Ref: Ro Med J. 2021;68(Suppl6)
DOI: 10.37897/RMJ.2021.S6.2

Modern diagnostic technologies and molecular therapies in oncogenetics

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ABSTRACT

Advances in cancer genomics research in recent decades have shown that different types of genomic changes cause neoplasms. The last genome was sequenced in 2018, and the information obtained from this technology brought valuable data for research and personalized management of these patients. By the second half of the last decade, it was becoming clearer and clearer that, although the number of genetic changes that could be achieved through targeted therapies was expanding rapidly, there was no genomic technology to identify them all at once. The mismatch repair (MMR) is represented by epigenetic mutations or phenomena in one of the genes of the MMR complex (most commonly MLH1, MSH2, MSH6 and PMS2). As a result, there is an accumulation of DNA abnormalities, especially in the mononucleotides repeats (MNRs) or dinucleotide repeats in the genome, which are known as microsatellites. A correct genetic diagnosis of the cancer patient helps to make the whole case management and directs the multidisciplinary medical team towards targeted molecular therapies.

Keywords: molecular therapy, oncogenetics, cancer, tumor genomic profiling, genotyping, MMR testing

TUMOR GENOMIC PROFILING (TGP)

"*All cancers are genetic, but some cancers are more genetic than others*". (George Orwell)

Advances in cancer genomics research in recent decades have shown that different types of genomic changes cause neoplasms. In 2012, the 100,000 Genomes Project was launched in the United Kingdom, which aimed to sequence the genomes of 100,000 patients with rare diseases and cancers. The last genome was sequenced in 2018, and the information obtained from this technology brought valuable data for research and personalized management of these patients. To date, this project concludes that for approximately 50% of cancer cases there is a choice of targeted molecular therapy or that patients may be included in clinical trials for further research. The 1+Million Genomes initiative (1+MG)

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Article History:
Received: 27 November 2021
Accepted: 22 December 2021

was launched in 2018 and is a partnership between the European Union and the U.K. that aims to sequence at least 1 million genomes in Europe, to collect data that will be used in national strategies for disease prevention, personalized medicine and in the development of new targeted molecules for diseases with genetic determinism, including neoplasms (1).

Some cancers show the same changes in one or more genes (95% of chronic myeloid leukemias have a reciprocal translocation between chromosomes 9 and 22, resulting in the BCR-ABL1,2 fusion genes), other cancers have a wide range of genetic changes that induce malignancy. In contrast, although many of these changes show tumor-specificity (e.g., BRAF mutations occur frequently in papillary thyroid carcinomas and cutaneous melanomas), they may also occur with lower frequencies in many other cancers (e.g., BRAF mutations are present in 2% to 20% of non-small cell lung cancers (NSCLC), colorectal adenocarcinomas, pediatric low-grade astrocytomas, and multiple myelomas). This variety of genetic events requires special technologies and methodologies in diagnosing cancer, as more and more genetic changes become clinically actionable through targeted molecules (2,3).

GENOTYPING

"*We've been asking ourselves a question whose answer has been sought since 1950. How many mutations does it take for a normal cell to turn into a cancer cell? The answer is - few. For example, about 4 mutations per patient are needed on average to trigger liver cancer, while for colorectal cancer about 10 mutations are needed*". (Dr. Peter Campbell)

A distinctive feature of several classical oncogenes (e.g., EGFR, RAS, BRAF, PIK3CA) is that they do not require the finding of the entire coding sequence to identify the most important activation mutations. In contrast, a subset of critical point mutations of the oncogene (and small indels) affects the "hotspot regions". For example, finding only 10 to 20 bases in each gene can test 80% to 99% of known mutations in EGFR, BRAF and KRAS genes. This phenomenon is well suited to methods such as mass spectrometry genotyping and PCR. As a result, these technologies have emerged as notable alternatives to conventional Sanger sequencing - in terms of cost, production rate and sensitivity - for assessing the specificity of "driver" mutations in oncogenes. However, such technologies also have disadvantages, including limited sensitivity (a minimum of about 10% tumor fraction), limited extent (number of genes and mutations), and an inability to detect multiple categories of genomic changes. The shortcomings of these technologies are similar from the qualitative point of view to those of Sanger sequencing and present significant limitations on the use of such platforms as clinical tools for comprehensive genomic profiling of the tumor (4,5).

NEXT GENERATION SEQUENCING

By the second half of the last decade, it was becoming clearer and clearer that, although the number of genetic changes that could be achieved through targeted therapies was expanding rapidly, there was no genomic technology to identify them all at once. Prior to the advent of next-generation sequencing, up to three separate profiling and genotyping platforms were required to detect underlying mutations, copy number variations (CNVs), and translocations in a tumor specimen.

Since 2005, several DNA sequencing technologies have emerged, which were completely different from the capillary-based instruments used to analyze the first human reference genome. These next-generation massive parallel sequencing (MPS) technologies have allowed an unprecedented depth and breadth of genomic investigations that have transformed the knowledge of cancer genomics.

MPS technologies have three major advantages compared to conventional techniques.

