNAs, other non-coding RNAs, and DNAs. Our high-through investigations identified around 6000 recognized proteins, with over 85% of these recognized miRNAs residing in brain cell-derived exosomes, indicating the exceptional compatibility of exosomes in encapsulating molecules with diverse physical and biological characteristics (13).

The chemical compositions of exosomes are significantly affected by the kinds and conditions of their parent cells. We noted that stimulated microglia, the resident immune cell population in the Central Nervous System (CNS), emit exosomes full of cytokines, while neural stem/progenitor cells (NPCs) generate exosomes plentiful in growth factors (14). These exosomes may permeate the blood-brain barrier into the peripheral circulation. Consequently, the unique profiles of illness-associated chemicals in exosomes separated from bodily fluids are regarded as prospective markers for diagnosing illnesses (15).

Exosomal cargos may be passively encapsulated; however, new research has also found several sorting mechanisms that actively incorporate molecules into exosomes. The endosomal selecting complex needed for transport (ESCRT) is identified as an essential carrier for the translocation of proteins into exosomes (16). Ubiquitylated proteins are identified by ESCRT-0 and directed to the endosomal membrane, while ESCRT-1 and ESCRT-2 facilitate bud development with the chosen proteins, and ESCRT-3 finalizes vesicle scission (17). Additionally, an ESCRT-independent process contributes to protein sorting into exosomes, primarily facilitated by ceramides, tetraspanins, or heat shock proteins. Nucleic acids are compartmentalized into exosomes with the aid of RNA-binding proteins (18). The ESCRT-2 complex, in conjunction with tetraspanin-mediated microdomains, the miRNA-induced silencing complex (miRISC), and phosphorylated argonaute 2 (AGO2), identifies certain RNA patterns and aids in their entrapment by exosomes. Consequently, although the mechanisms behind cargo sorting into exosomes are mostly unclear, it is apparent that cargos may be incorporated into exosomes by both random and selective processes (18).

Application of exosomes

Extracellular vesicles execute several roles in biological circuits. Microvesicles are involved in cell signaling, apoptotic bodies convey the contents of dying cells to healthy cells, and exosomes facilitate interactions between cells in many physiological and pathological processes. This text presents instances of exosome function in its native form (19).

Proteins on exosome surfaces

Exosomes are pivotal in the induction and inhibition of the immune system, capable of modulating immune system reactions through antigen presentation on their outermost layer (20). Exosome membranes merge with MHC–antigen complexes, eliciting antigen-specific T-cell responses that contribute to the beginning and development of inflammation. The exosomal receptors on the surface CD86 and lymphocyte function-associated antigen 1 (LFA-1) initiate inflammatory processes that stimulate immune system cells (21). Exosome surface proteins contribute to immune repression; exosomes from tumor cells expressing programmed death-ligand 1 (PD-L1), a regulatory checkpoint molecule, may block cytotoxic T-cell activity and enable the immunological evasion of tumor cells. In addition to surface proteins, exosomes

have been shown to transport protein, DNA, and RNA cargos that may elicit immune system reactions and other biological processes (22).

Transportation of cargo

The exosomal transfer of proteins, nucleic acids, and lipids from donor to recipient cells signifies a recently recognized mechanism of communication between cells. The delivery of exosomal cargo, particularly nucleic acids, might influence the behavior of the destination cell (23). The miRNA content of mesenchymal stem cell (MSC)-derived exosomes facilitates myocardial healing, acting as a substitute for MSCs (24). Exosomes produced from MSCs have a greater capacity to avoid hypertrophy or destruction compared to the MSCs themselves in vitro, attributable to variations in miRNA content among the exosomes and their original cells (25). The exosomal transport of water-soluble cytokines, growth factors, and hormones serves as an essential system for intercellular interaction, facilitating interaction over extensive distances to influence systemic immune responses. The lipid content of exosomes seems to possess many activities, including the regulation of metabolism and immunological responses in recipient cells (26).

The onset of metastasis in cancer development is facilitated by the epithelial-mesenchymal transition, which involves the conversion of sticky epithelial cells into migratory, aggressive mesenchymal cells (27). Exosomes originating from mesenchymal cells possess a unique payload compared to those from epithelial cells, and these mesenchymal-derived exosomes may facilitate migration. Exosomes inside the tumor environment may facilitate tumor proliferation by transporting proteins, lipids, and nucleic acids that induce immune suppression. Fatty acid cargo from tumor-derived exosomes may inhibit the immune system reactions of recipient dendritic cells (28).

Application of biomarkers

Exosomes are found in several biofluids, potentially offering a novel means to assess cellular state in both normal physiology and the emergence of disease disorders (29). Exosomes and other EVs may be profiled for their cargo by an easy liquid biopsy of bloodstream fluids, urine, or saliva, facilitating applications in evaluation, prediction of disease progression, and chemoresistance biomarkers (30). Although various cell types secrete exosomes, data indicates that exosome production elevates in certain pathological situations, including cancer. Exosomes exhibit stability in many bodily fluids and protect biomolecules from destruction. Pathological situations may manifest cellular alterations in the physiological constituents of exosomes produced by cells (31). The modified cargo of exosomes may be detected and evaluated using transcriptomics, proteomics, and lipidomics studies; hence, variations in the quantities of certain compounds might be advantageous for biomarker usage (32). Various laboratories have demonstrated variable expression of miRNAs in cancer cells and their exosomes in vitro; also, some research done on ex vivo and animal cancer models have corroborated alterations in miRNA expression levels. Various miRNAs are under development, involving miRNA-200-5p, miRNA-378a, miRNA-139-5p, and miRNA-379 for lung cancer, as well as miRNA-21 for oesophageal squamous cell carcinoma, which has been proposed as non-invasive detection biomarkers (33). Furthermore, exosomal proteins have biomarker potential for several malignancies. In oncology, the prompt identification of a neoplasm is crucial for successful treatment (34). A comprehensive understanding of the mechanisms governing exosomal cargo organizing and production, along with their transport via specific bodily fluids and stable levels throughout physiological conditions and various disease situations, would yield more robust biomarkers for tracking the start of disease and progression. Moreover, additional research is necessary to confirm the specificity and sensitivity of exosomal biomarkers in comparison to conventional cancer markers (35, 36).

Principal methods for the isolation of exosomes

Various exosome separation techniques have been established depending on their size, morphology, density, and surface protein composition (37). The significant similarity in physical and chemical characteristics among exosomes and non-exosome vesicles resulted in the presence of many non-exosome vesicles, including microvesicles and apoptotic bodies, in “exosome specimens” generated using established methods. Consequently, unless stated contrary, the word “exosome” in this article denotes a composite of tiny extracellular vesicles, including exosomes, apoptotic bodies, microparticles, and microvesicles (38).

Ultracentrifugation

Ultracentrifugation is the benchmark method for exosome separation, employed in 80 percent of exosome processing procedures. Ultracentrifugation requires little preparation of the sample and is cost-effective, with the exception of the initial expense of the equipment. Nonetheless, it is labor-intensive and attains only modest exosome purity (39). Ultracentrifugation segregates specimen constituents according to density; yet, there exists considerable overlap in the density areas of several EV kinds. Centrifugal forces segregate samples into distinct layers; high-density particles descend to the bottom of the tube, whilst low-density fragments ascend to the

top. The ultracentrifugation rates utilized for exosome separation vary from 100,000 × g to 210,000 × g. Enhancing the velocity may facilitate extractions but poses a risk of compromising the exosomes (40, 41).

