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Molecular genetic research in oncology – when, what and why

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Molecular tests, integral to clinical oncology, are routinely employed for diagnosing hereditary cancer syndromes. Healthy carriers of cancer-predisposing mutations can avail rigorous medical surveillance and preventive measures. Germ-line mutation-induced cancers often necessitate significant treatment strategy modifications. Personalized cancer drug selection, based on actionable mutations, is now a therapy cornerstone. The administration of inhibitors like EGFR, BRAF, ALK, ROS1, PARP, and other cytotoxic/targeted drugs is guided by molecular tests. Tumors invariably shed fragments (single cells, clusters, DNA, RNA, proteins) into various body fluids. The liquid biopsy, analyzing circulating DNA or other tumor-derived molecules, offers potential for non-invasive cancer monitoring, drug-sensitizing mutation analysis, and early cancer detection. Specific mutations and expression markers can effectively diagnose cancers of unknown primary origin (CUPs). Systematic tumor molecular portrait cataloging is likely to reveal new medically relevant DNA- and RNA-based markers. Pharmacogenetics understands how genetic variations influence individual drug responses. This knowledge aids in predicting positive patient responses to specific drugs, contributing to cancer therapy personalization. Integrating molecular tests, including pharmacogenetics, into clinical oncology has opened new diagnosis and treatment avenues. It has enabled cancer therapy personalization, improved early detection, and provided valuable tumor biology insights. As our cancer genetics understanding continues to grow, these tools will undoubtedly play an increasingly important role in improving patient outcomes.

Keywords: pharmacogenetics, oncology, liquid biopsy, cancer therapy personalization.

INTRODUCTION

Molecular genetic diagnostics, has revolutionized oncology by facilitating the detection of individual biological molecules. This approach has enabled a more precise and personalized treatment strategy, shifting the paradigm from a one-size-fits-all model to targeted therapies. The potential of molecular genetic tools was first acknowledged by oncohematologists, who recognized the diagnostic value of specific chromosomal translocations in various leukemias and lymphomas. For instance, the Philadelphia chromosome, a specific chromosomal abnormality that results from a translocation between chromosome 9 and 22, is a well-known hallmark of chronic myeloid leukemia [1]. Similarly, the translocation t(14;18), resulting in the overexpression of the BCL2 gene, is commonly observed in follicular lymphomas [2]. The advent of user-friendly methods of molecular analysis has revolutionized the field of molecular oncology. A significant breakthrough in this regard was the invention of the Polymerase Chain Reaction (PCR) by Kary Mullis in 1983 [3]. This technique, which allows for the amplification of specific DNA sequences, has had a profound impact on clinical DNA testing. PCR-based techniques are highly compatible with clinical routines. They can be used for a wide range of applications, including the detection of specific gene mutations associated with cancer, the identification of minimal residual disease, and the prediction of response to therapy [4]. Immunohistochemistry (IHC), a technique that enables the visualization of specific antigens within tissue, has played a pivotal role in the field of personalized oncology.

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One of the most significant applications of IHC is in the determination of the level of expression of the estrogen receptor (ER) in breast cancer tissues [5]. The ER status of a breast cancer, determined by IHC, is a critical factor in guiding the treatment strategy. ER-positive breast cancers, which express the estrogen receptor, are typically sensitive to endocrine therapy. Thus, IHC has revolutionized the treatment of breast cancer by enabling the tailoring of endocrine therapy based on the ER status of the tumor, determined through a laboratory test [6]. In addition to its role in breast cancer, IHC has also been instrumental in other areas of oncology. For instance, in colorectal cancer, IHC is used to test for Microsatellite Instability (MSI), a condition that leads to an increased mutation rate within the cancer cells. MSI status, determined by IHC, can influence the choice of therapy. Colorectal cancers with high MSI (MSI-H) have been found to respond well to immunomodulating therapy with immune checkpoint inhibitors [7].

