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Review Article

Next-generation sequencing technology in cancer

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Abstract: Next-generation sequencing (NGS) technology has revolutionized cancer research and treatment by enabling comprehensive analysis of genetic mutations, alterations, and expression profiles. It allows for the identification of cancer-driving mutations, helping to develop targeted therapies. NGS provides detailed insights into tumor heterogeneity, resistance mechanisms, and clonal evolution. Its high-throughput capacity facilitates large-scale studies, improving our understanding of cancer genomics. By enabling personalized treatment plans based on individual genetic profiles, NGS holds promise for more effective and tailored cancer therapies. Early reviews on cancer genomics often lacked comprehensive coverage of emerging technologies. They missed in-depth analysis of NGS advancements, their impact on cancer research, and clinical applications. The review addresses this gap by thoroughly examining NGS methods, their role in identifying genetic mutations, and their potential in personalized cancer treatment, thus providing essential insights into the evolving landscape of cancer genomics. The article covers technological advancements and bioinformatics approaches for NGS data analysis. It focuses NGS applications in research and diagnostics, particularly for solid cancer diagnosis. The review highlights specific cancer types, including hereditary breast cancer, melanoma, prostate cancer, thyroid cancer, lung cancer, and colorectal cancer. It explores NGS's contribution to understanding the genetic basis of these cancers and its potential for enhancing personalized diagnosis and treatment strategies. This review rectifies early lacunas by providing a comprehensive and updated examination of NGS technology, addressing gaps in previous analyses, and emphasizing bioinformatics approaches for NGS data analysis, which is crucial for interpreting vast genomic data accurately. The review meets the current need for a thorough understanding of NGS's role in personalized cancer treatment and research.

Keywords: Next-generation sequencing (NGS), Cancer genomics, Personalized treatment, Bioinformatics analysis, Solid cancer diagnosis, Targeted therapies