Differential centrifugation is a type of ultracentrifugation method that uses several sedimentation cycles to isolate target exosomes from cellular detritus, bigger vesicles, and proteins (42). The technique requires regular user interaction to eliminate supernatants and pellets and to initiate spin cycles. Exosomes may be lost throughout the multiple extractions of supernatant and the movement of samples between tubes; hence, exosome attrition is anticipated, prompting the application of larger sample amounts at the outset of processing to achieve the required yield. Notwithstanding these limitations, differential centrifugation is a reliable method that routinely yields exosomes of intermediate both purity and quality (43).

Ultrafiltration

Ultrafiltration is characterized by the application of minuscule pores (about 100 nm in diameter) and may be employed to segregate exosomes according to size. Ultrafiltration techniques are expeditious, with a single filtration cycle lasting from seconds to 30 minutes, facilitating high efficiency (41). Ultrafiltration separates vesicles by exerting force to propel sample fluid across membranes with about 100 nm holes. Membranes with varying pore diameters might be utilized in subsequent stages to eliminate further undesirable particles (44). The procedure is more rapid than ultracentrifugation; nevertheless, the applied pressure may harm exosomes owing to shear stress and might lead to exosome depletion from membrane adherence and obstruction caused by particle buildup, hence diminishing exosome yield and extending processing time. Membrane cleaning methods have been established to address these challenges (45).

Exosome ultrafiltration methods encompass arranged filtration, conjunction filtration, centrifugal ultrafiltration, and tangential flow filtration. Sequential and tandem filtration are dead-end techniques that are executed with a syringe (46). Sequential filtration involves numerous filtration phases, everyone utilizing a distinct molecular weight threshold, while tandem filtration integrates several filters within a single needle. The size-exclusion thresholds for exosomes generally range from twenty to two hundred nm; in tandem filtration, exosomes are retained in an intermediary membrane (47).

Precipitation

Precipitation techniques are extensively used in the characterisation of electric vehicles. A global study indicated that precipitation is the favored method for EV RNA investigation. Precipitation techniques employ volume-excluding polymers to sequester water molecules and precipitate less soluble constituents from the solution (48). Biological substances are removed from the solvent-containing areas inhabited by these polymers and become concentrated when their solubility limit is surpassed, resulting in precipitation. This strategy is frequently employed with other isolation techniques. This approach yields more output; nevertheless, it is constrained by the reduced quality of the goods (49).

Size-exclusion chromatography

Size exclusion chromatography (SEC) is the most delicate chromatography technology, extensively utilized in the separation as well as purification of biopolymers, including proteins and polysaccharides (50). The isolation of exosomes by SEC maintains vesicle integrity and biological activity while achieving a high yield. The SEC technique segregates biomolecules according to variations in hydrodynamic radius as they traverse an inert, low-adsorption resin composed of a hollow bead matrix inside a column (51). The particles exceeding pore size elute initially, however, smaller fragments and molecules infiltrate the pores to differing extents according to their size, resulting in prolonged elution times as particle or molecule size diminishes (52). To achieve high-resolution particle size, it is essential to examine processing parameters like column measurements, bead wrapping, resin type, velocity, and system capacity. This technique may be used to differentiate samples over a spectrum of viscosities, ranging from low-viscosity urine and cells grown in medium to high-fluidity plasma. Nonetheless, pretreatment of materials using ultracentrifugation or ultrafiltration is essential to get extracellular vesicle preparations devoid of protein and lipoprotein contaminants (53).

Exosomes and oncological biology

Exosomes stimulate tumor cell growth

Recent research indicates that cell-derived exosomes significantly contribute to tumor development and advancement (54). Recombinant epidermal growth factor (EGF) administration may enhance the absorption of exosomes generated from oral squamous cell carcinoma (OSCC) cells into OSCC cells (54). Conversely, the suppression of the EGF receptor (EGFR) or the use of EGFR blockers, such as erlotinib and cetuximab, eliminates exosome absorption by OSCC cells. Exosomes generated from cells have been demonstrated to promote growth, movement, attack, stemness, and chemoresistance in OSCC cells. These chemicals may initiate signaling cascades and enhance tumor cell growth by attaching to receptors on the tumor cell membrane (55).

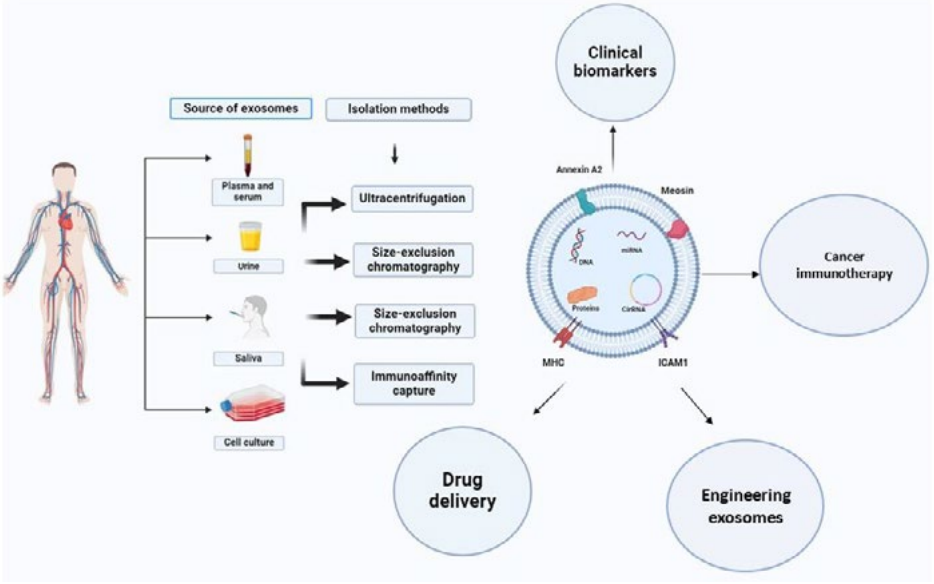

Fig 1. Schematic representation of exosome sources, isolation methods, and their uses. Exosomes include various proteins and nucleic acids that are characteristic of the kind and condition of the originating cells. The distinctive double-layer membrane of exosomes safeguards the biomolecules they transport, allowing these exosomes, which contain specific chemicals, to persist in diverse bodily fluids for extended periods, facilitating their detection and extraction. These exosomes may be modified for several therapeutic uses.

Exosomes facilitate the spread of malignant cells.

Comprehensive studies on the tumor environment indicate that exosomes generated by cancer cells play a significant role in essential aspects of cancer development, such as blood vessel formation, premetastatic niche formation, extracellular matrix development, epithelial-mesenchymal transition (EMT), cancer stem cell proliferation, and persistence against treatment (56). Research established that exosomes produced from cancer-associated fibroblasts (CAFs) are a significant factor in the advancement of ovarian cancer (OVCA). This work demonstrated that the circRNA molecule hsa_circIFNGR2 may clarify the new role of CAF-derived exosome circIFNGR2 in ovarian cancer cell development and metastasis (57). Metastatic organ tropism continues to be one of the most significant enigmas in cancer genesis since the formulation of the "seed and soil" concept. Researchers discovered that EGFR present in exosomes released by gastric cancer cells may be transported to the liver and incorporated into the plasma membrane of liver stromal cells (58). Translocated EGFR has been demonstrated to efficiently stimulate hepatocyte growth factor (HGF) by suppressing the expression of miR-26a/b. Furthermore, the increased paracrine HGF interacts with the c-MET receptor on migratory cancer cells, creating a conducive environment for the establishment and multiplication of metastatic tumor cells (58).

