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Next generation sequencing: a new era in comprehensive cancer management

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More than three-quarters of all mortality are related to non-communicable diseases (NCD). There were 1,67,256 new cases with 1,16,598 deaths from cancer in 2022 in Bangladesh and 1,56,775 new cases were detected in 2023.[1] The most common malignancies are those of the mouth, throat, oesophagus, stomach, breast, cervix uteri and lung in Bangladesh. In men, oesophagus, lung and oropharynx cancer rank as the top three. In women, oesophagus, cervix uteri and breast cancer are most prevalent.

Unfortunately, in majority of cases, cancers are diagnosed in late stages. It is well established that cancer can be largely cured if detected in an early stage. The main reasons for this late diagnosis are lack of awareness, social stigma, lack of enough facilities (specialist doctors and logistic support) in most part of the country. Traditionally, cancer management is considered as by involvement of surgery (surgical oncology), radiation (radiation oncology) and chemotherapy (medical oncology). Never the less, diagnosis or diagnostic oncology was not only perceived but not recognized as the most important field in medicine. Whereas, cancer prevention and early detection is the most desirable steps that should be emphasized. With the advent of targeted therapy (monoclonal antibodies and tyrosine kinase inhibitors) and immunotherapy, the importance of molecular tests for cancer management is ever increasing.

Cancer genomic profiling is based on next-generation sequencing (NGS) of cancer DNA and RNA. The test result will provide the information on diagnosis, risk assessment, treatment option, response to drug, detection of minimal residual disease and relapse. Till recently, such tests were not available for routine purposes. Obviously, the samples were mostly sent to abroad and many of the cancer patients go to the neighbouring countries for treatment, which drain our foreign reserve and neglected the development of this advanced field for fighting against cancer. With a humble beginning, our primary goal is to make these facilities/ technologies available in BIRDEM General Hospital and secondarily, initiate a comprehensive cancer care in our country.

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Past

English biochemist Frederick Sanger won the Nobel Prize in chemistry twice, the first time in 1958 for his work on the structure of the insulin molecule and then in 1980 for determining the base sequence of nucleic acids.[2] The emergence of cancer genomics has begun a new era of personalized and targeted therapies. The Human Genome Project was launched in 1990 and completed in 2003.[3] The advent of NGS technology has dramatically increased the efficiency of genome decoding and accelerated studies on cancer genomics. Cancer genome sequencing efforts began in 2005 including The Cancer Genome Atlas (TCGA). In 2007, the first whole-human genome sequence was generated through NGS.

Present

Cancer genome sequencing in clinical settings currently uses sequencing of i) whole genome (3.2 billion base pairs or gigabase long), ii) whole exome (about 1.1% or 30 megabase of DNA), iii) DNA methylation (60-80% of

genomic DNA)[4], iv) RNA transcriptome (median 16342 gene)[5] and v) targeted cancer related gene(s). However, protein-coding exons make up only 1.2% of the human genome. Limited information is available on somatic mutations in non-coding regions such as untranslated regions, introns, promoters, regulatory elements, non-coding functional RNAs, repetitive regions and mitochondrial genomes, which make up 98% of the human genome. Structural variants and viral incorporation into cancer genomes have not yet been widely investigated.

The evolution of sequencing technologies can be stratified into 3 distinct phases or generations.[6]

The first-generation was Sanger sequencing in the 1980s, which served as the cornerstone for DNA sequencing. It has some limitations, like, slower in processing, expensive, low through put.[7] However, the first human genome (in the Human Genome Project)[3] was sequenced with this technique taking thirteen years to complete with a staggering three billion dollars.

The second-generation was massively parallel sequencing through platforms such as Illumina and Ion Torrent, which revolutionized high-throughput sequencing capabilities. Currently, the third-generation technology have long-read and single-molecule sequencing abilities. Long-read sequencing analysis has revealed structural anomalies such as extensive deletions, gene fusions and various chromosomal rearrangements. Additionally, it has highlighted medium-scale structural abnormalities comprising complex combinations of local duplications, inversions and microdeletions.