Molecular tests have become an indispensable component of standard patient management in oncology, particularly in two key areas. Firstly, the identification of individuals with hereditary cancers, such as BRCA1 and BRCA2 mutations linked to breast and ovarian cancer, has become a routine practice in clinical oncology. This allows for early detection and preventive measures in individuals at high risk [8]. Secondly, numerous molecular tests, help select the most effective treatment based on the molecular characteristics of tumor tissues or other biological parameters of malignant disease. These tests can predict the likelihood of disease recurrence and guide decisions about whether chemotherapy is necessary in addition to hormone therapy [9].

There are also additional applications of molecular diagnostics in the developmental stage. For instance, modern molecule-oriented techniques, such as liquid biopsy, which virtually have no sensitivity limit, are being intensively explored for monitoring residual cancer disease and early tumor detection. This technique analyzes circulating tumor DNA (ctDNA) in the blood to detect minimal residual disease following treatment, monitor response to therapy, and detect relapse at the earliest possible stage [10]. Moreover, DNA and RNA assays, such as next-generation sequencing (NGS), can assist in differentiating between tumors of distinct histologic origin. This is particularly useful for diagnosing cancers of unknown primary site (CUPs), a condition where metastatic cancer is identified, but the location of the primary tumor is unknown [11].

Lastly, we underscore the role of Pharmacogenetic studies in predicting drug toxicity and effectiveness. These studies, conducted on DNA and/or tumor cells, have emerged as a powerful tool in the field of personalized medicine [12]. By examining specific genes that encode for drug-metabolizing enzymes, transporters, or targets, pharmacogenetic studies can help predict an individual's response to a particular drug, both in terms of its therapeutic effect and potential toxicity [13].

The aim of this article is to provide a comprehensive overview of the role and impact of molecular-genetic research in oncology. It seeks to elucidate the 'when', 'what', and 'why' of various investigative techniques, their practical applications, and their implications for patient management. The article will delve into key areas such as hereditary cancer syndromes, predictive markers, circulating tumor fragments, carcinoma of unclear primary origin, and pharmacogenetic studies. The ultimate goal is to enhance understanding and stimulate further research and discussion in this critical area of oncology.

Hereditary cancer syndromes

Hereditary cancer syndromes are characterized by a collection of genetic abnormalities that significantly increase the risk of cancer. Notably, this risk is often organ-specific, enabling the implementation of targeted diagnostic and preventive measures for individuals carrying these germ-line mutations. Compared to traditional genetic diseases, hereditary cancers are considerably more prevalent. For instance, the population frequency of breast or ovarian cancers linked to BRCA1/2 gene defects is approximately 1:500 and can even reach 1:40 in certain founder populations [14]. In the Ashkenazi Jewish population, the incidence of BRCA1/2 mutation status is particularly noteworthy. Approximately 2.0% of individuals of Ashkenazi Jewish descent carry a pathogenic variant in one of these two genes, usually one of three specific variants (BRCA1: c.68_69del AG; BRCA1:c.5266dupC; BRCA2:c.5946delT), called founder mutations [15]

There are several hereditary cancer syndromes, each associated with specific types of cancer. Hereditary Breast & Ovarian Cancer Syndrome (HBOC) is associated with mutations in the BRCA1 and BRCA2 genes, leading to a significantly increased risk of developing breast and ovarian cancer [16]. Lynch Syndrome (Hereditary Non-polyposis Colorectal Cancer Syndrome) is characterized by an

increased risk of colorectal cancer and other types of cancer at a young age, and is associated with mutations in several genes, including MLH1, MSH2, MSH6, PMS2, and EPCAM [17]. Familial Adenomatous Polyposis (FAP) is associated with mutations in the APC gene, leading to the development of numerous polyps in the colon and rectum at a young age, significantly increasing the risk of colorectal cancer [18]. Cowden Syndrome (CS) is associated with mutations in the PTEN gene, leading to an increased risk of developing several types of cancer, including breast, thyroid, and endometrial cancer [19]. Peutz-Jeghers Syndrome is associated with mutations in the STK11 gene, leading to the development of characteristic pigmented spots on the lips and in the mouth, as well as polyps in the digestive tract, and an increased risk of developing several types of cancer, including gastrointestinal, breast, and gynecological cancers [20].