First, they have led to an exponential decline in the cost of sequencing, making this technology widely accessible to many researchers and laboratories around the world. For example, in 2007, single-cell genome (DNA) sequencing using the Sanger method cost $ 70 million; by 2010, the cost of genome sequencing using MPS had dropped to about $ 50,000, and in 2013, genome sequencing could be done in a commercial or research environment for less than $ 5,000. Currently, in Romania, the cost of whole-genome sequencing (WGS) is between 2000 and 3000 €.

Second, the development of MPS has provided substantial increases in both the sensitivity and scalability of sequencing, thus allowing a deep mapping of thousands of genes. The first MPS method reported a 100-fold improvement in flow rate over Sanger sequencing; in 2013, it was possible to sequence more than 10 human genomes in a single day, using an Illumina HiSeq sequencing system.

Third, these technologies offer the ability to detect several types of changes in the cancer genome (basic mutations, indels, changes in the number of copies, and rearrangements). MPS technologies vary in enzymatic reactions and signal detection methodologies, but share common methodological attributes that have produced the innovative improvements mentioned above compared to capillary sequencing (5,6).

MMR TESTING AND COLORECTAL CANCER

The mismatch repair (MMR) is represented by epigenetic mutations or phenomena in one of the genes of the MMR complex (most commonly MLH1, MSH2, MSH6 and PMS2), which leads to the dysfunction of the enzymes produced by them. As a result, there is an accumulation of DNA abnormalities, especially in the mononucleotides repeats (MNRs) or dinucleotide repeats in the genome, which are known as microsatellites. Because DNA damage cannot be repaired, there is a change in the size of microsatellites, which can promote deletions or insertions of genes (InDel) and, consequently, of proteins encoded by them. Moreover, these insertions and deletions induce a change in reading frame and the production of truncated or altered proteins and, therefore, an increase in the expression of neoantigens. This is one of the reasons why MSI-MMR tumors are more immunogenic than other types of tumors.

MICROSATELLITE INSTABILITY – MSI TESTING IN COLORECTAL CANCER

Microsatellite instability (MSI) is a molecular marker for some colorectal cancers (CRCs) in which short tandem repeats are prone to mutations along with DNA sequences. This is due to a deficiency in the DNA repair system due to a germline or somatic mutation in the mismatch repair (MMR) gene. Germline mutations lead to Lynch syndrome (LS), while epigenetic inactivation results in sporadic colorectal cancer (CRC) tumors.

MSI testing is used to classify colorectal tumors as MSI – with high microsatellite instability, MSI-high (MSI-H), MSI – with low microsatellite instability, MSI-low/ MSI-L, and microsatellite stabile tumors – MSS. MSI-H tumors account for about 15% of all colorectal cancers and have the best prognosis, the highest survival rate, so MSI testing is considered a prognostic biomarker. The same test is used to identify Lynch syndrome in patients with a family history of CRC (7,8,9).

Previous studies have shown a predictive role for testing MSI in the chemotherapy process, the emergence of controversial findings in recent studies have not convinced many authors to recommend it as a routine examination to assess the therapeutic response. MSI testing remains an excellent prognostic and diagnostic tool for CRC tumors (10,11).

Due to controversial findings from studies on the prediction of the role of MSI in the response to chemotherapy, the European Society of Medical Oncology (ESMO) recently did not consider MSI as a predictive marker for chemotherapy. However, the association between the MSI status and the response to chemotherapy remains another promising field in cancer clinical and molecular research(12).

RAS AND BRAF TESTING IN COLORECTAL CANCER

The RAS/ RAF/ MEK/ ERK signaling cascade, known as the mitogen-activated protein kinase (MAPK) pathway, plays a key role in cell proliferation, differentiation, survival, and apoptosis.

Mutations that activate BRAF are usually mutually exclusive with KRAS mutations, represent 5-15% of the mutations found in CRC and are associated with a poor prognosis in stage II, III and IV. A study conducted in 2018 reported an average survival rate for patients with KRAS, NRAS and wild-type BRAF of 49.2, 36.2, 30.1 and 22.5 months, respectively.

BRAF protooncogene is located on chromosome 7 and consists of 18 exons; the typical mutation consists of a change of valine to glutamic acid at codon 600 (BRAF V600E), corresponding to almost 95% of the observed mutations. This change, identified in up to 7% of cancers, results in a constitutively active protein, similar to what happens in tumors with KRAS mutations. The pathognomonic mutation for sporadic CRCs in the BRAF gene (V600E) helps to differentiate sporadic CRCs from LS-associated CRCs.

Double mutations of RAF and RAS are rarely detected at the same time: in a report that includes a total of 2530 patients from three randomized studies in metastatic CRC (COIN, PICCOLO and FOCUS), there were only eight cases (0.3%). Although BRAF and KRAS work together in the EGFR pathway, their mutations result in different patterns of gene expression, with even greater heterogeneity found in CRC with BRAF mutations, as shown in a study of 218 BRAF-V600E colorectal mutated tumors. Two subgroups are identified: one subgroup expresses a high activation of KRAS/ mTOR/ AKT/ 4EBP1/ EMT, and the second is characterized by cell cycle disorders. Both were independent of microsatellite instability, PI3K mutations, and sex. These two subgroups may correlate with different responses to BRAF and MEK inhibitors (2,13,14).

