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Biomarkers of human health.

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Abstract

Biomarkers in recent times have gained immense clinical and scientific curiosity. They are biological marker explained as changes in composition of cells or fluids of body which are helpful to understand various chronic diseases in terms of screening, diagnosis, prognosis, therapeutic management and chances of recurrence. Hence, they serve as indicators of diseases and can be measured either *in vivo* by imaging or *in vitro* by laboratory techniques. Different kinds of biomarkers are available with lots of advantages as well as disadvantages in the field of medical science. They help to understand the complete range of disease from the beginning phase till the end. They help in predicting risk factors for diseases, screening of diseases and monitoring the patient. They are fuel for drug research and development. Biomarkers evaluation involves tools that can help in understanding the disease or health, reason, diagnosis, disease advancement, relapse or result of treatment and hazard evaluation. These may include estimations straightforwardly on natural media (amniotic liquid, cerebrospinal liquid, plasma, blood, peritoneal and pleural fluid, saliva, serum, seminal fluid, sweat and urine) or estimations, for example, brain imaging which don't include direct testing of organic media however measure alterations in the function or integrity of the nervous system.

Keywords: Biomarkers, Cancer, Diagnosis, Screening, Prognosis.

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Introduction

The biomarkers have been several times defined in literature with considerably overlying definition. Houlka BS (1990) defined biological markers as "cellular, biochemical or molecular alterations that are measurable in biological media such as human tissues, cells or fluids." The National Institutes of Health in 1998, defined biomarker as a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention [1]. As per the World Health Organization (WHO) biomarker are defined as any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease [2]. Biomarkers evaluation incorporate techniques and tools that can help in understanding the disease or health, reason, diagnosis, disease advancement, relapse or result of treatment and hazard evaluation. These may include estimations straightforwardly on natural media (amniotic liquid, cerebrospinal liquid, plasma, blood, peritoneal and pleural fluid, saliva, serum, seminal fluid, sweat and urine) [3] or estimations, for example, brain imaging which don't include direct testing of organic media however measure alterations in the function or integrity of the nervous system [4]. A biomarker can be physical, chemical, or biological [5]. These may be cells, molecules, genes, gene products, hormones or enzymes. They may be produced in response to infection or disease or diseased organ (tumor) by the body. Biomarkers have always been utilized by physicians, scientists and epidemiologists, to acquire knowledge about human health and diseases. The term biomarker is used in preclinical research and clinical diagnosis moderately [6]. Biomarkers include everything from basic chemistries to more complex investigations of body fluids, cells and various tissues like for fever, body temperature and for stroke risk, blood pressure is a biomarker. The levels of cholesterol and triglyceride are marker for cardio-vascular disease. Similarly, C-Reactive Protein (CRP) is a biomarker for inflammation. They are the most specific and measurable indicators of modern science [7]. The laboratory-measured biomarkers are still being developed [7]. They help in predicting risk factors for diseases, screening of diseases and monitoring the patient [8]. The cholesterol levels help the doctors to predict chances of occurrence of a heart attack. When a patient is administered anti-cholesterol drug, the cholesterol level monitoring during follow-up helps to know if the medication is working, how much it is lowering the cholesterol and to what extend it reduced the risk of having a heart attack. Similarly, diabetic patients can test HbA1c levels. Liver Function Tests (LFT) indicates liver damage and Prostate Specific Antigen (PSA) indicates chances of prostate cancer and disease stage. They are also used to treat cancer patients and many other disease conditions [9,10].

Biomarkers History

The concept for use of biological markers for detecting and improving therapy of disease starts from the very early times of medical sciences. In the fourteenth century, examination of urine i.e. uroscopy for signs of disease was done by doctors [11]. In 1960, scientists found that few Chronic Myelogenous Leukemia (CML) patients have short chromosome 22 associated with their cancer. This defect is called as Philadelphia chromosome. This occurs by transfer of genes between chromosomes 9 and 22 resulting in creation of the 'oncogene' BCR-ABL. The BCR-ABL leads to the production of a protein due to elevated tyrosine kinase activity. Therefore, this biomarker helps to identify candidates likely to be benefitted by the use of inhibitors of tyrosine kinase [12]. The biomarker terminology is over 30 years old. The term biomarker was first used by Karpetsky, Humphrey and Levy, as the serum RNase level was not a biomarker either for the presence or extent of the plasma cell tumor" [13]. Urine for many decades was used as biomarker because it can be easily examined. Sushruta, the "Father of Ayurvedic Surgery," said that because of sweetness, the urine of diabetes patients attracted ants [13]. The origin of biomarkers is old but the speed of progress over the past 2500 years was somewhat slow [14]. One of the most renowned biomarkers discovered in the mid 1980's is HER-2 gene and receptor. About 20%-30% patients of breast cancer demonstrated HER-2 receptor over-expression [15]. It indicates higher chances of poor outcomes and a new drug target site [15].