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1. INTRODUCTION

Cancer originates at the genome level and follows an evolutionary progression. This process is distinguished by the build-up of diverse genetic alterations, including somatic mutations, changes in the number of copies of genes (copy number changes or SCNAs), and structural variations (SVs). These alterations may occur with or without a basis in heritable variables (germline variants) and epigenetic factors [1-3]. Through examining familial cancer cases and studying cancerous tissues [4], the appreciable quantity of hereditary and acquired mutations was discovered in well-established genes that limit tumor growth (RB1, TP53, and APC), along with changes in the number of gene copies[5]. The analysis of copy numbers has identified distinct oncogenes and their associated activators that facilitate cancer development, such as HER2/ERBB2 [6]. Genomic mutational profiling is now used to diagnose disorders, assess treatment sensitivity, detect residual sickness, and predict prognosis. In addition, this cancer therapy has explicitly targeted certain recurring driver mutations. Advancements in NGS technology and bioinformatics/computational methodologies have facilitated the thorough examination of various analyses of cancer genomes involving the use of targeted sequencing, whole-exome sequencing (WES), RNA sequencing (RNA-Seq), and whole-genome sequencing (WGS) in genetic analysis [7,8]. Significant endeavors have been made to analyse and examine various cancer genomes worldwide. Significant initiatives include TCGA, which stands for the Cancer Genome Atlas (TCGA), and the International Cancer Genome Consortium (ICGC), which are two organizations focused on studying the genetic makeup of cancer [9]. These projects aim to examine the diversity and genetic modifications in cancer thoroughly. The major focus of cancer genome sequencing initiatives, both on a global and local scale, is to target the WE gene. Much mutational data for various prevalent and rare malignancies in protein-coding genes or areas has been collected. The systematic investigation has uncovered several newly discovered cancer genes, pathways, and activities associated with the cancer genome. A recent research called "Three Saturation" has exhaustively investigated most driver genes, often mutated in cancer [10,11]. At present, it is critical to prioritize investigating rare genetic mutations with a low occurrence rate. However, it is necessary to confirm their accuracy via functional study [12.] Genetic abnormalities, including point mutations, copy number variations, and rearrangements, may impact cancer development. Most of these changes are somatic, occurring in cancer cells rather than the patient's reproductive cells [13]. Medications such as gefitinib and erlotinib, EGFR inhibitors, which hinder the activity of the epidermal growth factor receptor kinase (EGFR), greatly impact the survival rate of cancer (Lung cancer) patients who have EGFR mutations. However, those who have a normal EGFR do not have the same effects from these drugs [14-16]. Hence, genome-based cancer diagnostics is becoming vital in ascertaining therapeutic alternatives. In recent decades, there have been appreciable progressions in experimental and informatics techniques used for genome investigation. These developments have been facilitated by DNA and RNA microarrays and capillary-based DNA sequencing, often referred to as Sanger sequencing [17]. These innovations made it easier or more convenient to examine mutations occurring in the exons and variations in the number of copies of genes, resulting in the identification of several significant modifications in the genome of cancer cells [18]. The task of identifying and diagnosing changes in the genetic makeup of cancer cells continues to be a challenging task. Advancements in sequencing techniques now enable the identification of previously unknown changes in the order of chromosomes (chromosomal rearrangements) and microbial infections. These approaches allow for detecting abnormalities in the number of copies of genetic material (copy number alterations) with exceptional precision [19,20]. Recent advancements in DNA sequencing technology are revolutionizing the examination of several human illnesses. NGS technology has revolutionized the science of cancer genetics by offering remarkable speed and throughput at lower prices. This technology enables the thorough sequencing of whole cancer genomes to detect specific mutations and new disease genes, thereby providing a basis for extensive studies on the diversity of cancer. The therapeutic ramifications of this crucial component of cancer biology are significant. However, until recently, the lack of technology capable of analysing the complexity of cancer genomes has hindered complete investigation. Cancer development is increasingly associated with widespread changes in cells' epigenetic and RNA characteristics. Cancer is now acknowledged as a complex network that includes changes at the genomic, epigenetic, and transcriptome levels, eventually leading to the final tumor phenotype. NGS has greatly transformed the paradigm of carcinogenesis. Due to their expensive nature, the sequencing methods used in the Human Genome Project and their succeeding years were constrained in their capacity to describe cancer genomes. The original Sanger technique was the basis for creating other sequencing platforms, which were improved via amplification, sequencing, and detection stages. Over the last several years, the commercialization of second-generation technology has greatly improved the capabilities and applications of DNA, RNA, and chromatin analysis. Emerging technologies, such as single-DNA-molecule sequencing systems, are presently being developed or brought to the market. Furthermore, the rapid increase in genetic data accumulation necessitates concurrent progress in bioinformatics and computational tools to aid data handling and analysis. The rapid and profound breakthrough in the advancement of sequencing technologies in recent years has significantly improved our comprehension of human genetics and genome. NGS is widely used for many sequencing applications, including whole-genome sequencing, WES, transcriptome sequencing, targeted area sequencing, epigenetic sequencing, and other methodologies [21]. The rapid expansion of NGS technology offers significant prospects for its use in genetic counselling, risk evaluation, and the administration and therapy of diseases. One of the therapeutic applications of this approach is cancer molecular diagnosis [22]. The study of genomics has been significantly transformed by NGS, leading to a substantial improvement in our understanding of the genome's structure, function, and dynamics. Scientists have used this innovative technology to conduct a thorough investigation into the intricacies of genetic information in novel ways. NGS has become a pivotal instrument for researchers in several fields, including basic biology and clinical diagnostics, due to its cost-effectiveness and efficient handling of massive volumes of data [23]. NGS allows for the simultaneous sequencing of millions of DNA fragments. This high-throughput capability is crucial for metagenomics, where samples often contain various microorganisms. NGS can generate large amounts of data, providing a comprehensive overview of microbial communities [24,25]. NGS has facilitated comprehensive genome sequencing and streamlined research in transcriptomics, epigenomics, metagenomics, and other omics disciplines [26]. Cancer, a

