

## Epigenetics – Role as Biomarker in Cancer Diagnosis

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### Abstract

Cancer is a disease either caused by genetic mutation or epigenetic modifications at the transcription level. Disease diagnosis at the early stage is a challenging step to be taken care for improving the outcome of patient survival. Latest advances has renewed interest and ended in the development of different potential biomarkers. Biomarkers of cancer include DNA, RNA, proteins, lipids, sugars, small metabolites, cytokinetic and cytogenetic parameters and the entire tumour cells found in the body fluid. Biomarkers should be thoroughly investigated for diagnosing the disease accurately, and to aid in the selective targeted therapies to improve the disease outcome. This review gives a brief description on various biomarkers for diagnosis, prognosis and therapeutic purposes, and how the epigenetic biomarkers act as cancer biomarker effectively.

**Keywords:** Hypomethylation; Microarrays; Malignant tumors; Chemotherapy

### Introduction

Epigenetics alludes to change in the gene expression levels without bringing any alteration in DNA sequence. DNA methylation and Histone modifications are considered as significant epigenetic mechanisms that confer the heritable changes in cellular phenotype [1-5]. These play a vital role in DNA based processes like Replication, Transcription and DNA repair. Consequently, genomic alterations or abnormal expression in chromatic regulators can have profound effect leading to induction of Cancer [6,7].

### DNA methylation

Hypermethylation of CpG islands located in Promoter regions of tumor suppressor genes is considered to be important mechanism for gene inactivation. Hypomethylation refers to the reduced levels of global DNA methylation which promotes the different types of malignancies leading to cancer [8-10].

### Histone modifications

Histone acetylation involves the regulation of chromatin structure leading to the increased or decreased levels of gene transcription. HAT and HDAC are the enzymes involved in the addition and removal of acetyl groups from lysine residues on the histone N-terminal tails. Histone methylation is carried out by conserved proteins known as HMTs which facilitates the addition of methyl groups to the amino terminals of histone proteins and is related to different biological processes ranging from transcriptional regulation to epigenetic silencing [11-14].

DNA methylation is linked with many key processes like telomeres, centromeres, X-chromosome inactivation, and suppression of repetitive elements, genomic imprinting and carcinogenesis. There are two types of abnormal DNA methylation associated with human malignancies. Global hypomethylation is often associated with chromosomal instability and loss of imprinting whereas

hypermethylation occurs at CpG islands located in Promoter regions and often associated with inactivation of tumor suppressor genes [15-20].

Epigenetic aberrations have an impact on the stages of tumorigenesis, eventually promoting the neoplastic cells with increase in pathogenicity. Identification of those alterations can be used as prognostic biomarkers for diagnosing Cancer at the early onset of the disease. These biomarkers will be helpful for characteristic patients whose malignancies are sensitive to particular cytotoxic chemotherapies that can hold guarantee for anticipating from which patients will be benefited from newer agents targeted at oncogenes [21-28].

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### Biomarkers

Discovery of novel biomarkers in cancer research is the future challenging step. A biomarker is defined as a biological entity found in tissues, blood, or other body fluids that indicates normal or abnormal process of a condition or disease [29-35]. Different types of cancer biomarkers are developed for screening the individuals at risk or for the detection of the presence of a specific type of cancer or for the prediction of the tumour's outcome or help in recognizing the specific drug treatment for a patient [35-56].

### Types of biomarkers

Biomarkers are mainly classified into three categories (Figure 1):

Diagnostic and prognostic biomarkers are often called as quantifiable traits that facilitate doctors to aid in the best treatment for the patients. These biomarkers exist in various forms; ancient biomarkers comprise assessing the patients with radiological techniques and using tumor specific antigens.

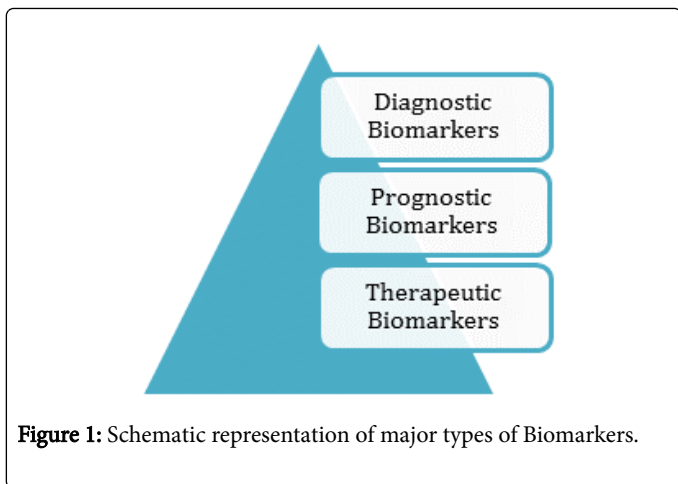


Figure 1: Schematic representation of major types of Biomarkers.