Characteristics of an ideal biomarker

An ideal biomarker is one which explains the occurrence of a moderate proportion of disease in the community. It must have many qualities for being used clinically. Firstly, the biomarker testing must be easy and safe to be conducted, non-invasive if possible, utilizing external body fluids preferably. The assessment of biomarker should be done by simple laboratory test which are rapid and specific. Secondly, it should be specific and useful to identify different classes and causes of disease. Thirdly, for early detection it should be sensitive. Lastly, the sensitivity and specificity of biomarker needs to be high for reducing false results [16].

Classification

They are classified as

Imaging biomarkers: Computerized axial Tomography (CT), Magnetic Resonance Imaging (MRI) and Positron Emission Tomography (PET)

Imaging technology is involved with the developing newer biomarkers. Imaging techniques can be helpful in detecting and treating diseases at an earlier stage and thereby reduce the economic burden pressuring today's healthcare. CT, MRI PET and nuclear imaging are widely used for imaging and are now being introduced in newer dimensions [17]. They can be highly helpful in slowly progressing diseases like Alzheimer's disease, lymphoma, non-small cell lung cancer, and rheumatoid arthritis [18,19]. MRI is helpful in Alzheimer's disease and multiple sclerosis treatments while PET is useful in Parkinson's disease by scanning dopamine transporters [20]. Functional imaging advances provide an insight of metabolism and biological pathways occurring in tumor by studying blood flow and vascular permeability. Therefore, it allows clinicians to examine biological activity at molecular as well as cellular levels. They are useful for monitoring drug's pharmacokinetics and pharmacodynamics during clinical trials [21].

Imaging biomarkers have advantages like mostly non-invasive and produces intuitive, multidimensional output. They yield both qualitative and quantitative data. They are mostly comfortable for patients. In combination with other information sources, they are helpful to clinicians in making diagnosis. Radiation exposure and high cost are limitations of these markers [22].

Molecular biomarkers

An emerging research area is to identify molecular biomarkers which help to distinguish normal physiology from pathological condition. Molecular biomarkers can be referred as non-imaging biomarkers. It measures the levels in serum, plasma, cerebrospinal fluid and biopsy. The nucleic acids-based biomarkers like gene polymorphisms or mutations and gene expression estimation are also included [23]. Molecular biomarkers are commonly used to monitor treatment. They are useful in diagnosis, prognosis and risk evaluation of disease. They are also helpful in food safety assessment. Molecular biomarkers can be identified at different levels right from functional protein synthesis to the deposition of byproducts. By encoding amino acid sequence present on gene, protein formation starts [24,25]. By altering the DNA, epigenetic affects the synthesis of mRNA and finally proteins. The complex of mRNA, long and short non-coding RNAs and microRNA, is the next site for changes at molecular level. The complex itself as well as the metabolites formed can be used as biomarkers. Next newer field in is the estimation of lipids and its byproducts [26,27]. There are many high throughput laboratory techniques to analyze these biomarkers. These techniques include genomics, proteomics, transcriptomics, lipidomics and metabolomics. The advancement in these techniques helps in identifying molecular biomarkers in many research areas [28,29].

Biomarkers can be classified into three types based on molecular and genetic methods.

Type 0 also known as natural history marker. They are disease severity and depict the pathogenetic processes. They predict the outcome independent of therapy [30]. The best examples are count of CD-4 T-cell and HIV 1 RNA plasma level. The first demonstrates disease severity on organ and immune system while the second shows viral burden indicating the level of infection [30].