Exosomes and the immunological response

Exosome components secreted by tumor cells during development may suppress the normal functioning of the immune system, therefore evading assault and elimination by the host immunological response (59). This inhibitory action is primarily accomplished by modulating immune cell activity, facilitating immune cell death, and diminishing the population of immune cells (60). The exact mechanism by which exosomes contribute to tumor growth is unclear, complex, and dual-natured, necessitating more elucidation. Exosomes may mediate the interaction between innate immune cells and tumor cells, influencing tumor growth either positively or negatively. A previous study concentrated on exosome-mediated interaction among tumor cells and macrophages, neutrophils, mast cells, monocytes, dendritic cells, and natural killer cells (61).

Novel uses of exosomes in cancer detection and treatment

Exosomes used as diagnostic instruments

As previously stated, exosomes are found in many biofluids, providing a chance to assess cellular health in both normal and diseased settings. Exosomes in biopsies (liquid, plasma, serum, urine, saliva) have been utilized to assess and ascertain a patient's diagnosis, outlook, progression, and chemoresistance condition (62). Moreover, increased exosome production has been seen in other complicated diseases, notably cancer. Pathological states may be assessed by the analysis of exosomes generated by cells. Transcriptomics, proteomics, and lipidomic analyses may identify and examine discrepancies in the quantities of certain molecules. Exosomes have been explored for their potential in the early detection and prediction of cancer and cardiovascular illnesses (63, 64).

The use of exosomes in blood samples for cancer diagnosis has recently garnered significant interest.

In a retrospective investigation, scientists integrated artificial intelligence with surface-enhanced Raman spectroscopy to effectively identify exosomes in the initial stages of malignancies, including lung, breast, colon, liver, pancreatic, and gastric cancer (65). The work incorporates a classification algorithm to discern plasma exosome signaling patterns for determining their existence and tissue of origin. The conclusive complete decision model demonstrated an accuracy of 90.2% and a sensitivity of 94.4%, accurately identifying tumor organelles in 72% of positive individuals (66). Exosomes are becoming known as prospective biomarkers for diagnosing colorectal cancer owing to their extensive biological signatures and remarkable durability. A new study emphasizes the development of an exosome enriching platform using a 3D porous sponge microfluidic chip, which achieves an exosome capture rate of over 90% (66).

In comparison to conventional biomarker detection techniques, using exosomes for illness diagnosis has the following advantages: Exosomes possess excellent sensitivity due to their capacity to transport several biomarkers. High precision: Exosomes may transport cell-specific indicators, resulting in enhanced specificity (67). Non-invasive: Exosome extraction may be accomplished using standard bodily fluid techniques, eliminating the need for invasive procedures like tissue sampling or puncture. The harvested exosomes may be preserved for extended periods using cryopreservation and other techniques to enable future investigation. Despite the many benefits of using exosomes for illness detection, some problems persist Standardization concerns: The absence of standardized protocols for exosome gathering, processing, and identification may lead to variability in findings across various labs. (2) Technical challenges: The existing detection technology for exosomes is insufficiently advanced, necessitating the development of novel detection methodologies. (3) Sample volume concern: Due to the insufficient comprehension of exosomes, a substantial number of samples are required for validation and corroboration (68, 69).

Therapeutic application of exosomes

The employing of exosomes in cancer therapy primarily relies on two approaches: (1) their function as drug carriers for encapsulating pharmaceuticals; (2) their role as bioactive compounds (e.g., miRs) to modulate the tumor environment and impede tumor development and spread (70). Experimental investigations indicate that employing exosomes as drug carriers enhances medication inhibition and bioavailability while minimizing negative effects. RNA nanoparticles of this kind may enhance targeting efficacy and facilitate the delivery of therapeutic agents to targeted cancerous cells (71). Reports indicate that RNA nanoparticles may be utilized to provide chemical ligands (such as folic acid), pharmaceutical agents, or RNA aptamers on the exosomal surface. The above combinations may transport small-molecule chemical medicines to a target using chemical ligands or RNA aptamers (72). Binding RNA particles to particular tumor cells may minimize medication dosage and adverse reactions, facilitating targeted cancer therapy. Moreover, RNA nanoparticles altered on exosomes may associate with siRNA, miR, RNA aptamers, or ligands to selectively suppress cancerous cells (73).

Ongoing clinical studies using exosomes

Exosomes have been examined in therapeutic studies across several settings and uses in recent years. An assessment of the contemporary research landscape from the GlobalData database indicates that the primary domains in which exosomes are being investigated for therapeutic treatments are cancer (54%), central nervous system (13%), infectious illness (13%), and immunology (8%) (29). Among the 420 exosomal drugs currently in clinical development, over 65% are in the initial stages of development and have not yet been submitted for independent new drug review by the U.S. Food and Drug Administration (74). Only 6.4 percent, 7.3 percent, and 2.3 percent are in Stage 1, 2, and 3 clinical trials, respectively. As of now, only two items have been introduced for therapeutic use. Patisiran is intended for the treatment of polyneuropathy associated with hereditary transthyretin-mediated amyloidosis (hATTR) in adults. Patisiran is an innovative RNA interference (RNAi) medication aimed at decreasing levels of both wild-type and mutant transthyretin (TTR) for the treatment of individuals with hereditary transthyretin-mediated amyloidosis, a genetic neurological and cardiac condition (75). Ibudilast is a further readily available item associated with exosomes. It is a somewhat nonselective phosphodiesterase inhibitor that has been available in Japan for approximately twenty years for the treatment of asthma. Recent findings indicate its anti-inflammatory effect in both the peripheral immune system and the central nervous system through glial cell modulation in relapsing-remitting and/or secondary advanced multiple sclerosis (76).

The use of exosomes for the treatment of neurological and cardiovascular illnesses has garnered significant interest. Although there is now no efficacious therapy for neurological disorders like Alzheimer's and Parkinson's diseases, certain bioactive chemicals found in exosomes may facilitate neuronal development and repair, potentially offering a novel therapeutic approach for these conditions (77). Scientists have discovered that neurotrophic substances inside exosomes may facilitate neuronal development and repair. Exosomes were injected into the brains of mice, resulting in the

promotion of neuronal development and repair, as well as enhancement of cognitive performance (77). In cardiovascular research, diseases such as coronary heart disease and myocardial infarction are among the primary causes of mortality globally. Bioactive substances, including microRNA-132, transported by exosomes produced from mesenchymal stem cells, facilitate angiogenesis in myocardial infarction (78). Researchers have discovered that miR-126 present in exosomes may enhance endothelial cell proliferation and angiogenesis while diminishing vascular permeability. They encapsulated miR-126 into exosomes and administered it to the myocardial infarction location in mice. Exosomes were discovered to enhance the development and regeneration of cardiomyocytes, hence improving cardiac function in mice (79).