The Progress in human genome technologies like high-throughput DNA sequencing, mass spectrometry, and microarrays has led to the development of Cancer related biomarkers by comparing the sequence and expression levels of DNA, RNA and Protein. Genetic and genome based methodologies, for example, the investigation of gene expression pattern given by micro-array technology has significant role in diagnosis and prognosis of Cancer and many other diseases [57-63].

**Cancer biomarkers**

Cancer is defined as a group of diseases including genetic modifications that include point mutations, gene rearrangements and gene amplifications, bringing about changes in molecular pathways regulating cell growth, survival and metastasis [64-70]. When these types of changes appear among large number of patients suffering with specific type of tumor, then such progressions can be utilized as biomarkers to diagnose the targeted therapies (Figure 2) [71,72].

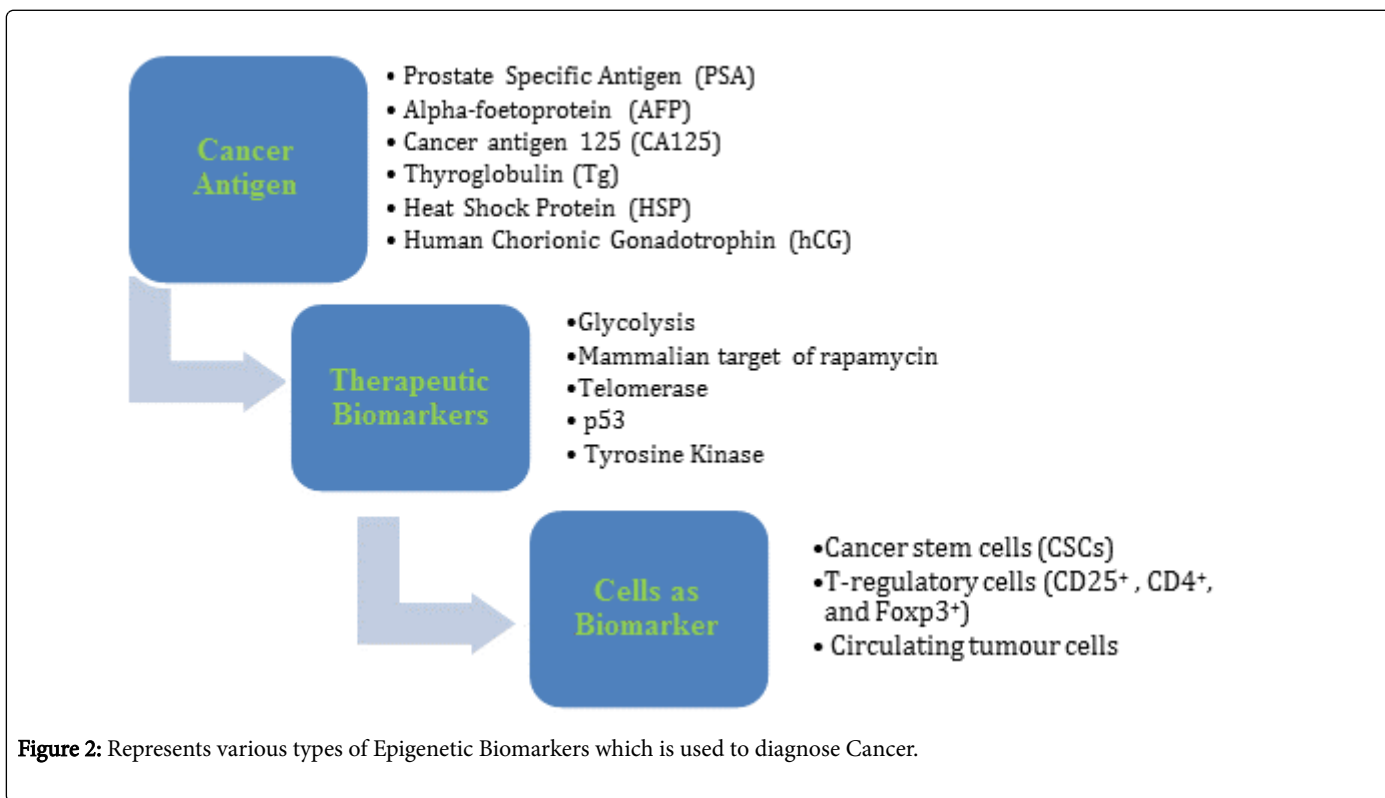


Figure 2: Represents various types of Epigenetic Biomarkers which is used to diagnose Cancer.

**Cancer antigen based biomarkers**

Tumor cells secrete few macromolecules into the extracellular fluid of which some proteins continue to persist in the blood stream which tends to act as potential serum biomarkers [73,74].