The third-generation sequencing involves sequencing single molecule of DNA without amplification. The advantages are little potential bias or inaccuracy produced by amplification step, speed of sequencing and reduced cost. Using a completely different principle to Sanger sequencing, third-generation technologies can decode much longer stretches of DNA fragments, called long reads. Techniques include PacBio's Single-Molecule Sequencing in Real Time and Oxford Nanopore Technologies.

NGS became an indispensable diagnostic tool for diagnosis and treatment decision in current clinical practice.[8] Tissue analysis benefits from support through methods like comprehensive genomic profiling (CGP) and liquid biopsies. However, precision medicine in oncology presents some hurdles, like, cost-benefit balance and widespread accessibility, especially in low- and middle-income countries. The key issue is how to effectively extend NGS to all cancer patients, thus, empowering treatment decision-making.

NGS enables detailed analysis of genetic material from various sources, like, genomic DNA (gDNA), RNA, cell-free DNA (cfDNA) and circulating tumor DNA (ctDNA). NGS marked an advancement in personalized medicine by the identification of somatic driver mutations, resistance clone, quantification of tumor mutational burden and germline mutations. Thus, helping clinicians choosing appropriate treatment for patients, whether the choice is chemotherapy, targeted therapy and/or immunotherapy. Liquid biopsy refers to the non-invasive analysis of tumor derived material, such ctDNA, circulating tumor cells (CTCs), RNA and exosomes, present in bodily fluids like blood, urine or cerebrospinal fluid.

Cancer genomic profiling depends on the integration of three important laboratory subspecialty like immunology, histopathology/surgical pathology and molecular diagnostics. Therefore, advanced technologies in these three fields are required to be implemented. Last but not the least, our doctors are directly or indirectly involved in cancer patient care and management would require update their expertise with the indication for such tests, result interpretation and targeted therapy. It is a new horizon in medicine which we cannot leave aside but embrace its full potential advantages. There is no better time under the present circumstances that our health sector needs to rethink seriously about it.

Future

The primary goal will be enhancement of sequencing technology by making it faster, cheaper and integrated with epigenetics (methylation profile) and transcriptome (expression), which will improve our understanding of genotype-phenotype correlation in health and disease.

Using NGS, the genetic information is analyzed, validated and clinically interpreted by a panel of multidisciplinary experts. A personalized treatment regimen is designed based on the unique genetics of the tumor and the patient's normal genome. In addition, the patient's family may benefit from knowing the risk of hereditary cancer and taking appropriate intervention.

NGS technology can be applied to decipher the genomic data of all living organisms, we almost exclusively use it for clinical and medical research purposes.

Usages

1) NGS assay can be used for whole exome sequencing. The entire coding region of all exons (coding sequence) of an organism including any cell types can be sequenced, in human, that is about 1% of the genome and is more often used in research. 2) NGS can also be performed at transcriptome level which includes entire assembly of RNA transcripts in a given cell type including mRNA, rRNA, tRNA, micro-RNA and non-coding RNA. Unlike DNA sequencing, this is called RNA sequencing. Specially designed mRNA sequencing is also often used to detect fusion genes. 3) The most commonly used NGS assay for cancer patients is targeted panel sequencing which usually interrogates dozens or hundreds of targeted genes. Such targeted NGS assays are usually designed for a disease or a category of diseases, for example, a panel designed for myeloid leukemia or a panel designed for lung or breast cancer. 4) The clinical application of NGS also includes tests for tumor mutation burden and microsatellite instability, and variants/mutations from circulating cfDNA (in plasma or other body fluids), also called "liquid biopsy".[9,10] It is known that many solid tumors shed their DNA, which may end up in the bloodstream or other body fluids. To a certain extent, such DNA can serve as representative samples for the primary tumors. Testing such DNA can generate information on mutations similar to that obtained from tissue biopsies. This is especially useful for tumors of unknown origin, difficult/risky to biopsy or if the tumor is already removed.

Clinical applications

NGS can be used to 1) classify tumor genotype (including cancer of unknown primary), 2) determine hereditary implication, 3) risk assessment, 4) treatment decision (any single or combination therapy including targeted drugs, for example, monoclonal antibodies and tyrosine kinase inhibitors), 5) estimate the prognosis, 6) predict minimal residual disease and recurrence, 7) monitor treatment response and 8) avoid unnecessary treatment cost and consequences.

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