condition marked by diversity, is largely caused by the build-up of DNA mutations. Advanced sequencing technology will significantly influence cancer diagnosis, treatment, and care. Utilizing NGS, the genomes of humans and several cancer genomes can be successfully sequenced, which enables the reveal of a complete blueprint of knowledge of the standard human genome and a deep comprehension of the mutations of cancer genomes across different kinds of cancer and by this, a more thorough comprehension of the molecular process of cancer development and the logical basis for treatments can be directed by molecules [27]. In near future, it would be possible to analyse the genetic makeup of every patient to determine if they have a normal or malignant condition. Conventional genome sequencing can uncover the patient's genetic makeup, their susceptibility to hereditary cancer, and their body's responses to medications, and this helps to identify individuals at high risk and to determine which chemotherapy treatments are least likely to cause negative side effects with the highest probability of success. Cancer genome sequencing allows for monitoring unique molecular genotypes associated with the illness and aids in developing or selecting targeted medicines.

2. NGS TECHNOLOGY

The primary benefit of this NGS technique lies in its capacity to rapidly handle vast quantities of data, which is achieved by enabling the simultaneous sequencing of many specific regions of the genome across different samples, thus facilitating the identification of concurrent mutations within the same cycle. Another significant benefit of frequent tumor sequencing is the reduced time required to analyse the data, reducing the time needed for clinical reporting. In addition, unlike traditional sequencing methods, NGS studies need less DNA/RNA input. Various genetic abnormalities, such as single/multiple-nucleotide changes, modest additions and removals, differences in the number of copies of genes, and fused gene sequences, can be accurately and sensitively detected all at once. NGS is more sensitive than Sanger sequencing, with the ability to identify allele frequencies of 2% –10% compared to 15%–25% for Sanger sequencing [28]. NGS allows for quantitative evaluation of the mutant allele, which depicts the distinct steps involved in the NGS process, from extracting nucleic acids to annotating genetic variations. Currently, three primary firms provide the available NGS systems: Roche, Illumina, and Life Technologies (Thermo Fisher Scientific, based in Waltham, MA, USA). Each currently known system uses distinct sequencing chemicals and applies various signal detection methods. The Roche 454 platforms use pyrosequencing, a method that detects the incorporation of nucleotide bases by a chemiluminescent signal. [29] There is an inverse relationship between the signal intensity and the number of bases added during homopolymer readings. Most NGS equipment primarily uses the sequencing by synthesis technique, which entails using the DNA strand that requires sequencing as a template, generating a complementary strand, and then determining the sequence of the template strand [30]. The Illumina MiSeq and HiSeq sequencers use four nucleotides labelled with fluorescent tags [31]. These markers, in combination with optical imaging, enable the visualization of the synthesis of the complementary strand. The anticipated error rate with Illumina technology is 0.4%, a whole number without any fractional or decimal parts [32]. Life Technologies uses unlabelled nucleotides and an optical methodology. Sequencing via synthesis entails using minuscule wells linked to a semiconductor device. DNA undergoes clonal