Exosomes and their applications must adhere to GMP, irrespective of their therapeutic reason. A GMP-grade exosome production method entails the use of superior quality ingredients, cells, culture environments, manufacturing technology, and proficient staff, all under carefully regulated and meticulously overseen settings (80). Moreover, separation and quality control release assessments are needed post-production to guarantee that the finished product meets the highest requirements before administration to patients for its intended application (81). The European Medicines Agency and the US FDA have organized seminars and recognize that recent advancements in cellular and molecular biotechnology have resulted in the creation of innovative medicines. Both authorities have issued suggestions and classifications for the manufacture of advanced therapies. As the emerging area of exosomes expands in treatment and diagnostics, further suggestions, standards, and requirements for investigators and producers are necessary to ensure the safety of modern therapies for patient usage (82, 83).

Prospective advancements of exosomes in medicinal applications

Advancements in accuracy gene editing technology will enable the accurate alteration of exosomes by genetic engineering, facilitating more exact control in the future. A prior work presents the application of precision gene editing technology to accurately change exosomes for therapeutic impacts on Parkinson's disease. Genetic engineering enables the incorporation of therapeutic carriers into exosomes, which are then introduced into cells. The essay examines the potential applications and obstacles of exosomes in neuroscience (84, 85).

Cell engineering technology may regulate the cells that produce exosomes, hence influencing their release volume and quality. Separate research presents cell engineering techniques to regulate the cells from which exosomes originate, therefore influencing the amount and quality of exosome release (86). A scientist investigates the use of human umbilical cord mesenchymal stem cell exosomes to enhance angiogenesis via the Wnt4/β-catenin pathway and presents the potential applications of cell genetic engineering in regulating exosome release (87).

Nanotechnology has significant potential to enhance the understanding and influence of cell physiology and disease. Recent studies highlight the application of nanotechnology to manipulate the dimensions, form, and exterior features of exosomes to affect their physiological and pathological impacts (88). The paper examines the prospect of employing nanotechnology to modify the features of exosomes for use in cancer therapy. In summary, exosomes are extracellular vesicles with significant potential and are crucial in both physiology and disease. Altering it in various manners allows for the regulation of its biological functions and stability, allowing comprehensive investigation and application of its purpose and mechanism of action. As technology advances, exosome alteration technologies will see greater application.

CONCLUSION

Exosomes provide significant advancements in drug transport, noninvasive illness detection, therapy, and several other domains due to their therapeutic potential and distinctive biological roles. In comparison to liposomes, nanoparticles, microspheres, microemulsions, and other artificial pharmaceutical delivery systems, exosomes have distinct natural benefits as possible indicators for prognosis and disease diagnosis, drug delivery vehicles, cell-free therapies, and cancer vaccines. Despite the potential of exosomes in the diagnosis and treatment of numerous illnesses, obstacles persist in their transition from laboratory research to clinical use. Initially, there are constraints regarding vesicle extraction and the assessment of whether the separated exosomes are suitable candidates for therapeutic use. This may result from several variables, including discrepancies in separated particle quantity, morphology, methods, circumstances, and the source of separated exosomes. A significant restriction is the heterogeneity in exosomal quantity and purity associated with isolation methods. Although some isolation procedures may offer superior purity and yield compared to others, variations in technique, machinery, and human error complicate the determination of the best suitable approach. Additionally, there are discrepancies in cargo loading efficiency, including electroporation sonication, and extrusion. Although exosome research is still in its early developmental phase, a more comprehensive knowledge of subcellular components and processes related to exosome production and targeted cellular

interactions will illuminate their physiological roles and therapeutic applications. Exosomes undoubtedly constitute a potential instrument in medicine and may provide solutions to several contemporary medical difficulties.

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Authors' contributions

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A Review of Urological Abnormalities After Kidney Transplantation and their Management

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Abstract:
Urological issues in renal people with transplants include more than just posttransplant consequences. These issues contribute significantly to patient death and morbidity, resulting in long-term consequences for graft viability. Finally, transplantation is a key component of the urological network; hence, the transplant team ought to be ready for predictable and unforeseen urological difficulties in both the immediate and future. These mostly comprise surgical urological problems (urine leaks, ureteral stenosis, and vesicoureteral reflux) and bladder outlet blockage. A great deal has been achieved in the therapy of urological problems in the past few decades, owing mostly to developments in endourologic procedures. The purpose of this study is to outline the therapy of urological disorders following a renal transplant in light of present knowledge.

INTRODUCTION

End-stage kidney disease (ESKD) is a very serious illness that necessitates expensive and time-consuming treatment. Effective treatment for ESKD can considerably enhance the satisfaction of life and longevity; nevertheless, impediments, such as access to care, reactive rather than proactive therapy, and inequities in minority groups, severely influence ESKD patient treatment (1-3). Currently, kidney transplantation (KT) is unquestionably more effective for the management of ESRD in comparison to dialysis (4). The five-year survival rate alongside KT, irrespective of whether the donor is living or deceased, is over twice that of dialysis, whereas dialysis expenses three times more. Because of the longer lifespan and superior standard of life it offers, more KT are carried out every day within the globe (5). It is important to remember that KT is a medical procedure that, can lead to consequences (6). With present established criteria, better surgical methods and the introduction of suppressive medicines have resulted in record-low instances of posttransplant complication and organ loss (7). Urological disorders (UDs) account for the majority of surgically related issues, happening at rates ranging from one to fifteen percent following transplantation (8, 9). Nevertheless, criteria differ, making a reliable assessment of the frequency problematic. Also, UDs are not restricted to postoperative; these issues have been shown to have an influence on transplant viability and remain a major source of patient mortality and morbidity (9, 10). An examination of the research reveals that the primary emphasis when reporting urological diseases after surgery has been on postpartum UDs including urine leakage, ureteral stenosis, and vesicoureteral reflux (VUR) (11). As a result, while designing this study, we felt it was vital to address posttransplant UDs from a wider point of view. The purpose of this study is to outline the treatment of post-kidney transplantation urological issues in light of present knowledge (12, 13).

A review of UDs related to kidney transplantation

Problems in the early post-transplant phase can be roughly classified as vascular, urinary, fluid storage, and wound recovery issues (14, 15). Vascular problems include bleeding, thrombosis, aneurysm, dissection, and stenosis, whereas urological concerns mostly entail leaks and/or blockage of the accumulating network

(16). The majority of lengthy hospital stays are caused by initial surgical urological problems (17). Older donor age and the recipient's previous cardiac episodes are risk factors for UDs (18). Additional significant hazards are extended dialysis times and poor bladder capability (19). Yet, it is uncertain if living donor or dead donor transplants are associated with urological problems. Routine perioperative ureteral stenting has been proven to drastically minimize the risk of UDs (20).

Ultrasonography is the primary imaging technique for transplant assessment in the initial post-transplant duration, particularly when vascular issues, fluid accumulation, or blockage are suspected (21). Aside from being harmless, it can offer some further details on the function of the graft through measurement of the intra-renal conductivity index (22). Ultrasound and/or computed tomography-guided needle aspiration, accompanied by biochemical and bacterial investigation, is critical for determining the specific cause of fluid accumulation (23, 24). A fluid level of creatinine that is much higher than that in the blood implies a urine leak, compared to a lymphocele with serum-like values. Gram stain and cultures are crucial since any fluid accumulation may become contaminated (25).