**Prostate specific antigen (PSA)**

Prostate specific antigen (PSA) is probably the most widely considered biomarker in prostate cancer. It belongs to the family of Kallikrein genes and is produced by both normal and neoplastic prostate epithelial cells. PSA is available in small quantities in the serum of normal men, and is frequently raised in the vicinity of prostate cancer [75-79].

**Alpha-foetoprotein (AFP)**

Alpha-foetoprotein (AFP) is a well-known diagnostic biomarker utilized in hepatocellular carcinomas (HCC). But it is not specific to detect in the early stages of hepatocellular carcinoma. It is the major serum foetal protein found in mammals, and is actively produced and secreted by the liver hepatocyte. Due to the fact that the levels of AFP may be elevated in serum from patients with other chronic liver diseases; AFP cannot be used for screening in patients suffering with hepatitis C or cirrhosis [80,81].

**Cancer antigen 125 (CA125)**

CA-125 antigen is known to be the primary serum tumour marker used in ovarian cancer for prognosis, response to chemotherapy and disease progression. The CA 125 antigen is found out to be a

membrane glycoprotein delivered by tissues derived from coelomic epithelium expressed by most epithelial ovarian cancers. The Major drawback in using the CA125 test as a screening tool is its lack of sensitivity and its inability to detect early stage cancers. It may also be elevated in other malignant cancers, including those originating in the lungs, fallopian tubes, breast, endometrium, and gastrointestinal tract [82-87].

### Thyroglobulin (Tg)

Thyroglobulin (Tg) is an organ-specific tumour marker; associated with patients possessing differentiated thyroid cancer that emerge from the follicle cells which increases the levels of thyroglobulin in the blood. It is considered to be a large glycoprotein stored in the follicular colloid of thyroid gland which acts as pro-hormone in the intra-thyroid synthesis of thyroxine (T4) and triiodothyronine (T3) [88-90].

### Heat shock protein (Hsp)

Hsp has gained importance due to its implication in tumour progression and response to therapy which has led to the development of targeted therapy by the usage of Hsps as immunological adjuvant in anticancer vaccines. Heat shock proteins (Hsps) are overexpressed in various human cancers and are involved in cell cycle related processes. It might be due to the stimulation of the Hsp induction by physio-pathological features of the tumour microenvironment [91-95].

### Human chorionic gonadotrophin (hCG)

Human Chorionic Gonadotrophin (hCG) is a hormone normally produced by placenta, which are found to be high in the blood of patients with certain types of ovarian cancers, testicular and choriocarcinoma. The elevation of serum levels of hCG and its metabolites cannot be considered for prognosis as it is estimated that  $\beta$ hCG might directly modify the growth of the cancer, leading to a worse outcome. Pregnant women are tested with the presence of hCG levels and may not be useful as a marker under this condition [96-100].

### Therapeutic biomarkers

Cytotoxic chemotherapy and radiotherapy are considered to be the best medications available for malignancy; however, these may cause serious side effects causing damage to normal cells along with the tumor cells. Recent advancements in understanding the underlying mechanism Cancer has led to the development of targeted therapies which may inhibit the growth of the tumor cells interfering in their molecular pathways leading to apoptosis. For example, Imatinib and Erlotinib drugs can inhibit the activity of Protein tyrosine kinases targeting the Epidermal Growth Factor Receptor (EGFR). Other targeted therapies like antibody bevacizumab will act upon a growth factor that stimulates tumor blood vessel growth [101-105].

### Glycolysis

Most of the malignant tumours depend on glucose for their development. When human tumour cell lines were studied with varying degrees of glycolysis, it has been observed that there is an inverse relationship between the rate of glycolysis and damage induced by chemotherapeutic drugs and radiation. Studies have shown that 2-DG selectively sensitizes tumour cells to ionizing radiation, without causing damage to normal cells. Thus, Clinical trials in brain tumour patients using a hypofractionated radiotherapy protocol combined with

2-DG proved to be successful. Combination therapy has resulted in minimal acute toxicity and late radiation effects and there is significant increase in survival rate and improvement in quality of life has been reported [106-110].

### Mammalian target of rapamycin (mTOR)

Mammalian target of rapamycin (mTOR) is a serine-threonine protein kinase which fits in with the PI3K [phosphoinositide 3-kinase (PI3K)-related kinase] family, assumes a noteworthy part in regulating cell development and proliferation. When mTOR is activated, the phosphorylation levels of its downstream targets p70S6K and 4EBP1 are promoted, which leads to increased levels of ribosome biogenesis, translation, inhibition of autophagy and reorganization of the actin cytoskeleton. Studies have shown that the (PI(3)K)-PTEN-mTOR signaling pathway is aberrantly activated in many tumours, resulting in dysregulation of cell growth and proliferation. Loss of PTEN mRNA or protein production in tumour tissue can be considered as Biomarkers to evaluate the activation of the pathway. Proliferative marker Ki-67 is used to evaluate the Inhibition of mTOR by rapamycin, which can measure the presence of phosphorylated form of the ribosomal protein S6, and its therapeutic effects on tumour cells [110-114].