Hereditary cancers often exhibit unique clinical features, including early onset, the presence of multiple neoplasms, and a preference for specific histological patterns. The advent of genetic testing has revolutionized the management of hereditary cancer syndromes, facilitating early detection, personalized treatment strategies, and improved patient outcomes.

According to the National Comprehensive Cancer Network (NCCN) guidelines, molecular genetic testing for hereditary cancer syndromes is indicated when there is: a personal or family history suggestive of a hereditary cancer syndrome; early-onset cancer; multiple primary tumors; rare cancers; or several family members with the same or related forms of cancer. The guidelines also recommend genetic testing when the results can potentially impact risk management and treatment [21].

Traditionally, genetic testing is focused on single-gene analysis of specific high-risk genes. However, the advent of next-generation sequencing (NGS) has revolutionized this approach. NGS allows for the simultaneous testing of multiple genes, thereby facilitating a comprehensive analysis of cancer susceptibility genes [22]. This is particularly beneficial in cases where several genes may be implicated in a hereditary cancer syndrome, or where the genetic cause of cancer is complex and multifactorial. Multi-gene panel tests, which use NGS technology, can identify pathogenic mutations across a range of genes in a single test [23]. This not only increases the efficiency of genetic testing but also broadens our understanding of the genetic basis of hereditary cancers. As such, NGS and multi-gene panels have become a frontline approach in the identification of individuals with cancer predisposing gene variants.

In addition to multi-gene panels, whole exome sequencing (WES) by NGS is another powerful tool in the study of hereditary cancers [24]. WES involves sequencing all the protein-coding genes in a genome (known as the exome), allowing for a comprehensive analysis of cancer susceptibility genes [24]

Importantly, the use of NGS in genetic testing has the potential to identify new genes associated with hereditary cancers. As our understanding of the genetic landscape of cancer continues to grow, it is likely that more genes associated with hereditary cancer syndromes will be discovered. This underscores the clinical significance of NGS and multi-gene panel and WES analysis in hereditary cancer predisposition.

Effective treatment based on the genetic characteristics of the tumor

The advent of precision oncology has underscored the importance of tailoring cancer treatment based on the genetic characteristics of the tumor. Specific genetic alterations within the tumor, such as mutations, amplifications, or translocations, can drive cancer growth and progression [25]. These genetic aberrations can be identified through techniques such as NGS, allowing for a comprehensive genomic profile of the tumor.

Tumors harboring mutations in the KRAS, NRAS, or BRAF genes have been shown to respond to specific targeted therapies [26]. KRAS mutations are among the most common oncogenic alterations in human cancers. For a long time, these mutations were considered ‘undruggable’ due to the molecular structure of the KRAS protein, which made it difficult for drugs to bind effectively. However, recent advances in drug development have led to the creation of effective inhibitors against specific KRAS mutations [26]. One such mutation is the G12C mutation, which is common in lung and colorectal cancers. The development of KRAS G12C inhibitors represents a significant breakthrough in the treatment of cancers with this mutation. AMG 510 (Sotorasib) is the first FDA-approved specific KRAS G12C inhibitor that works irreversibly. It blocks KRAS in an inactive GDP-bound state. Preclinical studies of AMG 510 have shown that it inhibits phosphorylation of extracellular signal-regulated kinase (ERK), a critical downstream effector of KRAS, and induces

long-lasting complete tumor regression in mice bearing KRAS p.G12C tumors [27].

Similarly, BRAF mutations, particularly the V600E mutation, can be targeted by BRAF inhibitors. The V600E mutation results in a constitutively active BRAF kinase that promotes cell growth and proliferation. BRAF inhibitors work by selectively inhibiting the activity of the mutated BRAF kinase, thereby slowing down tumor growth [28].

NRAS mutations, on the other hand, can be targeted indirectly through MEK inhibitors. The NRAS protein is part of the RAS/RAF/MEK/ERK signaling pathway, which regulates cell growth and survival [29]. Mutations in NRAS can lead to the continuous activation of this pathway, promoting uncontrolled cell proliferation. MEK inhibitors work by blocking the activity of MEK, a protein downstream of NRAS in the signaling pathway, thereby inhibiting the growth of tumors with NRAS mutations. Research has shown that AML cells with NRAS mutations are dependent on continued oncogene expression, both in vitro and in vivo [30]. MEK inhibitors, such as PD0325901 or trametinib, have been used in preclinical studies to treat primary Nras-mutant AMLs. These treatments significantly prolonged survival and reduced proliferation but did not induce apoptosis, promote differentiation, or drive clonal evolution.