Ureteral stenosis

Ureteral blockage happens to between two and ten kidney transplant patients after surgery, typically during the first several weeks or months. Prompt evaluation and treatment are critical to preventing graft rejection (26). A number of the patient, donor, and operational characteristics have been studied as indicators of ureteral blockage after donation (27). Allografts with over two renal arteries from donors over the age of sixty-five are at higher risk (28). The researchers hypothesized that many renal veins may be associated with "not enough inferior pole perfusion, generating relative ischemia to the ureter" (29). Extending ischemia duration and ureteroneocystostomy without ureteral stent implantation have also been linked to restriction development (30). Since the kidney transplant is denervated, the newly transplanted kidney patient seldom experiences signs of ureteral blockage until urinary tract restoration is performed by pyeloureterostomy or ureteroureterostomy to the native ureter (31). As a consequence, recipients often exhibit an undetectable reduction in kidney activity and a drop in urine production (32). Individuals can also be upset by a dull aching or fullness over the allograft as a result of peritoneal discomfort. The majority of ureteral restriction in kidney transplantation happens within three to six months following surgery, which is the primary objective of present medical care (33). Pathogenesis includes ureteral ischemic necrosis, ureteral stones, scar constitution that results in fibrosis around the ureter, chronic urinary system infection, AR, DGF, CMV, BKV infection (34). Numerous techniques, such as robotic surgery, endoscopic procedures, and stenting, are used to treat ureteric restrictions (35). Stenting involves inserting a wire mesh tube through a catheter into the ureter to keep it open and let urine flow (36). This is a short-term management choice. Robotic operation is a minimally invasive process that reconstructs the ureter and reattaches it to the renal system through small cuts (37). Balloon dilation, endoureterotomy, and laser endopyelotomy are examples of endoscopic techniques. For brief, nonischemic restrictions, balloon dilatation works well (38). A method of minimally-invasive surgery having a high rate of achievement is endoureterotomy (39).

Urine leakage

Urine leakage is most likely during the first two weeks after operation. Urine leakage is the most prevalent urologic problems following kidney transplantation, besides ureteral stricture, symptomatic vesicoureteral reflux (VUR), urolithiasis, bladder outlet blockage, and lymphocele-induced urinary tract blockage (40). A blocked urethral catheter, which causes significant intravesical tensions in the context of a freshly developed ureteroneocystostomy, must be recognized as soon as possible since it is an effectively repaired reason for urinary fistula (41). The most frequent urine leakage location is the ureteroneocystostomy (42). It is often caused by ischemia necrosis of the distal ureteral portion of the ureterovesical anastomosis (43). As a result, it is critical to avoid ureteral devascularization during transplant nephrectomy and to construct a watertight ureterovesical connection (44). Urine leaks during KT cause edema, discomfort, higher drain output, and deterioration of graft performance (45). These individuals must be closely monitored, sepsis excluded, and a Foley catheter placed. Drain fluid and Foley discharge must be checked (46). The magnitude of the urine leak following a kidney transplant determines the therapy, which may involve catheters, stenting, and radiographic draining (47). Minor leakages are frequently addressed with a long-term Foley's catheter, however, larger leakage may necessitate percutaneous nephrostomy, antegrade or retrograde stenting, and radiographic drainage (48).

Vesicoureteral reflux (VUR)

VUR is one of the most common urological consequences of KT. It is characterized as an irregular movement of urine rearward from the bladder to the kidney or, in severe situations, up to the kidney pelvis (49). In the majority of people, VUR can be inherited or acquired, after obstruction or breakdown of the bladder musculature, as well as malfunction of the

nerves that govern bladder evacuation (50). Clinical characteristics vary from asymptomatic to ESRD and seem to be closely connected to the seriousness of backflow and the frequency of urinary tract infections (UTIs) (51). The method used for ureteral insertion and bladder health at the point of donation is undoubtedly important variables in the occurrence of after-surgery VUR. The link between post-transplant VUR and a higher likelihood of UTIs is undeniable. However, the transplant society has yet to achieve a consensus on the ideal pre-emptive method, clinical significance, care, and influence on permanent allograft performance and longevity. The primary goal of VUR care is to minimize infections of the kidneys and destruction. Furthermore, doctors should strive to avoid UTIs while minimizing permanent evaluation and therapy processes. Treatment might be without surgery (e.g., urotherapy, antibiotic treatment), least-invasive (endoscopic injection), or surgery (ureteral reimplantation). Aside from VUR in the renal transplant, the existence of VUR in the native kidneys in cases of reflux nephropathy raises the risk of UTI, particularly in more severe reflux. Methods such as native nephrectomy in high-grade reflux or distal ureter closure in poor-quality reflux after surgery have been found to have a protective impact.

Bladder Outlet Obstruction (BOO)

A BOO is a condition that occurs where the passage of urine is either partially or fully obstructed. This causes signs such as decreased urine flow, pain in the pelvis, and bladder irritation (52). Persistent BOO can cause consequences including infections, bladder stones, diminished urinary tract function, and kidney disease. Urinary retention caused by BOO is a serious urological issue that can arise in the early as well as late stages following KT, especially in middle age and elderly men (53). Early evaluation and management of BOO are critical for maintaining allograft performance (54). Proper long-term transplant performance is contingent on low-pressure urine preservation and efficient bladder evacuation (55). In urinary retention, higher intravenous pressure caused by detrusor muscle tension has a negative impact on graft activity (56). This might result in leaking from the ureterovesical anastomosis or serious infections via the ureteral pathway, particularly in the initial days following KT (57). In over time, intravesical pressure rises owing to extended detrusor rigidity caused by BOO, and this elevated tension affects graft operation by inducing hydronephrosis (58). Luckily, investigations such as KT recipients have indicated that individuals who have permanent renal insufficiency owing to BPH demonstrate better renal function in both early and late operative examination (59).

Persistent UTI following KT

urinary tract infections (UTIs) are the most prevalent kind of infection after KT (as much as 47 percent). Furthermore, many individuals develop asymptomatic bacteriuria (ASB) soon after KT (60). Although most facilities treat asymptomatic bacteriuria within 1-3 months after KT, there is emerging evidence that this could be unneeded. The incidence of UTI appears to be higher with ureteral stents, especially during the initial month following the transplantation (61). Early catheter extraction lowered the incidence of urinary tract infections with no increasing risk of urine leakage and ought to be conducted within three weeks of following KT (62). Nevertheless, even after catheter extraction for UTI, the chance of recurring UTIs persists greater in individuals who previously suffered from catheterization-associated UTI (63). Any person with chronic or persistent urinary tract infections following KT ought to be thoroughly evaluated for anatomic or functional urinary tract deficiencies as previously described (64). Female sex, immunosuppression, previous severe rejection, CMV infection, re-transplantation, polycystic kidney disorder, Type 2 diabetes, and VUR in the native kidneys all increase the probability of urinary tract infections following KT (65). Prophylactic antibiotics ought to be supplied in the emergency condition and for initial recurrence, with special attention paid to screening for functional or anatomical allograft diseases (66).