proliferation on minuscule particles. The semiconductor chip measures the change in pH caused by the release of protons when nucleotides are introduced individually. The expected error rate for the Ion Torrent approach varies between 1.8% and 1.9%, with the majority of errors occurring in identifying homopolymer segments [33]. NGS procedures include a range of techniques that entail the examination of both tumor DNA and RNA. DNA sequencing may be categorized into three main types: WGS, WES, and targeted sequencing. WGS is a process that analyzes an organism's whole set of genetic information, which necessitates a significant amount of DNA for accurate results. To get precise identification of clinical mutations, it may be essential to have a sequencing coverage of 100 to 200 times [34]. However, this may be expensive and time-consuming. Typically, 30 to 60 times coverage is used to detect structural rearrangements. WES specifically targets the genome's coding sections (exons), constituting around 2.5% of the human genome. It finds genetic changes, common or unusual, connected with a disease or phenotype. WES offers a more streamlined and expedited option than whole genome sequencing (WGS) [35]. This method often utilizes oligonucleotide probes, which selectively attach to certain DNA areas, leading to an augmentation of exonic sequences. Targeted sequencing is a precise method that selectively analyzes genes linked to a certain ailment [36]. This technology is precise and efficient for clinical laboratory use, significantly reducing costs. RNA-Seq allows for identifying factors that include alternative gene splicing, post-transcriptional changes, gene fusions, mutations/single nucleotide polymorphisms (SNPs), and abnormalities in gene expression. Before further processing, the obtained RNA is purified and then transformed into complementary DNA using reverse transcription [37]. Furthermore, NGS may be used to examine changes in epigenetic factors, including modifications in promoter methylation, microRNAs, and the levels of other small RNAs. It is critical to acknowledge that, there are currently no diagnostic panels especially tailored for this objective. Life Technologies is currently developing disease-specific kits [38]. The kits provided consist of the Ion AmpliSeq Colon and Lung Panel version 2, BRCA1/2 Panel, AML Panel, and RNA Lung Fusion Panel. However, Illumina's primary objective is to create cancer-panel kits that possess extensive utility and provide insights into genes linked to various forms of cancer, such as TruSeq Amplicon and TruSight Cancer [39]. The advent of contemporary NGS technologies, including Illumina, Pacific Biosciences, and Oxford Nanopore, has profoundly revolutionized the field of genomics. These techniques enable the simultaneous sequencing of a substantial quantity of DNA fragments, ranging from millions to billions [40,41]. The ability to do so has opened up new opportunities for comprehending microbial diversity, epigenetic modifications, gene expression, and genetic variability. NGS has been essential in finding mutations that cause diseases, discovering novel targets for therapy, and providing insights into intricate biological processes, such as tumor heterogeneity and developmental processes [42]. Introducing new massively parallel or next-generation methodologies has significantly decreased the cost of sequencing per base and greatly boosted sequencing rates compared to classic Sanger sequencing and other sequence analysis methods [43,44]. NGS is expected to greatly facilitate the comprehensive analysis of the cancer cell genome and the progress of molecular pathology and tailored treatment for cancer patients.