### Telomerase

Telomerase is an enzyme known as reverse transcriptases, which uses RNA as a template for producing DNA and it contains both RNA and protein components. The enzyme is sole responsible for protecting the cell from degradation and death by the maintenance of telomere. Thus it can be treated as one of the best diagnostic markers for human cancer, related to malignant tumours, thus making it an ideal target for chemotherapy [115-119].

### p53

The p53 gene is known to be a tumour suppressor gene which prevents the uncontrolled multiplication of abnormal cells. Radiation and many other anticancer drugs cause damage to the DNA of cancer cells, which activates the p53 gene leading to apoptosis. During treatment, an intact wild type p53 gene is essentially required to stimulate programmed cell death of a cancer cell. Thus p53 gene is a well-studied potential biomarker for predicting prognosis and patient's response to therapy [120,121].

### Tyrosine kinase

Tyrosine kinases belong to group of enzymes that regulate various cellular processes like cell growth, differentiation, migration, and apoptosis that aids in tumour development and progression. Thus targeting protein Tyrosine kinases as a therapeutic biomarker is an attractive approach to inhibit the tumour growth. For example, tyrosine kinase inhibitor Gefitinib and Trastuzumab have proved to be anti-cancer agents [122-124].

### Cells as Biomarker

#### Circulating tumour cells (CTCs)

Circulating tumour cells can be considered as a powerful biomarker to predict the disease progression and response to therapy. Increase in CTCs at any time during therapy is an indication of progression, whereas decreased number of CTCs shows the effectiveness of the

therapy. Studies have shown that they can be considered as standard tumour markers (e.g., Ca27-29) in predicting prognosis [124].

### T-regulatory cells (CD4+, CD25+ and Foxp3+)

T regulatory cells (T-regs) are considered to be important in inducing and maintaining peripheral self-tolerance, consequently preventing immune pathologies. They are assumed to control both natural and acquired immune responses. T-regs is well known as a surrogate immune marker of cancer progression; also acts as a predictor of response to targeted therapies. The presence of FoxP3+ cells within tumours has proven to predict the prognosis, metastatic ability and invasiveness of some tumours by modulating the ability of the immune system to target tumour cells [125-127].

### Cancer stem cells (CSCs)

Cancer stem cells are subpopulation of cells that have the capacity to self-renew and to generate the more differentiated progeny which make up the bulk of a tumour. Studies have shown cancer stem cells (CSCs), tumourigenic cancer cells, or tumour-initiating cells, can give rise to new tumours when transplanted into immuno-deficient animals. Therefore, it is utmost importance to identify CSCs for every possible tumour which may lead to new therapeutic avenues [128-131].

### Epigenetic biomarkers

It is well known that, in cancer cells, genes are either altered by mutations, or through epigenetic modifications to chromosomes that change gene-expression patterns. Epigenetic modifications are supposed to occur either through DNA methylation of genes or by acetylation, methylation, or phosphorylation of histones and other proteins around which DNA is wound to form chromatin [132-134]. Recent advancement in the field has led to the awareness of the epigenetic changes driving neoplasia to be used as significant cancer biomarkers. Studies have shown that the activity of DNA methyltransferases (DNMTs), are altered in tumour cells and are known to be associated with several developmental abnormalities [135-140]. Epigenetic changes can be either through Hypermethylation or Hypomethylation of genes. Hypermethylation markers can be utilized for detecting both primary and metastatic cancers. For example, hypermethylation of p16 promoter in the circulating serum DNA is found in recurrent colorectal cancer. These biomarkers have been proved to be useful for identifying patients sensitive to specific cytotoxic chemotherapies and may help in predicting which patients benefit from newer targeted agents directed at oncogenes.

### Conclusion

Epigenetic alterations have been associated with the development and progression of human cancer. Studies have shown that epigenetic modifications are reversible, in contrast to genetic mutations. This has led the researchers to focus on developing epigenetic drugs in treatment of cancer patients. Although, advancements in epigenetics research has led to improved disease outcome of patients with certain forms of lymphoma and leukemia's by using the drugs that alter DNA methylation and histone acetylation, more attention should be paid for optimizing and validating the methylation markers in clinical trials. Therapeutics designed to reverse the epigenetic alterations in cancer cells, along with diagnostic and prognostic assays based on gene-

methylation patterns, are promising new avenues for future progress in patient care.

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