DISCUSSION AND CONCLUSION

Drs. Joseph Murray, a plastic surgeon, and Hartwell Harrison, a professor of urology, performed the first effective kidney transplant. For many years, physicians were the main surgeons who performed kidney transplants (67). Currently, the surgical function of the urological in kidney transplantation is becoming less crucial due to, among other reasons, lower training in vascular procedures for speciality urologists (68). Yet, because of special difficulties connected to the genitourinary tract, urological involvement in KT remains crucial, and the urologist's knowledge of surgeries on the urinary tract is usually indispensable (69). UDs are prevalent following a KT, although they can be decreased by the application of ureteral stents, early identification, and quick management. According to the criteria, problems including urine leakage, ureteral stenosis, lymphocoele, lithiasis, urethral stricture, and vesicoureteral reflux happen in two percent to thirty percent of all patients (70). The bulk of them are ureteral problems caused by anastomosis errors. A large number of urological problems (82.5%) were treated using an endourological technique rather than surgical intervention (71). From 2002, regular placement of a ureteral stent has resulted in a substantial decrease in leakage of urine or fistula. Ureteral stenting,

while reducing the amount of urine leakage and initial blockage owing to anastomotic edema, might result in UTIs or urethral damage with hemorrhage (72). Consequences following KT might be difficult to control because of the increased risk from prior procedures and the necessity for continued immunosuppression in frequently comorbid individuals (73). Considering recent advancements in endoscopic urology, a minimally invasive treatment for urological problems may be a suitable choice. Furthermore, a thorough postoperative urologic evaluation before KT is required to establish risk factors before the operation. Finally, UDs following KT might have serious repercussions, including persistent graft malfunction and, ultimately, allograft removal (74). Effective transplantation is thus heavily contingent on both a complete urologic work-up before transplantation and the early identification of problems following operation (75). Notwithstanding a dearth of large-scale prospective studies, endoscopic therapy options provide minimally invasive choices for individuals with a range of UDs following KT (76).

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The Role of Personalized Medicine in Oncology and Targeted Therapy

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Abstract:
Cancer, the second foremost worldwide cause of mortality, affects individuals both physically and emotionally. Traditional therapies, including surgery, chemotherapy, and radiation, have detrimental consequences, necessitating the pursuit of more targeted methods. Precision medicine facilitates more tailored therapies. The introduction of next-generation sequencing technology, as well as the growing frequency of large-scale tumor molecular profiling programs throughout the world, have changed cancer diagnosis and treatment. With the increasing availability of comprehensive genetic tests in medical and scientific settings, healthcare practitioners face complex hurdles in understanding and translating data. The article encapsulates the existing and forthcoming strategies for the implementation of precision cancer therapy, emphasizing the obstacles and potential ways to enhance the understanding and optimize the therapeutic value of molecular profiling outcomes.

Background

Cancer is a collection of illnesses characterized by various aetiologies and prognoses. Consequently, cancer therapy, whether therapeutic or palliative, utilizes a wide array of approaches. In recent years, the molecular comprehension of cancer has significantly advanced (1). The clarification of the arrangement of DNA as its genetic makeup sparked progress in biomedical research, leading to personalized medicine (PM), whereby the newfound comprehension of the relationships between genes and diseases may be used for diagnosis and treatment (2). The new paradigm in oncology has justified mechanism-based strategies that may enhance or supplant non-specific techniques, including cytostatic chemotherapy, radiation, and surgery, with approaches expected to yield better cure rates, cost-effectiveness, and diminished toxicity (3). Cancer is the second foremost reason for mortality globally, impacting not only the person's physical condition but also their mental well-being, psychologically undermining their whole support network. One of the primary exacerbating elements in this procedure is that standard therapies, including surgeries, chemotherapy, and radiation, although effective, are intrusive procedures that induce several negative consequences, even when accounting for the kind of malignancy and the patient's particular characteristics (4, 5).

In the last two decades, there has been a notable rise in scientific papers using "personalized medicine" in their titles, amounting to 115,464 papers generated by March 2024, with more than 103,198 produced post-2010. Nonetheless, the heightened use of the moniker does not align with a widespread comprehension of the notion (6). Numerous efforts have been undertaken to provide a complete description of precision and PM. The notion of PM suggests a less broad approach that emphasizes enhanced accuracy in diagnostic categorization and therapy alignment (7). PM aims to actualise the medical idea of delivering the appropriate medication to the suitable patient at the optimal moment and is notably linked to the genetic

categorization of individuals into more precise disease categories. The notion has emerged as a vital element within the burgeoning life science sector (8). PM emerges as a novel strategy in this complex situation. It tailors medical therapy to each individual's specific genetic composition, circumstances, and habits, to achieve the best possible results. Precision medicine is a significant success in oncology since it provides focused solutions to chronic difficulties like cancer stem cells and treatment resistance (9). The Prime Minister aims to enhance effectiveness and mitigate adverse effects by tailoring medicines to the distinct genetic and molecular characteristics of each individual's malignancy (10). This PM strategy is particularly efficacious in overseeing the complexities of malignant cells' communications with their environment, hence enhancing therapeutic effects. Furthermore, PM possesses the ability to change detection approaches, particularly for malignancies that often go untreated in the early stages. The use of personalized biomarkers may allow for earlier detection and a more detailed evaluation of medication success. Because cancer has a propensity to metastasize, requiring a multi-targeted approach, PM is an important component of cancer therapy (11, 12). This article aims to evaluate the present and prospective possibilities of precision medicine in oncology. This approach may transform cancer therapy by enhancing its effectiveness and safety.

Understanding PM in Oncology

The notion of cancer-agnostic individualized therapy informed by molecular profile is attractive; yet, its effective practical application presents significant hurdles that need careful study. A significant practical obstacle is the intricate, multistep process of aligning specific medicines with identified molecular changes (13). The procedure begins by determining whether the patient's overall health permits the use of molecular profiling, the appropriate time to initiate molecular profiling throughout the patient's treatment, the necessity of a re-biopsy of the tumor lesion or a liquid biopsy, and finally, that diagnostic genetic analyses should be performed (14). Subsequent actions involve NGS analysis, bioinformatic analysis of information, variant calling, and the practical evaluation of discovered genetic changes, tasks managed by physicians and geneticists. Every distinct phase in this complex procedure has particular obstacles and hazards, which are elaborated upon in other articles within this series (15).

The conclusion of the Human Genome Project (HGP) enabled scientists to decipher and analyze an individual's genetic code and determine genetic susceptibilities to certain illnesses (16). This pivotal event transformed the viewpoint on health from responsive to preventive. Currently, researchers are striving to get a full comprehension of bodily functions across several omics levels and to delineate how genetic predispositions are influenced by exposure to environmental factors (17). Collectively, this knowledge will enable researchers and physicians to more accurately anticipate patient responses to certain treatments. CDx assays serve as invaluable instruments in tailored therapeutics by evaluating genetic features that determine a patient's responsiveness to certain treatments (18). This method may significantly influence patient treatment. The revolution consists of the transition from a doctor choosing a general medicine, which is mostly exploratory for the patient, to one that precisely addresses the illness using PM (18, 19).

This review discusses the domains of personalized medicine and precision medicine, together referred to as PM. While the phrases are often used interchangeably nowadays, both denote the use of distinct patient features to choose the optimal therapy; the discipline was once termed customized medicine. As it gained popularity and grew prevalent in science, media, and culture, the phrase started to embody a misperception. Numerous individuals erroneously believed that the "personalized" approach included the creation of distinct remedies for each person (20). The scientific community, particularly the National Research Council, advocates for the application of precision medicine to supersede the ambiguous term personalized medicine, therefore elucidating the true objective of the discipline (21). Nonetheless, individualized medicine is becoming acknowledged by all people. Historically, stage II and III tumor clinical studies have assessed

results through histology; however, the histological examination may not sufficiently represent the impacts of gene-targeted medicines or immunotherapy (22). PM methods evaluate patients' circulating DNA (liquid biopsy), immunological indicators, and other biological characteristics to determine effectiveness and guide therapy options. Genomic indicators have shown to be the most successful thus far; however, new biomarkers, such as protein analysis and transcriptomics, are presently being developed and evaluated (23). Numerous molecular abnormalities have been detected by sequencing and high-throughput innovations, resulting in the authorization of targeted medications by the FDA. The precision medicine paradigm has recently been dominated by immunology and its association with genome research, since genetic features, including mismatch repair gene deficiencies, are major drivers of checkpoint inhibition efficacy (24).