Type 1 also known as biological activity marker. They respond to therapy. This marker activity is evaluated in relation to early phase clinical trials [31]. *In vitro*, triple-drug antiretroviral combinations were found superior to single as well as double-drug combinations. In clinical trials the former showed better response by increasing CD4 T-cell counts and decreasing HIV 1 RNA plasma levels. Such results have increased the approval of antiretroviral agents like protease inhibitors. The absence of type I marker due to lack of activity or inadequate dose, helps to stop trial of newer agent [32].

Type 2 also known as surrogate marker of therapeutic efficacy. They are either single or combination of many markers. They account for the efficacy of an agent. The ultimate aim is to find a correlation between early change in marker level and outcome [33]. Their main use in phase 2/3 trials is as efficacy endpoints.

Another class of biomarkers are those which are helpful to take decision during early stages of drug trials, like Pharmacodynamic (PD) biomarkers are for drug effect [34]. Biomarkers can be described as diagnostic biomarkers based on drug development. They help to define a disease like myocardial infarction is diagnosed by estimating levels of cardiac troponin [35,36].

A prognostic biomarker helps to classify patients by chances of risk for disease to occur or progress. These help to evaluate the normal course of disease in a patient without any treatment [37]. Therefore, they are related to outcomes. E.g. Her-2/neu over expression in breast cancer patients is related with poor prognosis [38].These biomarkers classify patients by chances of responding a particular therapy. The prognosis may be good or bad or adverse [39]. E.g., in breast cancer receptors of estrogen and progesterone help to understand the prognosis of endocrine therapy and Her-2 to Herceptin [40].

DNA as biomarker

Increase in serum DNA concentration is related to different types of cancers and diseases like sepsis and autoimmune disorders. Mutation in oncogenes, mismatch-repair genes and tumor-suppressor genes can be used as DNA biomarkers. Mutations in gene encoding tumor suppressor p53 are associated with more than half of sporadic cancers [41]. Other cancer-related gene mutations, like RASoncogene, RB1 (Retinoblastoma gene) and APC (Adenomatous Polyposis Coli gene) also have the potential as markers for prognosis or selection of therapy [42].

Circulating DNA as biomarker

When cells first have mutations in the target genes and turn cancerous, the symptoms may not present for months or years, leading to difficulties for clinicians in making early and accurate diagnosis. The effective cancer therapy needs early detection. Therefore, development of reliable methods mainly using non-invasive methods for early cancer detection is very important. Mandel and Metais reported in 1948 the presence of circulating DNA in the bloodstream. Leon *et al*. [43] reported for the first time that the plasma levels of circulating DNA were very high in cancer patients in comparison to healthy controls. It was suggested that lysis of tumor cells may give rise to circulating DNA in cancer patients [44].

mRNA as biomarker

The mRNA biomarkers are being searched in different fields of life science [45]. In pharmacogenomics it was applied successfully to establish specific drugs treatment prediction [45]. Analysis of mRNA gene expression is also useful in differentiating types or stages of diseases. Thus, various forms of heart disease, neuropsychiatric disorders or cancer can be differentiated by analyzing of specific genes expression [46,47].

miRNA as biomarker

miRNAs are 20-22 nucleotides, non-coding RNA molecules which are involved in mRNA post-transcriptional processing. Therefore, they regulate physiological and metabolic processes [48] and effect entire cellular physiology, tissue differentiation and organ development. Many studies had shown that miRNA is differentially expressed during disease or injury in target tissue. miRNAs due to short length are less sensitive to RNase [49-52].They are being studied for use in cell-free body fluids for detecting organ injury [53-55].Cellular miRNAs relate with various conditions like inflammation, differentiation, diabetes and cancers [56]. The cancer cells release miRNA into circulation and therefore, it can be used to know the type of cancer [57]. By analyzing miRNA profiles, gastrointestinal cancer can be differentiated from non-gastrointestinal cancer. Plasma has highest number of miRNAs, followed by saliva. Urine and pleural fluid have no miRNA. Altered levels of miRNAs in body fluids like bile, sputum, urine, feces, cerebrospinal fluid and saliva have been found in patients with alcoholic liver disease and cancer. Park *et al*. [58] studied that miR-125a and miR-200a are expressed in saliva of patients with Oral Squamous Cell Carcinoma (OSCC). The miRNAs detection in saliva is non-invasive and rapid tool for diagnosis of oral cancer. miRNA-biomarkers the advantage over protein-based biomarkers due to their relatively simple molecule that can be easily detected.

lncRNA as biomarker

This group of long non-coding RNAs (lncRNAs) have more than 200 nt. They are being studied in cancer research. H19 is the first known lncRNAs. These are markers for tumors of liver, liver metastases, esophagus, colon and bladder. There occurs absence of methylation of promoter segment there occurs rise in lncRNA which indicates tumor tissue presence.