In the context of melanoma, studies have also shown the potential of MEK inhibitors in treating NRAS mutant melanoma [31]. However, all these findings are from preclinical studies and more research is needed to validate these results in clinical settings.

Rearrangements in the anaplastic lymphoma kinase (ALK) and c-ros oncogene 1 receptor tyrosine kinase (ROS1) genes have emerged as significant actionable targets in cancer therapy [32]. These genetic rearrangements are frequently observed in non-small cell lung carcinoma (NSCLC), a common type of lung cancer [33].

The presence of these rearrangements is associated with distinct clinical and pathological features. Patients with these rearrangements are often younger, have a milder or no history of smoking, and exhibit adenocarcinoma histology (34). Moreover, these rearrangements have been found to contribute to the metastasis of NSCLC by promoting cell migration and invasion [34].

Targeted therapies, such as ALK inhibitors (Crizotinib; Ceritinib; Alectinib Brigatinib; Ensartinib; Lorlatinib), have been developed to specifically act against tumors harboring these genetic rearrangements. The use of ALK inhibitors has led to significantly improved survival benefits [35]. However, the clinical benefits of ALK inhibitors are almost universally limited by the emergence of drug resistance. Therefore, continued research into new drugs and combination therapies is required to improve outcomes in NSCLC. The ultimate goal is to enhance the efficacy of these targeted therapies and improve the prognosis for patients.

Furthermore, mutations in the BRCA1/2 genes, particularly in the context of Hereditary Breast and Ovarian Cancer Syndrome (HBOC), have been effectively targeted by Poly (ADP-ribose) polymerase (PARP) inhibitors [36]. These inhibitors, such as olaparib (Lynparza), rucaparib (Rubraca), and niraparib (Zejula), are particularly effective against tumors carrying mutations in the BRCA1 and BRCA2 tumor suppressor genes [37]. PARP inhibitors work by blocking the activity of PARP, a protein that helps cells repair damaged DNA. In the presence of BRCA1/2 mutations, cells are already deficient in one mechanism of DNA repair. By blocking PARP, these drugs further hinder the cell's ability to repair DNA damage, leading to the accumulation of DNA damage and, ultimately, cell death [37]. The FDA has approved four PARP inhibitors to treat cancers with BRCA1/2 mutations [38]. These drugs have shown significant clinical outcomes in treating BRCA1/2 deficient cancers. Treatment with rucaparib improves how long some people with metastatic prostate cancer live without their cancer getting worse. Among patients whose tumors had BRCA mutations, progression-free survival was 11.2 months in those treated with rucaparib and 6.4 months in those who were treated with any of three other commonly used drugs for this form of prostate cancer [39]. Ongoing preclinical and clinical studies are exploring how to combine the PARPi with immuno-oncology drugs to further improve clinical outcomes [38]

The use of genetic testing and targeted therapies based on the genetic characteristics of the tumor represents a paradigm shift in cancer treatment. By matching the treatment to the genetic makeup of the tumor, precision oncology aims to improve the efficacy of therapy, minimize side effects, and ultimately, enhance patient outcomes. However, challenges remain, including the development of resistance to targeted therapies and the need for ongoing monitoring of the tumor's genetic landscape to capture the emergence of new alterations. As our understanding of the genetic basis of cancer continues to grow, so too will the potential of precision oncology to transform cancer treatment.