Current and upcoming molecular techniques for PM

Integrating PM with other diagnostic procedures in clinical settings

Initially, it is essential to acknowledge the vital connections between research and routine diagnostic procedures in precision oncology (25). Extensive research studies may reveal new clinically relevant indicators, which might then be used as a groundbreaking standard diagnostic tool to enhance patient outcomes. Numerous tumor-specific molecular abnormalities, such as protein amplification, mutations in driver genes, or modifications, have been established as reliable predictors of response to targeted therapy, and novel markers are constantly developing (26). As a result, clinical molecular pathology assessment has become a key lab tool for understanding tumor behavior and directing therapy choices. Standard tests like as immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) are critical tools in precise medicine, with various markers discovered utilizing these procedures (27). IHC may detect modifications at the protein level due to genetic abnormalities, primarily gene amplifications, with specific DNA rearrangements or point mutations, such as EML4-ALK rearrangement in non-small cell lung cancer (NSCLC) (28).

Since the occurrence of druggable gene abnormalities and indicators that predict cancer growth, NGS technologies have increasingly replaced traditional methods, like single-gene testing and focused mutational systems, in regular biological pathology studies (29). Traditional methods have improved their accuracy and sensitivity in detecting actionable alterations that benefit from related targeted medicines, as well as mutations that cause resistance to particular drugs. Nonetheless, these systems demand that each marker be pre-defined for detection, requiring a personalized test for each analyte (30). NGS allows for the simultaneous investigation of several genome modifications, such as genetic mutations, copy number variations (CNV), modifications, and gene combinations (31, 32).

Thus, it provides a more accurate, economical, and tissue-conserving approach to tumor analysis compared to sequential single-biomarker evaluations, particularly considering the genetic complexity associated with malignancies (33). Several studies comparing the usefulness of NGS to reverse transcriptase based-(RT-PCR) focused mutation systems, single-gene testing, and various other traditional approaches indicate equivalent precision and selectivity in finding frequent druggable chromosomal aberrations in current practice (34). The declining prices and faster processing times of NGS, enhancements in computational biology assessments, and the standardization of databases to improve the clinical assessment of genomic results make the shift to extensive genome-wide analysis through NGS in patient trials compelling in the context of personalized oncology (35).

Contemporary utilization of next-generation sequencing methodologies

NGS may be confined to a small collection of genes, focus on the coding sections of the genome, or conduct a thorough analysis of the complete tumor genome, including intronic regions. The selection between these approaches is based on many parameters, including the intended use of tumor testing (clinical appointments versus research), required outputs, technical effectiveness, and cost (31). To yet, WES and WGS remain primarily within the investigation sphere,

focused on gathering comprehensive genetic data for translational studies that may improve our knowledge of cancer biology over the years (36). The practical relevance of assessing all genes within large sections is questionable; yet, the benefits of concurrent multi-gene screening by NGS and the minimal additional costs related to adding more genes stimulate the adoption of more broad genomic assessment in clinical scenarios (37). A key issue is that, despite extensive tumor sequencing and genotype-matched studies identifying curable mutations in driver genes in up to 40% of persons, only a much lower number (10-15%) are treated with genotype-matched medicines (38).

Several variables are at play, but the main difficulty is the absence of licensed or experimental medicines that correspond to particular driver changes. Recruiting individuals with unusual molecular disorders for research studies creates challenges and slows the discovery of novel treatments. In addition, intratumoral variety (e.g., trunk versus extended alterations) and the evaluation to identify whether a certain genetic change acts as a "true" driver in a particular tumor may significantly affect the success of the related treatment (39). Hess et al. have shown that numerous somatic hotspot modifications, formerly considered "drivers" in cancer, may actually be recurring passenger mutations occurring in highly changeable parts of the genome (40).

Improvements in sequencing technology, including whole genome sequencing, enabled concurrent detection of single nucleotide modifications, changes in copy number, and changes in a structure such as gene fusions, hence enhancing the diagnostic effectiveness of relevant discoveries in tumor samples (41). The new research analyzed 2,520 examples of metastatic cancers and their matched healthy tissue to underscore the necessity for complete genetic profiling. Whole genome sequencing (WGS) was used to record the genetic alterations observed in each cancer metastasis, thereby elucidating the genomic anomalies in the metastasis of 22 solid tumors, with 62 percent expressing at least one identifiable mutation (41, 42).

Recent sequencing methods have expanded the length of reading sections beyond several kilobases, thereby boosting the ability to find complex structural differences in the genome (43). Research using genomic DNA from individuals with various brain malignancies successfully identified SNV, CNV, and methylation patterns concurrently by a low-pass whole genome sequencing method employing long-read sequencing. In oncology, the bulk of long-read sequencing efforts has focused on RNA sequencing, which has revealed novel combining and splicing variants related to tumor development or resistance to therapy. At present, the incidence of mistakes of long-read techniques is excessive for somatic variation detection; nevertheless, the transition to these approaches for tumor evaluation might give an integrated approach for genomic tumor characterization and better-personalized therapy management (44, 45).

Biomarkers

The quality, amount, and accessibility of tissue specimens from individuals with cancer pose hurdles to the successful application of customized treatment. The chemical treatment of formalin-fixed, paraffin-embedded tissues may modify genetic material, and reduced cancer levels in specimens may impair the accuracy of assays or lead to incorrect alteration identifications (31). Furthermore, using preserved samples or lesions from a particular time point shouldn't effectively depict intratumoral variability across geographic or temporal dimensions. The collecting of many tumor specimens is hampered by the requirement for intrusive procedures that risk patient safety and cost large resources (46-48).

The evaluation of circulatory tumor-specific biomarkers is a growing area that might address several difficulties with tumor specimens. This comprises circulating tumor cells (CTC), circulating tumor DNA (ctDNA), and other RNAs, proteins, or chemicals found in bodily fluids like as plasma, urine, and intraperitoneal or cerebral spinal fluid. Liquid biopsies are readily accessible utilizing less-invasive technologies that provide a continual and dynamic evaluation of tumor-specific assessment, and predictive or diagnostic signs (49, 50). Next-generation sequencing can be used to analyze plasma CTCs and ctDNA, supplying a greater understanding of the tumor's genetic architecture than traditional tumor tissue evaluation, because it spans several areas of the

tumor (including both original and spread locations) and resolves intratumoral variability (51).

Currently, there are no FDA-approved diagnostics for identifying and assessing these conditions, and their therapeutic value is uncertain (52). MiRNAs are important because of their longevity and frequency in the bloodstream, and their fingerprints are being studied as markers for diagnosis and prognosis in a variety of kinds of tumors, particularly for the detection of minimum remaining illness. The primary obstacles, nevertheless, continue to be the unpredictability and absence of repeatability in research outcomes caused by unclear data gathering and processing procedures. There is an essential demand for academic consistency to maximize the possibility of liquid samples in clinical situations, and numerous projects are presently in the works to fulfil this requirement (53, 54).