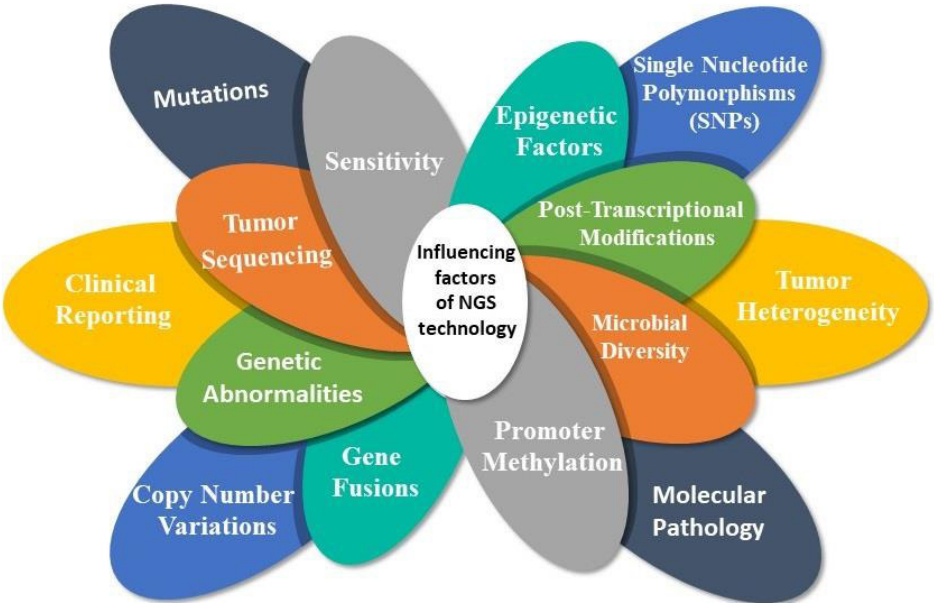

Fig 1: Influencing factors of NGS technology

3. BIOINFORMATIC APPROACHES FOR NGS DATA ANALYSIS

The management, analysis, and interpretation of DNA or RNA sequences produced by NGS requires computer algorithms. To get a deeper understanding of biological phenomena, the initial sequencing data generated by NGS devices must undergo a series of steps, including processing, analysis, and interpretation. It is the implementation phase of bioinformatics approaches, which include diverse computational tools, algorithms, and techniques for pre-processing, aligning, finding variations, measuring and studying gene expression, evaluating differences in expression, and doing additional specific research [45]. After data processing, several computational approaches like De novo assembly, reference-based mapping, and transcriptome analysis are used to retrieve essential biological information. Contemporary bioinformatics techniques facilitate the identification of genetic variations, such as single nucleotide polymorphisms (SNPs), copy number variations (CNVs), and structural variants. Integrative studies combine NGS data with other genomic and functional data sources to examine gene expression and regulatory networks.

4. NGS APPLICATIONS IN RESEARCH AND DIAGNOSTICS

NGS has profoundly transformed scientific research and clinical genomics by allowing for efficient and simultaneous analysis of many samples. The significance of NGS in translational medicine stems from its increased ability to simultaneously analyze many samples and its sophisticated computational tools for organizing data and vast collections of reference libraries. These tools aid academics, medical practitioners, and pharmaceutical researchers in understanding the genetic underpinnings of disorders. Various Population genome sequencing programs, such as 1000 Genomes, ExAC, ESP6500, UK 100K, Indigenome, and gnomAD, have produced substantial NGS data [46]. GnomAD is notable for being the biggest and most commonly used [47] of all the datasets provided. The collection comprises exome and genome sequencing data from a combined population of 140,000 people. This database is widely used for calculating allele frequencies in uncommon diseases, identifying disease genes, and comprehending the biological significance of genetic differences[48]. Consequently, establishing information databases has facilitated the development of comprehensive and restricted sequencing panels crucial for clinical research and diagnosis [49]. Moreover, comprehensive genetic panels are advantageous in clinical research, particularly cancer genetics.

5. NGS ANALYSIS FOR SOLID CANCER DIAGNOSIS

Detecting notable changes in cancer-associated genes in solid tumor samples improves the precision of patient diagnosis and prognosis. It also aids in identifying the suitable targeted medicines required to enhance the treatment for individual cancer patients within the context of customized medicine. The NGS research on solid cancer thoroughly examines the advancing molecular approach to cancer, emphasizing the advantages over conventional diagnostic approaches.

5.1. Hereditary breast cancer

Hereditary breast cancers (HBCs) account for around 5%–10% of all breast cancer cases, with approximately 30% of these cases resulting from mutations in the BRCA1 and BRCA2 genes. These genes contain the genetic instructions required for the synthesis of proteins that play a critical role in preventing the growth of tumors by repairing DNA and keeping the genetic material stable [50]. Genetic mutations in these specific genes greatly increase the likelihood of getting HBC. Therefore, it is highly recommended that individuals who have received a diagnosis of breast cancer at a young age or have a significant family history of the illness should consider receiving genetic counseling and undergoing BRCA-gene testing. Traditional DNA sequencing methods, such as direct Sanger sequencing, are labor-intensive and expensive due to the large size of the BRCA1 and BRCA2 genes, which consist of 23 and 27 exons, respectively. Denaturing high-performance liquid chromatography (DHPLC) has been used and proposed as a technique to expedite molecular analysis. The high efficacy of NGS approaches in detecting Genetic alterations such as point mutations and insertions/deletions (indels) may be seen in the BRCA1/BRCA2 genes. This technological progress has revolutionized the field of genetic

analysis by significantly decreasing both the duration and expenses involved [51-53]. NGS is currently suitable for regular diagnostic procedures since it provides superior speed and sensitivity compared to Utilizing DHPLC in conjunction with Sanger sequencing.

