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Diagnostic Biomarkers Related to Cancer Detection and Treatment:

A Review Article

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Abstract

Cancer is a serious problem affecting the health of all human societies. Thus the challenge is how the diagnosis and then who find effective treatment. Proto-oncogenes under abnormal condition turn out to be oncogenes throughout genetic mutation, which is risky and preventing normal cell division and existing. Common mutations of p53 gene controlled on the disability of genes for replication. Advance and new molecular approaches that target the tumor cell or the tumor microenvironment was needed. Many molecular techniques like PCR, gene expressions, FISH & CISH,epigenetic signatures and protein biomarker profiles, which in sequence allows to identifying the combination of biomarkers which may best recognize the presence or risk of cancer or check cancer therapies. Different techniques used to monitor the biomarker is essential to control the disease. Therefore, this study review aimed to clarification the most common methods used to diagnose the cancer related with some biomarkers.

***Keywords*— Molecular techniques, IHC, Cancer, Biomarkers**

I. INTRODUCTION

A. Cancer and Molecular Biomarkers Profile

The treatment of cancer is very difficult and within a decade it has become lethal in development countries (1). Therefore, cancer is a critical problem that lead to effect on the health of all human societies. At tissue level, cancer is a variety diseases thus consider the main confrontation for its diagnosis, after that effectiveness of therapy (2, 3).

Generally, cancer damages cellular relatives and leads to dysfunction of very important genes. Because the cell cycle will be quite affected then cause abnormal growth (4, 5). Under normal condition, Proto-oncogenes are responsible for cell division and growth, but during genetic mutation it becomes oncogenes, which are very dangerous for cell survival (6).

Cancer cell result from the abnormal molecular and cellular action, thus, the p53 gene associated with cancer, p53 abnormality occurs in sixty per cent of cancer patients. In normal circumstances , p53 responsible of cell division and death, angiogenesis, differentiation, and DNA metabolism. The DNA-binding position is the common mutations of the p53 gene controlled on disability of genes for replication (7).

Early detection of cancers is a good way for prevention and successful treatment in aggressive cancer which not easy to treat and has a poor prognosis. Thus needed to advance molecular techniques that deal with cells or the environment of tumors (8).

Molecular Genetics methods that used to detect cancer i.e. gene expressions, epigenetic, protein biomarker, which sequentially permit to identify the combination of markers which lead to detect the risk of cancer or existence or improve cancer therapies (9).

The term molecular profiling mean the means estimation of DNA, RNA, and proteins in cancer cells by molecular techniques which include:

B. Polymerase chain reaction (PCR)& Real -Time PCR

PCR used for amplifying and identifying specific nucleic acid sequences. typical PCR is an amplification of one piece or more pieces of a selected DNA sequence to create millions of copies of the template DNA are made and enable identified and analysis (10).

Real-time polymerase chain reaction (RT-PCR) is used to amplify and at the same time quantify DNA targeted molecule. Quantitative PCR used for specific sequences in a DNA or RNA sample, it enables quality and quantity measurement. The quantity one may be either an absolute

number of copies or a relative amount when normalized with other normalizing genes (11). RNA related biomarkers for example miRNA can be detected via RT-qPCR which converts RNA into complementary DNA for further molecular study (12).

The study has been done on breast cancer diagnostics related to her-2 biomarker using RT- PCR. In breast cancer cells, authors reported that her-2 over-expression gene and directly associated with aggressive actions (13). Over-expression of her-2 gene has a vital effect in the pathogenesis and progression of cancer and in 30% of breast cancer patients considered significant biomarker and target of therapy (14).

The action of survivin biomarker has been known in the poor prognosis, increase proliferation of cancer cells, and treatment outcomes (15, 16, 17). Thus by using RT- PCR , survivin expression has been considered as a target for tumors ,also it was related to HER2, VEGF markers (18,19).

C. Microarrays

It is considered a molecular technique for patterns of genes expression , also called "gene chips". The main challenge is to discover the genes active well in cancers and separate genes from others in cancer cells. This technique can detect gene expression in two different cells, like cancer cells and normal. Thus, should be used two different fluorescent dyes. Changing therapy decisions about cancer will depend on data obtained from microarray tests (20). The principle of this technique depends on the specific hybridization between the probes on the chips and the sample targets (fluorescent-labelled DNA, cDNA or cRNA molecules) (21) .