Biomarker in biological media

Amniotic Fluid (AF)

Acetylcholinesterase (AchE): It is mainly present in the CNS and a little amount of it is found in skeletal muscle, erythrocytes membrane, serum of fetus and placenta. Normally the amniotic fluid has no AChE. It may be present due to the Neural Tube Defects (NTD) in the fetus. Such defects are identified on ultrasonography and confirmed using AChE electrophoresis in amniotic fluid and AFP assay.

Alpha-Fetoprotein (AFP): AFP level is elevated in amniotic fluid in open NTDs and Down's syndrome. Maternal serum along with AFP may be used for screening for NTDs and Down's syndrome.

Sialosyl Tn (STN) and Zinc Coproporphyrin-1 (ZnCP-1): Their levels in maternal serum are used clinically for finding Amniotic Fluid Embolism (AFE).

Blood

Creatinine and BUN: They represent kidney insult but have certain disadvantages. Serum creatinine levels may alter after around 50% loss of renal function. Hence it may not be possible to detect an early acute renal injury. Creatinine levels depends on age, muscle mass, gender, etc. BUN too has a similar disadvantage and it depends on diet and hydration.

Serum Amyloid A (SAA): It is a blood biomarker for acute exacerbations of COPD. Data have demonstrated that there is increase in levels of acute-phase proteins such as SAA with renal cancers.

Troponin T and I isoforms: Troponin is the most sensitive and specific test for myocardial injury. These are superior in comparison to other biomarkers of cardiac disease like myoglobin and Creatine Kinase (CK)-MB. This is because the CK-MB and myoglobin are also released in skeletal muscle injury patients.

Cerebro-Spinal Fluid (CSF)

CSF can help in the diagnosis of wide neurological diseases such as subarachnoid hemorrhage and CNS infections.

Amyloid β42 and Tau protein (total and phosphorylated): These proteins are associated with the pathogenesis of Alzheimer's Disease (AD) and helps to differentiate it from other types of dementia. The diagnosis of AD also includes now imaging of brain and biomarkers in CSF.

Glucose: Decrease in CSF glucose levels is associated with mumps, herpes simplex virus, some enteroviruses, and lymphocytic choriomeningitis virus [58].

Lactate: CSF lactate levels are raised in epilepsy and haemorrhage [58].

Myelin Basic Protein: This marker is used in assessing disease activity in patients of Multiple Sclerosis. It is a major part of myelin. Raised CSF levels of myelin indicate occurrence of demyelination.

Protein: An elevated protein level is associated with inflammatory conditions such as Guillain Barré Syndrome, where levels are often above 1 g/L.

Peritoneal fluid

Amylase: This enzyme levels is raised in pancreatitis.

Neutrophils: Increased neutrophil count (>250/μL) is seen in peritonitis (bacterial, tuberculous, pancreatic or malignant).

pH: Below 7.0 is seen in bacterial infection.

Total protein: Higher than 30 g/L in the fluid is an exudate suggesting inflammatory or malignant ascites.

Pleural fluid

Adenosine deaminase: This marker was used earlier in the diagnosis of Tuberculosis.

Saliva

Saliva can be helpful for the diagnosis of diabetes, HIV, arthritis, various forms of cancer, and heart disease [59].

Seminal fluid

Acid phosphatase: Is used as a marker to detect prostatic fluid. Prostatic Acid Phosphatase (PAP) is used as marker for prostate cancer. Rape investigations often include detection of PAP in vaginal fluid [60].

Fructose: Very low fructose level in the semen of azoospermic human indicates that seminal vesicles and/or vasdeferens are absent.