5.2. Melanoma

BRAF mutations significantly influence between 40% and 70% of malignant melanomas. Based on data from the COSMIC database, 44% of melanomas have BRAF mutations, with a notably elevated occurrence of 97.1%, specifically at codon 600 of the BRAF gene [54]. Vemurafenib, a medication created by Roche, obtained authorization from the US Food and Drug Administration (FDA) in August 2011 to manage advanced or metastatic melanoma [55]. It and dabrafenib belong to a class of small-molecule kinase inhibitors that effectively block mutant BRAF. Detecting BRAF mutations using highly reliable techniques in real-time is critical. The efficacy of these therapies will be determined using polymerase chain reaction (PCR) and Sanger sequencing. Ihle *et al*. assessed various methodologies used to analyze BRAF mutations[56]. An analysis was conducted to assess the sensitivity, specificity, costs, workload, feasibility, and limitations of various techniques for methods as there are many accessible methods, such as allele-specific PCR methods that include the Cobas BRAFV600 test, pyro sequencing using the Thera screen BRAF Pyro kit, high-resolution melting analysis, immunohistochemistry, the NGS approach, and Sanger sequencing [57]. They recommend using a combination of high-resolution melting and VE1-antibody staining for the most effective strategy in analyzing the p. V600E mutation. This strategy would integrate the minimum detectable limit with a swift procedure that exhibits 100% sensitivity.

5.3. Prostate cancer

Prostate cancer (PC) is progressively emerging as the primary cause of cancer-related deaths among males in several nations. The disease's passive and active variations are probably caused by many genetic occurrences, as shown by the significant tumor heterogeneity. It is not feasible to accurately differentiate before commencing treatment, as there are distinctions between these two types. The majority of men who are diagnosed with prostate cancer often have a slow-growing illness that does not need rapid and intense therapy. However, if these individuals are unduly treated, it might harm their overall well-being [58]. The therapeutic outcomes vary significantly across patients, with some individuals exhibiting a rapid recurrence of symptoms after treatment. In contrast, others maintain a state of being free from the condition for a prolonged duration before experiencing a relapse. Recent breakthroughs in NGS technology have enhanced our comprehension of PC's biological and clinical diversity. Techniques like utilization of DNA-Seq, RNA-Seq, chromatin immunoprecipitation-Seq, and methyl-Seq methods have significantly improved our understanding of the main pathways involved in the development of PC, such as the AR-signaling, PI3K–PTEN–Akt, and RTK–Ras–MAPK pathways [59]. Fourteen Two trials have shown the feasibility of doing a thorough screening of prostate cancer patients as part of regular diagnosis [60,61]. Manson-Bahr *et al*. have shown the feasibility of DNA analysis from malignant tissue acquired by transrectal ultrasonography needle-core biopsy samples. They identified a mutation pattern in PC surgery tissues that was similar to patterns seen in the past. The pattern showed genetic alterations in the SPOP, TP53, ATM, and MEN1 genes, together with the fusion of TMPRSS2 and ERG. Furthermore, nonsensical genetic alterations were detected in the genes MAP2K5 and NCOR2. Lacono *et al*. conducted an initial retrospective study using NGS on 60 samples. The sample included 30 patients categorized as high risk and 30 persons categorized as moderate risk. The researchers discovered nonsynonymous variants and single nucleotide polymorphisms (SNPs) occurring at a frequency of 10% in the TP53, CSFR1, KDR, KIT, PIK3CA, MET, and FGFR2 genes. This finding provides evidence of their contribution to the development and aggressiveness of PC. However, conducting a regular diagnostic study of many genetic abnormalities in PC must be advised.