Challenges and prospects of PM in oncology

Although significant progress in precision oncology, critical constraints and obstacles of genome-guided treatment persist, necessitating resolution to facilitate larger and more effective clinical applications and optimize patient outcomes. Initially, during clonal development in carcinogenesis and cancer progression, malignancies acquire several pro-oncogenic genetic abnormalities (55). As a result, tumors vary and become more subclonal as the sickness progresses. As a result, treatment effectiveness in late cancer phases is essentially constrained owing to the considerable likelihood of underlying genetic features that enable malignancies to escape single-target customized therapy (56). Focusing on certain cancer-driver genes during the early stages of treatment may result in a more significant anti-cancer impact. In subsequent decades, the early implementation of tailored treatment strategies in medical care may give better therapeutic effectiveness (57).

Furthermore, our knowledge of how to assess the toxicity of detected genetic changes limits cancer precision. Cancers often obtain multiple passenger co-mutations that aren't necessary for tumor development (58). Furthermore, normal tissues show a buildup of genetic mutations with different clinical implications. Somatic alterations in blood cell production that grows with age are often identified in the clinical examination of circulating tumor DNA, therefore lowering the accuracy of the disclosed mutational patterns (58).

Furthermore, changes in other typical pro-oncogenic driver genes were found in several non-malignant diseases (59). Future improvements may address these constraints by using tailored models to assess the functional ramifications of detected genetic changes and the most relevant therapy targets at the RNA, protein, or physiological stages. Furthermore, the introduction of artificial intelligence-driven technologies may enhance the pathological and therapeutic interpretation of molecular testing (60).

Thirdly, from a pragmatic standpoint, project management in oncology is now hindered by several structural and technical constraints. The interval between the initiation of genetic testing and the implementation of personalized medications may last a few weeks (61). Therefore, in a primarily progressed oncological therapeutic scenario, a considerable percentage of persons are lost throughout the operation. Furthermore, the availability of recent tissue specimens is often critical for gathering accurate genetic data on the present molecular composition of cancer, owing to the modular formation and genetic causes of treatment tolerance that may evolve after past treatments for cancer (62). As a consequence, customized oncology is often dependent on the efficacy of sophisticated tissue collection and biopsy, which could impact the balance between risk and benefit for this therapy strategy. Future improvements in liquid biopsies by examining circulating tumor DNA may obviate the need for additional tissue-based analysis (63).

The primary barrier to global adoption of precision oncology is the financial strain of comprehensive genetic sequencing and, more importantly, the cost of personalized treatments (64). Unfortunately, the availability of genetic testing and personalized cancer therapy is now restricted to a small percentage of cancer patients in wealthy countries. In the long term, improved precision in selecting targeted cancer therapies through improved forecasting of therapeutic advantages may lower expenses in comparison to conventional unguided therapy by enabling patient treatment and averting hospital stays due to disease-

related adverse effects. A significant need exists for research specifically focused on efficiency evaluations of the precision oncology approach (65, 66).

CONCLUSION

The medical care of cancer patients is undergoing a notable shift towards personalized therapy through the application of molecular diagnostic technologies. Substantial restrictions remain that need to be resolved to enhance patient outcomes, notably the degree of cancer-specific variation in genes, the meaning and therapeutic classification of identified genetic anomalies, and current technology limits in molecular testing. A deeper understanding of complex molecular processes, achieved by integrating various genetic and functional investigations within an advanced personalized healthcare decision-making framework, as well as a better capacity to rapidly identify and monitor personal cancer-driving molecular changes via liquid biopsy specimens, will soon profoundly alter the present knowledge of tumor biology and therapies.

Authors' Contribution

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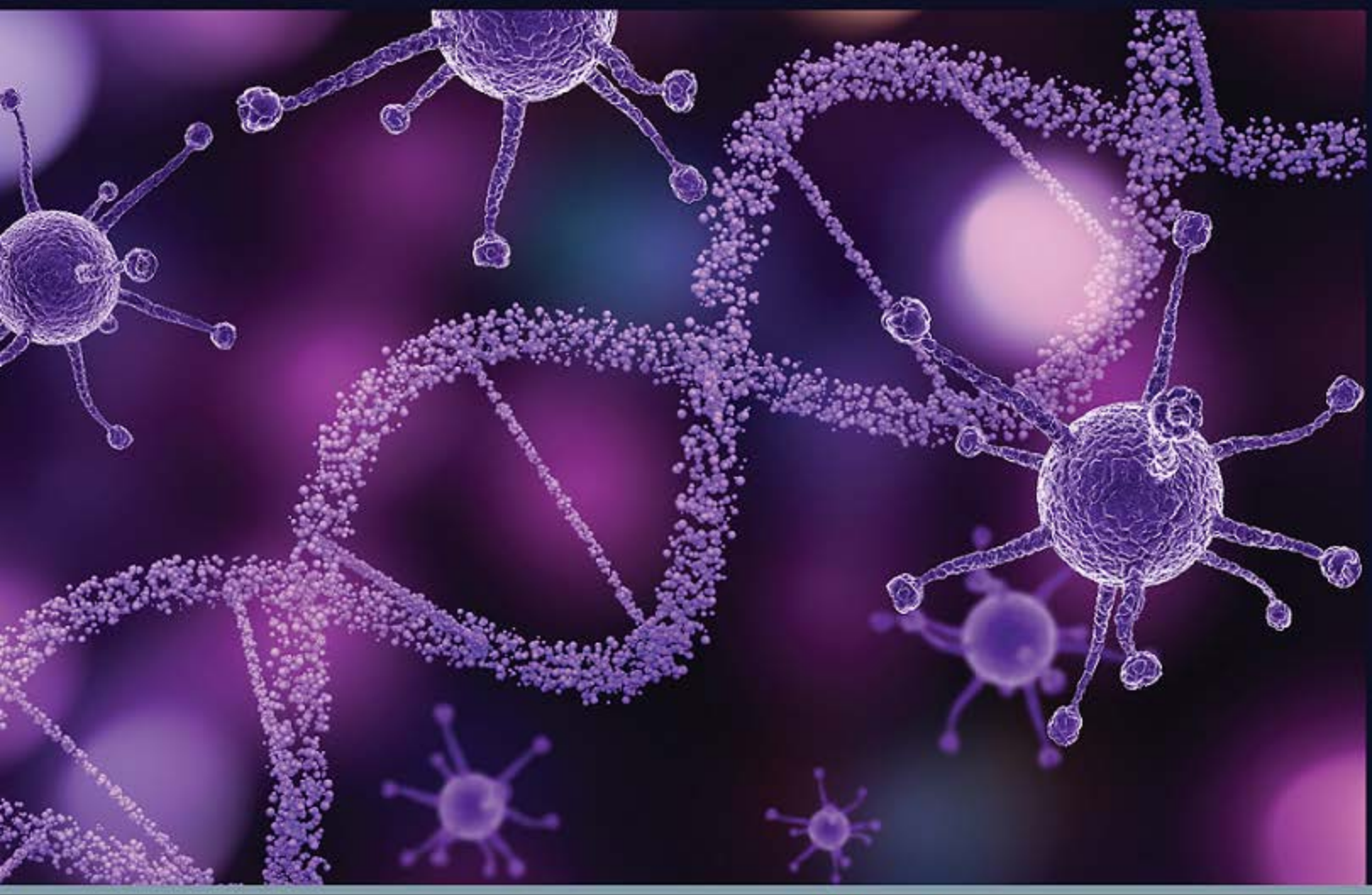

آینده علم پزشکی، شخصی محور است