Sweat

Chloride concentration: Sweat chloride >60 mmol/L is associated with Cystic Fibrosis (CF). In CF there occur mutations in gene encoding for Cystic Fibrosis Transmembrane conductance Regulator (CFTR) protein. This leads to dysfunctional epithelial chloride channels. Reabsorption of chloride and sodium ions is decreased through the duct of sweat glands. Hence there is production of very concentrated sweat with sodium and chloride >60 mM concentrations.

Urine

Hematuria: It is a very nonspecific marker and is associated with only severe renal injury.

Pyuria: Pyuria i.e. presence of WBCs in urine is a marker indicating UTIs. In glomerulonephritis and nephritic syndrome, there occurs pyuria, hematuria, proteinuria, and casts.

Proteinuria: Proteinuria means excess of serum proteins in urine. It indicates glomerular disease, proximal tubule damage, or a high serum protein level. It only occurs after severe renal damage and takes days to develop. Clinically, it is the most sensitive marker of Chronic Kidney Disease (CKD) progression, especially when combined with estimated Glomerular Filtration Rate (eGFR), but these

have limitations. Hence, new validated biomarkers are required to assess CKD progression.

Specific Gravity (SG): Elevated/lowered SG of urine can occur due to tubular injury, but can also be associated with dehydration.

Casts: Red cell, white cell and epithelial cell casts is seen with bleeding into the tubule, inflammation and degeneration of tubular lumen or necrosis of renal tubules, respectively.

Crystals: It is an indicator of infection, inflammation, or a metabolic disorder. These markers can be affected by diet and other factors independent of renal injury.

pH: It can be an indicator of renal failure. The pH is affected by sleep and varies before and after a meal. Continuous measurements of pH can provide useful information.

β2-Macroglobulin (β2M): Increased urinary β2M excretion is associated with an early tubular injury due to nephrotoxin exposure, cardiac surgery and renal transplantation. Its use is limited due to rapid degradation, instability in urine and at urinary pH<6.0. Cabre *et al*. reported that retinol binding protein 4 (RBP4) levels was also raised in individuals with reduced GFR and suggested that RBP4 levels is associated with kidney dysfunction in diabetic patients.

Ceruloplasmin: Its increase in urine suggests development of micro albuminuria in normoalbuminuric diabetic patients. Therefore, they can be used for Diabetic Kidney Disease (DKD).

Cystatin c: It is an inhibitor of cysteine protease and a marker of kidney injury. Serum levels of Cystatin c are used in kidney injuries, while urinary levels of Cystatin c is used to detect DKD.

Immunoglobulin G (IgG): Urinary IgG excretion is associated with progression of glomerular diffuse lesions.

Immunoglobulin M (IgM): Its presence in urine is associated with large pore in glomerular capillary wall.

Transferrin: Wang *et al*. indicated that transferrin excretion in urine was a good marker for demonstrating nephropathy occurrence. But transferrin excretion in urine is not specific for DKD as its increased levels is also associated with primary glomerulonephritis.

Biomarkers for cancers

Annually, >11 million cancer patients are diagnosed. Cancer involves modification in multiple genes expression which provides survival benefits and decreases proliferative changes in somatic or germinal cells. Many evidences suggest that cancer also involves 'epigenetic changes' such as DNA methylation and alterations in histone modifications. These lead to alterations in chromatin condensation which modifies various genes expression. Cancer cells show multiple genetic changes that include point mutations, gene rearrangements, and gene amplifications, culminating into derangement in cellular pathways regulating its growth, death and metastasis. An ideal and specific biomarker for various cancer types is still a big challenge. Cancer or tumor markers promote non-invasive and high-speed diagnosis of cancer and enhance early screening and detection. The increasing demand for cancer biomarkers is because of their ability to know cancer type and target in a patient-oriented manner. These markers can help in development of targeted therapies, predicting risk of cancer, help in screening, monitoring of the patient and foresee how the patient will respond to therapy. Efficient cancer markers are always in great demand as they can help in reducing mortality by improving diagnosis at an early stage and helping to individualized treatments of cancer patients.

Cancer biomarkers are expressed by cancer cells either due to etiology or as a result of malignancy. These are synthesized at a higher rate in cancerous cells. They can be present intracellularly or may be released into the circulation [61].