5.4. Thyroid cancer (TC)

Most thyroid nodules seen in the general population are harmless; it is critical to differentiate the ones that might potentially be cancerous precisely. Performing ultrasound-guided fine-needle aspiration (FNA) of the thyroid nodule, followed by cytological examination, is a commonly used diagnostic technique that can effectively distinguish between malignant and benign nodules in most instances. Around 25% of nodules cannot be identified using FNA cytology because of the insufficient diagnostic material available for comprehensive molecular characterization using conventional methods. Over the last several years, many research studies have been conducted to explore the possibility of using an NGS molecular test to improve the detection of TC [62-64]. The ThyroSeq, a pioneering gene panel, was developed in 2013 to detect 284 genetic mutations in 12 cancer-related genes specifically. A grand number of 228 thyroid samples, consist of neoplastic and nonneoplastic samples, were subjected to sequencing. It included 105 frozen samples; 72 samples were preserved using formalin, and 51 were obtained using fine needle aspiration (FNA). These examples include all fundamental types of TC [65.] This method identified seventeen specific genetic alterations in different types of thyroid cancer, with mutation rates ranging from 30% to 83%. In contrast, 6% of non-cancerous thyroid nodules had similar changes. In 2014, Nikiforov *et al*. validated the efficacy of ThyroSeq version 2, a novel panel capable of identifying gene mutations, when used with ThyroSeq RNA, a panel designed to detect gene fusions [66]. Both panels were tested on substantial thyroid nodules exhibiting follicular or oncocytic (Hürthle cell) features. The results indicated that the panels facilitated an accurate assessment of the cancer risk in these nodules [67]. The cytology categorized these nodules as the diagnosis of atypia of uncertain significance/follicular lesion. ThyroSeq version 2.1 involves the examination of 14 genes to identify specific genetic changes and 42 distinct kinds of gene fusions seen in thyroid cancer [68]. Simbolo *et al*. conducted a study using the Ion AmpliSeq Hot Spot Carcinoma Panel version 2 (Life Technologies)[69] to categorize sporadic medullary thyroid cancer according to diagnostic criteria [70]. Among the 21 patients, 13 had a somatic RET mutation. The research found that there were only 10 instances identified using both Sanger sequencing and NGS., whereas Sanger sequencing could not discover 3 cases. The results indicate that NGS has greater sensitivity. Overall, this research has shown that NGS can enhance the categorization of thyroid nodules. Furthermore, this will enhance the quality of patient treatment and enable physicians to circumvent expensive and hazardous diagnostic procedures.

5.5. Lung cancer

Lung cancer (LC) is the primary cause of cancer-related fatalities in developed countries and is often detected at an advanced stage. An extensive understanding of predictive biomarkers has facilitated the identification of lung cancer patients who are appropriate candidates for tyrosine-kinase inhibitors (TKIs) as a therapeutic intervention [71]. Evaluating EGFR mutations in clinical settings is crucial for efficiently managing patients with TKIs. Around 80% to 90% of EGFR mutations comprise either a small deletion in exon 19 or the L858R mutation in exon 21. Furthermore, other genetic alterations in exons 18 to 21 of the EGFR gene may be susceptible to TKIs. The T790M mutation in exon 20 requires further examination since it is linked to resistance to first-generation TKIs but susceptibility to third-generation tyrosine-kinase inhibitors (TKIs) [72-75]. ALK rearrangement is a marker of resistance to TKI. Currently, the FDA has only authorized medications for the treatment of LC that target two specific genes, namely EGFR and ALK. The ideal sample for molecular analysis is tissue preserved in formalin and embedded in paraffin. Sanger sequencing has been widely regarded as the most reliable method for identifying EGFR mutations for several years. In recent times, alternative molecular diagnostic techniques have been utilized, which include high-resolution melting, restriction fragment-length polymorphism, mutant allele-specific PCR, peptide nucleic acid-mediated PCR, pyrosequencing, immunohistochemistry with particular EGF R antibodies, and the Scorpion Amplification Refractory Mutation System [76.] Immunohistochemistry or fluorescence in situ hybridization remains the most dependable technique for investigating ALK rearrangements. Tumor tissues remain the preferred samples for molecular analysis, and noninvasive sampling is very attractive compared to tissue biopsy. Blood collection is a less intrusive alternative to tissue samples, particularly useful for severely sick patients or where tissue specimens are limited or inaccessible. Moreover, sampling at various intervals may aid in surveilling the tumor's genetic changes and forecasting the likelihood of early resistance or lack of response to therapy. NGS may use plasma DNA to detect cancer-related genetic changes that can inform LC treatment options. Plasma has the potential to indicate the disease state more accurately than a tumor biopsy[77-80]. In addition, plasma screening may detect EGFR treatment-resistant mutations that may indicate early clinical progression during therapy [81].