D. Sanger sequencing

It is referring to examine DNA strands to discover mutations by analyzing long, nearby sequencing reads. It is the process of selective incorporation of chain-terminating dideoxynucleotides by the enzyme (DNA polymerase) during in vitro DNA replication; By sequencing the analysis will detect changes in DNA or RNA by detecting the existence or not of an inserted or deleted sequence of gene segment (10).

Amplification by COLD-PCR (low denaturation heat) was used to detect KRAS and BRAF V600E mutations in HRM against to conventional PCR and sequencing that detected 57 KRAS-BRAF mutations in 117 colorectal cancer samples, while COLD-PCR detected 72 KRAS-BRAF mutations .This data increases the detection about 26.3%. Also, this technique does not require expensive equipment and time-consuming procedures, therefore it can be considered in diagnosis [22]

E. Hypermethylated promoters

Cancer cells are characterized by a Decline 0f about 5-6% of the total amount of 5-methyl cytosine in DNA methylation which has the main role in cancer development (23).

Hyperethylated considered as a new assay for the diagnosis and prognosis of cancer since many studies are focused on CpG (the regions of promoters). In cancer, 45-65% per cent frequently abnormal hypermethylation in CpG region have occurred (24, 25).

Only methylated Septin 9 (mSEPT9) DNA approved to examine the colorectal cancer by the detection of cfDNA in the serum of patients [26]. SEPT9 may relate to tumor progression of prostate cancer [27]. Recently, mSEPT9 cfDNA effectiveness in colorectal cancer was approved to use it routinely [28].

F. Fluorescent In situ hybridization (ISH)

It is mean localizes and determines a specific DNA or RNA sequence in a tissue section or in circulating tumor cells using a labelled CDNA or RNA or probe. ISH very effective to detect gene amplification, translocations , deletions, and fusions. In cancer cells, Gene fusions frequently occur because of genomic rearrangement or defect on mRNA processing (10).

Erlotinib, Gefitinib Inhibitors of the tyrosine kinase activity are widely used in Non-Small cell lung carcinoma (NSCLC) treatments. Because of the variety of genetic mutations according to the Epidermal growth factor receptor (EGFR) dysfunction, some patients are resisting to these treatments. Thus, the EGFR copy number detected by FISH technique is considered one of the biomarkers used to choose the correct therapy. FISH, using an appropriate probe against HER2, it identify extra copies of the gene, a sign that it is more likely to respond to Trastuzumab treatment.FISH is the food drug approved(FDA) method to detect inversions or translocations in the ALK gene. Under pathological conditions, the ALK gene breaks and fuses it is 3' (containing the tyrosine kinase domain) with the 5' of other genes (29).

G. Protein expression by Immunohistochemistry (IHC)

It is the most common methods used to detect proteins within tissues , it detects cellular biomarkers to identify diseases by staining with specific antibodies (30). IHC are performed on cancer cells and embedding tissue (fresh or fixed), so it simple, suitable, and low - cost test according to Rosaria , 2019 (29).

The principle depends on antibody binding to the antigens to evaluate the level of protein expression in tissues. Proteins related tumors consist of tumor specific Ag, tumor cell proliferation markers ,protein products of oncogenes ,tumor suppressor genes, and enzymes (10).

Also, several drug targets can be determined by using antibodies. The anti-ALK, anti-ROS1, anti-EGFR mutated, anti-BRAF V600E, anti-NTRK, and anti-PD-L1 were used in lung adenocarcinoma biopsies (31).

Many studies In Iraq investigated the IHC related important biomarkers for cancer diagnosis ,they cited that high protein expression of immune markers of FOXP3 and TGF-β in lung cancer (32,33) and recently VGEF, APE-1 in cervical cancer versus normal cases related lymphangiogenesis (34).

II. Conclusion

It is very important to know how to improve the early diagnostics and then take the decisions about certain targeted treatment to prevent progress the cancers. Different molecular methods, IHC , and epigenetic mechanisms led to determine new or novel cancer biomarkers , development and defective proteins in various diseases especially in cancers . These results could be valuable in detection ,treatment and reduce cancer progression.

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