Detection of increased serum levels of these markers suggests tumor activity. Cancer biomarkers are nonspecific and sometimes synthesized normally. PSA is normally synthesized in prostate but high serum levels occur in cancer of prostate. Cancer Antigen 125 (CA-125) is a biomarker for ovarian cancer with low sensitivity and specificity. High levels of CA-125 can be seen in patients with kidney or liver disease, pancreatitis. However, it can be used during treatment follow-up and predict a treatment failure with use of chemotherapeutic agents. Several tumor markers in combinations can provide risk predictions in people with high family history for the disease. Another biomarker, Carcinoembryonic Antigen (CEA) is increased in colorectal, lung, pancreatic or breast cancer patients. Smoking too raises CEA levels. CEA levels estimation is an effective way to determine adequacy of postoperative therapy in cancer of colon. Other biomarkers have lower specificity and cost effectiveness.

Telomeres and telomerase play pivotal role in initiating and progressing human cancers. The main role of telomere is to stabilize the ends of chromosomes. However, by various mechanisms, telomeres can become dysfunctional. This may lead to genomic instability thereby developing cancer. Because there exist significant variations in telomere length and telomerase activity between malignant and benign tissues, these have potential to be utilized as markers for diagnosis of cancer. They have several clinical applications as marker of tumor. They help in detection of cancerous cells in early stage where upregulated telomerase activity. They are prognosis indicator in cancer. They help to distinguish benign tumors and detect cancer cells in circulation.

Some types of cancer biomarkers

Alpha-Fetoprotein (AFP): AFP is used in diagnosis and prognosis of hepatocellular carcinoma and hepatoblastoma.

Significantly high levels of serum AFP are rarely reported in malignancies of pancreas, gastrointestinal tract, kidney lungs and breast.

BRCA-1, BRCA-2: They are used in diagnosis of breast cancer. BRCA1 helps to assess the response of different chemotherapy agents like anthracyclines.

Cancer Antigen 125 (CA125): CA125 is used in diagnosis and prognosis of epithelial ovarian carcinoma and fallopian tube cancer. Presently, it is the best and most superior biomarker for epithelial ovarian malignancies.

Cancer Antigen 15-3 (CA15-3): It is used in diagnosis and prognosis of breast cancer. Its sensitivity for the diagnosis of breast cancer is low as higher levels also occurs in benign breast diseases and acute and chronic hepatitis.

Cancer Antigen 19-9 (CA 19-9): It is diagnostic and prognostic marker for pancreatic cancer and bladder cancer. Its level is also elevated in 8% acute and chronic pancreatitis.

Carcinoembryonic Antigen (CEA): It is used in diagnosis and prognosis of colorectal cancer. CEA assay is helpful in managing breast, pancreatic, lung, prostatic and ovarian cancer patients.

Human Chorionic Gonadotrophin (HCG): It is used in diagnosis of germ cell tumors (ovarian, testicular).Serum HCG levels are rarely increased in non-trophoblastic cancers of breast, lung, pancreas and bladder.

Prostate Specific Antigen (PSA): It is diagnostic and prognostic marker of prostate cancer. PSA is not very effective for screening and early detection of prostate cancer as it is specific for prostate cells and not prostate cancer. But combination of PSA, digital rectal examination and transrectal ultrasound, is very helpful in early detection of prostate adenocarcinoma.

Thyroglobulin (Tg): It is used in diagnosis and prognosis of papillary and follicular thyroid cancer. Tg can be measured in serum, thyroid cyst fluids and other fluids/ tissue obtained by fine needle biopsy of thyroidnodules.

Conclusion

This is the period of science and technology and every day is a day of new invention. Present research is concentrated on such biomarkers which are associated with particular disease. Such tests are being developed that can monitor alterations of very low levels in biomarkers. Since 1980s, biomarkers are results of huge trials conducted for major diseases like heart and cancer disease. The discovery of new biomarkers for disease and measure them rapidly at an early stage will be boon for disease diagnosis. The monitoring of health status, onset of disease, prognosis and treatment outcome using non-invasive methods is a most desirable aim in health care delivery. Biomarkers play an important role in drug development process as well as research. Studying relationship between various biological pathways and clinical output is important to develop new treatment methods. We can hope a better future by developing new biomarkers helping us in early detection not only for cancer but for other diseases too.

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