5.6. Colorectal cancer

EGFR, a key factor in cancer development and survival, is the focus of many medications used in colorectal cancer (CRC) treatment. However, anti-EGFR therapy (cetuximab or panitumumab) only provides benefits to a limited number of patients with metastatic CRC. Therefore, accurately forecasting patient reactions is crucial for avoiding negative consequences and minimizing expenses. Ras proteins, including HRas, KRas, and NRas, have a vital function as downstream effectors in facilitating the transmission of EGFR, which transmits signals to the intracellular signaling cascade [82]. Patients with CRC individuals with mutations in the KRAS gene, namely at codons 12 and 13 in exon 2, exhibit resistance to treatment targeting the EGFR protein [83]. Therefore, KRAS is regarded as a predictive biomarker for assessing the efficacy of anti-EGFR treatment. A significant proportion of patients, around 40%–50%, who possess the typical KRAS exon 2 gene variant do not experience any benefits from these medications. It implies the possible existence of other genetic alterations in pathways associated with EGFR, which might have a significant impact. Recent findings suggest that resistance to anti-EGFR treatment may arise due to further mutations in KRAS and NRAS and changes in downstream genes such as BRAF or PIK3CA [84]. Both intra-tumoral heterogeneity (variations in genes inside a tumor) and inter-tumoral heterogeneity (differences in genes across different tumors) affect the probability of treatment failure and the development of drug resistance in CRC. The most recent update of the National Comprehensive Cancer Network guideline strongly advises the genotyping of tumor samples (either the primary tumor or metastasis) in all patients with metastatic CRC for RAS mutations, namely in exons 2–4 of KRAS and NRAS. Patients with a verified KRAS or NRAS mutation should not be administered Cetuximab or Panitumumab [85]. Real-time PCR and pyrosequencing are the most dependable techniques for evaluating these genes. However, both methods are time-consuming and have limited sensitivity. Many gene panels have been developed via partnerships with Illumina and Life Technologies to study various important genes related to CRC [86-89]. Indeed, using a multigene strategy is crucial to simultaneously capture a wider range of mutations, thus enhancing our understanding of CRC. In the future, the data collected from these NGS investigations is anticipated to be useful in creating new targeted drugs or prolonging the effectiveness of anti-EGFR therapy.

6. CONCLUSION

Cancer results from genetic alterations, including somatic mutations and structural variations. Advances in next-generation sequencing (NGS) and bioinformatics have revolutionized cancer research, enabling detailed genetic analysis and the identification of new cancer genes. NGS has improved diagnosis, treatment selection, and prognosis prediction. Projects like TCGA and ICGC have cataloged genetic alterations across cancer types, aiding research. NGS's rapid, accurate sequencing and reduced costs have expanded personalized medicine, improving cancer treatment and patient outcomes.

7. AUTHORS CONTRIBUTION STATEMENT

All authors have made a substantial, direct, and intellectual contribution to the work and approved it for publication. Dr. Anand Mohan Jha contributed to the data extraction, analysis, and article preparation. Prof Dr. Ammar A. Razzak Mahmood, Dr. Anil Kumar, Dr. John Abraham and P. Krubaa contributed by being a part of writing the article.

8. CONFLICT OF INTEREST

Conflict of interest declared none.

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