MGMT TESTING IN GLIOBLASTOMAS

Grade IV glioblastoma or astrocytoma is the most common primary malignancy of the central nervous system in adults. It has a very poor prognosis, with an average survival rate of less than a year. The current standard of care is surgical resection followed by radiation therapy in addition to alkylating agent temozolomide (TMZ) chemotherapy.

MGMT (O 6-methyl-guanine-DNA methyltransferase) is a DNA repair enzyme. This enzyme re-

pairs tumor cells from damage induced by the alkylating agent, thereby inducing resistance to alkylating agents chemotherapy. Epigenetic silencing of the MGMT gene by promoter methylation results in decreased MGMT protein expression, reduced DNA repair activity, and a potential increase in sensitivity to therapy. The methylation status of the MGMT promoter was widely assessed by the methylation-specific PCR method, which is both sensitive and specific.

MGMT promoter methylation has been reported to be elevated in lower-grade oligodendrogliomas and astrocytomas, in which it is variably correlated with 1p19q deletion and IDH mutations. The prognostic and predictive significance of MGMT promoter methylation status in these tumors has been demonstrated in some clinical trials.

THE IMPORTANCE OF ONCOGENETIC DIAGNOSIS

A correct genetic diagnosis of the cancer patient helps to make the whole case management and directs the multidisciplinary medical team towards targeted molecule therapies.

PARP inhibitors

PARP inhibitors or poly (ADP-ribose) polymerase inhibitors are a type of drug that targets cancer. PARP is a protein that helps damaged cells repair themselves. Some examples of PARP inhibitors are olaparib, niraparib and rucaparib. These molecules are currently used for breast, ovarian, fallopian tube, and peritoneal cancer, where patients have BRCA1 or 2 mutations.

Researchers initially used these drugs in cancers that have defects in the repair of cell damage, especially cancers with a mutation in the BRCA1 and BRCA2 genes. The BRCA1 and BRCA2 genes normally play an important role in repairing cells in the body. Cells are less likely to repair themselves if there is a mutation in one or both genes. People with BRCA mutant genes have an increased risk of certain cancers, including breast cancer, ovarian cancer, prostate cancer, and pancreatic cancer. Cancer cells with faulty BRCA genes already have a poor repair system, so blocking PARP with a PARP inhibitor means that the cells will no longer be able to repair themselves and be destroyed.

There are currently clinical trials with these PARP inhibitory molecules in cancers of the pancreas, lung, head and neck, glioblastoma, prostate, stomach, esophagus, uterus, cervix, kidney, and bladder (15).

Cell growth inhibitors

Cell growth factors are chemicals produced by the body that control cell development. There are many types of growth factors and they all work in different ways. Some growth factors direct the cells to their mature form, indicating them how to specialize, while others cause the cells to grow and divide into new cells, or signal the cells to stop developing or even induce programmed death, or apoptosis. Growth factors work by binding to receptors on the cell surface. This sends a signal inside the cell, which triggers a chain of complex chemical reactions.

Types of growth factors are: epidermal growth factor (EGF) - controls cell growth; vascular endothelial growth factor (VEGF) - controls the development of blood vessels; platelet-derived growth factor (PDGF) - controls the development of blood vessels and cell growth; fibroblast growth factor (FGF) - controls cell growth.

EGFR/ ErbB-1 inhibitors

Each growth factor works by attaching to the appropriate receptor on the cell surface. For example, EGF binds to the epidermal growth factor receptor (EGFR).

There are two major classes of EGFR inhibitors: tyrosine kinase inhibitors (TKI) (e.g., erlotinib, gefitinib): these bind to the tyrosine kinase domain in the epidermal growth factor receptor and stop EGFR activity (16,17,18); monoclonal antibodies (e.g., cetuximab, necitumumab): these bind to the extracellular component of EGFR and prevent the binding of the epidermal growth factor to its own receptor, thus preventing cell division.

EGFR inhibitors can be used in the treatment of various cancers such as: non-small cell lung cancer (NSCLC), pancreatic cancer, breast cancer and colon cancer.

CONCLUSIONS

The new powerful genome sequencing technologies and RNA expression research brought an immense progress in understanding of the genesis and pathways of cancers. Cancer show increased incidence in certain families, sometimes without fitting mendelian pattern; these cases may be due to single gene mutation with obscure penetrance. Even if the genetic basis of cancer susceptibility in general population is not completely revealed, identifying the persons who genetically belongs to high-risk class for the development of cancer represents the next target of the genetic research. A variety of mutational mechanisms are implicated in the concept of activation of oncogenes that underline sporadic cancers occurrence. Gene expression profiling is used in our days to guide the oncogenic therapy. All these aspects justify the presence of oncogenetics among the most important innovative technologies of the gynecological practice.

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Conflict of interest: none declared
Financial support: none declared