Liquid biopsy

Liquid biopsy, a non-invasive diagnostic tool, has emerged as a revolutionary technique in the field of oncology [40]. It involves the analysis of bodily fluids, primarily blood, to detect circulating tumor cells (CTCs) or circulating tumor DNA (ctDNA) [41]. As tumors grow, they shed cells and DNA fragments into the bloodstream, which can be detected and analyzed using liquid biopsy. This technique provides a comprehensive snapshot of the tumor's genetic landscape, enabling the detection of specific genetic alterations that can guide personalized treatment strategies [41]

This method, has shown promise in various clinical scenarios:

1. *Early Cancer Detection:* Liquid biopsy can detect cancer at an early stage, which is crucial for improving quality of life, survival rates, and reducing the financial burden of cancer treatments [42]. It has been utilized for the early detection of solid cancers and concentration of tumor DNA in the bloodstream can provide an indication of the cancer's advancement [43].

2. *Monitoring Treatment Response:* Liquid biopsies can be used to monitor cancer progression, track a patient's response to treatment [44], or as a surveillance method for individuals who have completed treatment but are at high risk of disease recurrence [45]. For instance, in individuals with non-metastatic pancreatic cancer, liquid biopsies have been demonstrated to diagnose surgically removable tumors [46].

3. *Tracking Minimal Residual Disease (MRD):* MRD refers to the presence of disease undetectable by conventional clinical and imaging methods [40]. Liquid biopsies can detect MRD, enabling the detection of circulating tumor DNA (ctDNA), circulating tumor cells (CTC), or tumor-specific microRNA. These liquid biopsy markers not only enhance our understanding of the disease but also pave the way for personalized medicine, where treatment decisions are tailored to the individual patient's disease profile.

Despite its potential, several challenges need to be addressed to fully realize the clinical utility of liquid biopsy:

1. *Sensitivity and Specificity*: Many liquid biopsy strategies being developed for early detection of cancer lack the sensitivity required to detect early-stage cancers. Additionally, the small amounts of tumor-derived components shed into the circulation can limit the detection of cancer at early stages (47].

2. *Standardization and Reproducibility:* There is a lack of preclinical and clinical standardization, which has so far hindered the development of an algorithm for precise tumor profiling [48].

3. *Technical Challenges:* Isolating circulating tumor cells (CTCs) can be technically more challenging than isolating cfDNA [48].

Liquid biopsy is a powerful tool, and significant advances in this technology have impacted multiple aspects of precision oncology, from early diagnosis to management of refractory metastatic disease [48]. The goal is not to select and refine a single approach to liquid biopsy. In fact, the synergy of multiple circulating biomarkers can reveal the specifics of a cancer. Future research may focus on fluids beyond blood, such as ascites, effusions, urine, and cerebrospinal fluid, as well as methylation patterns and elements such as exosomes. The FDA has approved several liquid biopsy tests, such as Guardant360 CDx and FoundationOne Liquid CDx, which check for multiple cancer-related genetic changes [41]. These tests can assist doctors in selecting the best treatments for some people with cancer. Liquid biopsy tests are currently used for non-small cell lung cancer, advanced breast cancer, colorectal cancer, and prostate cancer [41].

Liquid biopsy holds great promise for both healthy individuals and those diagnosed with cancer. For healthy individuals, it could serve as a routine prescreening method to identify those who may have early-stage cancer. For cancer patients, it provides valuable information about cancer cells that can help healthcare providers plan treatment and management.

Carcinoma of unclear primary origin (CUP)

The diagnosis of carcinoma of unclear primary origin (CUP) remains a significant challenge in oncology. Approximately 3–5% of patients with newly diagnosed metastatic disease have an unknown organ or tissue origin for these metastases [49]. In many cases, the inability to make the correct diagnosis is solely due to limitations in tumor imaging techniques. Even autopsy fails to identify the primary tumor in 15–45% of cases [49].

The diagnostic approach in patients with CUP largely relies on common clinical judgment, anatomical localization of metastases, gender of the patient, and habits such as smoking [50]. Immunohistochemistry, which uses a spectrum of tissue-specific markers, is the gold standard for the clinical analysis of CUP. However, it has several

limitations. Many expression-based markers are not sufficiently specific for a given tumor type. Some proteins are expressed at low levels and therefore cannot be detected by conventional antibody-based methods [51]. The range of diagnostic antibodies is limited to those marketed by biotechnology companies, and interpretation of immunohistochemistry results is subject to interlaboratory variation.

DNA- and RNA-based tests may offer advantages over immunohistochemistry. Certain mutations are highly characteristic of specific types of cancer. For instance, the presence of a TKI-sensitizing somatic mutation in EGFR in tumor tissue aids in diagnosing lung cancer [52], and detection of a BRCA1/2 germline mutation in a patient with adenocarcinoma of unknown primary site prompts consideration of breast or ovarian cancer as the most likely tumor variety [53].

RNA expression markers can outperform some immunohistochemical tests. The development of personalized PCR diagnostic tests, which can be performed in any molecular genetics laboratory without the need for industrial facilities, opens up new avenues for the diagnosis and management of CUP [54]. This advancement brings us closer to the era of personalized medicine, offering hope for improved patient outcomes.

Despite the challenges posed by CUP, these innovative methods offer a beacon of hope. They not only enhance the accuracy of diagnosis but also pave the way for personalized treatment strategies, thereby bringing us closer to the era of personalized medicine. However, the journey is far from over. The field continues to evolve, and further research is needed to overcome the existing limitations and to fully harness the potential of these advanced techniques.

Pharmacogenetic studies

Pharmacogenetic studies have emerged as a powerful tool in the field of personalized medicine, underscoring the role of genetic variations in predicting drug toxicity and effectiveness [55]. These studies, conducted on DNA and/or tumor cells, provide insights into how an individual's unique genetic makeup influences their response to drugs [55]. By examining specific genes that encode for drug-metabolizing enzymes, transporters, or targets, pharmacogenetic studies can help predict an individual's response to a particular drug, both in terms of its therapeutic effect and potential toxicity [53]. In the realm of cancer genomics, many studies have traditionally focused on acquired, somatic mutations [54;55]. These are mutations that are unique to tumor cells and occur in genes encoding proteins that play a central role in the hallmark processes that dictate malignant growth. They are acquired randomly following exposure to agents that have the potential to damage DNA in cells [54]. In the context of cancer, these somatic mutations accumulate in the cancer cells and are commonly used as drug targets [54].

However, increasing evidence shows that inherited germline genetic variations also play a key role in cancer risk and treatment outcome [56]. For example, variations in the DPYD gene can affect the metabolism of 5-fluorouracil, a commonly used drug in the treatment of various cancers. Patients with certain DPYD variants may experience severe toxicity when treated with standard doses of 5-fluorouracil [56].

These examples underscore the importance of pharmacogenetics in oncology, as understanding these genetic variations can lead to more personalized and effective treatment strategies, thereby improving patient outcomes and reducing adverse drug reactions.

CONCLUSION

We are currently in the midst of a transformative era in medical research. The advent of Next-Generation Sequencing has revolutionized the field, enabling the analysis of germline variants in DNA, somatic mutations, and RNA profiles. This has led to a continuous accumulation of data, paving the way for the identification of new hereditary syndromes, molecular targets for targeted cancer therapy, tumor-specific diagnostic markers, and pharmacogenetic options for a personalized approach.

However, it is important to acknowledge the challenges that come with this progress. The clinical integration of even relatively simple and straightforward assays, such as BRCA1/2 analysis or EGFR mutation testing, took years, and many questions remain unresolved. The complexity of these processes underscores the intricacies involved in translating research findings into clinical practice. Furthermore, the management of the huge influx of new candidate markers poses a significant challenge. These markers, represented by multiple rare and diverse molecular events, cannot be clinically validated on an individual basis due to their rarity and diversity. This raises questions about how clini-

cal medicine will adapt to accommodate this wealth of information. Despite these challenges, the potential benefits of these advancements are immense. They hold the promise of transforming patient care by enabling more precise diagnoses, more effective treatments, and ultimately, better patient outcomes. The profound impact of molecular diagnostics in oncology is undeniable, providing clinicians with invaluable tools for accurate diagnosis, prognosis, and therapeutic decision-making. As our understanding of the molecular underpinnings of cancer continues to grow, so too will the role of molecular diagnostics in guiding cancer management. This ongoing evolution underscores the dynamic nature of medical research and its potential to shape the future of oncology.

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