Review Article

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Cancer biomarkers - Current perspectives

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In the recent years, knowledge about cancer biomarkers has increased tremendously providing great opportunities for improving the management of cancer patients by enhancing the efficiency of detection and efficacy of treatment. Recent technological advancement has enabled the examination of many potential biomarkers and renewed interest in developing new biomarkers. Biomarkers of cancer could include a broad range of biochemical entities, such as nucleic acids, proteins, sugars, lipids, and small metabolites, cytogenetic and cytokinetic parameters as well as whole tumour cells found in the body fluid. A comprehensive understanding of the relevance of each biomarker will be very important not only for diagnosing the disease reliably, but also help in the choice of multiple therapeutic alternatives currently available that is likely to benefit the patients. This review provides a brief account on various biomarkers for diagnosis, prognosis and therapeutic purposes, which include markers already in clinical practice as well as various upcoming biomarkers.

Key words Biomarkers - cancer - cancer therapy - diagnosis - glycolysis - molecular targets - Pin 1 - prognosis

Introduction

Every cell type has a unique molecular signature, referred to as biomarkers, which are identifiable characteristics such as levels or activities (the abilities of genes or proteins to perform their functions) of a myriad of genes, proteins or other molecular features. Biomarkers are therefore, an objective measure or evaluation of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention[1]. This includes all diagnostic tests, imaging technologies, and any other objective measures of a person's health status. Biomarkers are subject to dynamic modulation, and are expected to enhance our understanding of drug metabolism, drug action, efficacy, and safety. These can also facilitate molecular definition of diseases, provide information about the course of disease and predict response to therapies.

More than 11 million people are diagnosed with cancer every year. It is estimated that there will be 16 million new cases every year by 2020[2]. Cancer is a cluster of diseases involving alterations in the status and expression of multiple genes that confer a survival advantage and undiminished proliferative potential to somatic or germinal cells[3]. Alterations primarily in three main classes of genes *viz.*, (proto) oncogenes, tumour suppressor genes and DNA repair genes collectively contribute to the development of cancer genotype and phenotype that resists the natural and inherent death mechanism(s) embedded in cells (apoptosis and like processes), coupled with dysregulation of cell proliferation events (Fig.). There is increasing

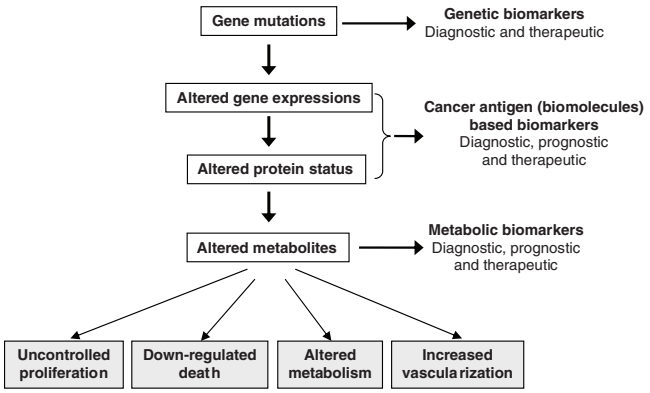

Fig. The process of carcinogenesis, showing opportunities of identifying biomarkers.

evidence to suggest that cancer is also driven by 'epigenetic changes' like DNA methylation and altered patterns of histone modifications, leading to alterations in chromatin condensation status thereby regulating expression of certain set of specific genes[4,5].

Technologies to recognize and understand the signatures of normal cells and how these become cancerous, promises to provide important insights into the aetiology of cancer that can be useful for early detection, diagnosis, and treatment. Biomarkers are therefore invaluable tools for cancer detection, diagnosis, patient prognosis and treatment selection[6]. These can also be used to localize the tumour and determine its stage, subtype, and response to therapy. Identification of such signature in surrounding cells or at more distal and easily sampled sites of the body *viz.*, cells in the mouth (instead of lung) or urine (instead of urinary tract) can also influence the management of cancer.

Cancer cells display a broad spectrum of genetic alterations that include gene rearrangements, point mutations, and gene amplifications, leading to disturbances in molecular pathways regulating cell growth, survival, and metastasis. When such changes manifest in majority of patients with a specific type of tumour, these can be used as biomarkers for detection and developing targeted therapies, besides predicting responses to various treatments[7-9].

Genetics, genomics, proteomics, many non invasive imaging techniques and other technologies allow measurement of several biomarkers. Currently, there is a greater understanding of the disease pathways, the protein targets and the pharmacologic consequences of drug administration. Therefore, application of biomarkers in the clinical practice is likely to result in advanced knowledge leading to a better understanding of the disease process that will facilitate development of more effective and disease specific drugs with minimal undesired systemic toxicity. Establishment of biomarkers requires a comprehensive understanding of the molecular mechanisms and cellular processes underlying the initiation of cancer, especially focusing on how small changes in only a few regulatory genes or proteins can disrupt a variety of cellular functions. A major challenge in cancer diagnosis is to establish the exact relationship between cancer biomarkers and the clinical pathology, as well as, to be able to non invasively detect tumours at an early stage. Similarly, identification of subtle changes in the genomics and proteomic status specific to malignant transformation will allow molecular targets to be used for developing therapeutics. This review is a brief account of the biomarkers employed currently in clinical oncology for diagnosis and therapy as well as potential ones that particularly hold promise as targets for therapy.

Diagnostic and prognostic biomarkers are quantifiable traits that help clinical oncologists at the first interaction with the suspected patients. These particularly aid in (*i*) identifying who is at risk, (*ii*) diagnose at an early stage, (*iii*) select the best treatment modality, and (*iv*) monitor response to treatment[6]. These biomarkers exist in many different forms; traditional biomarkers include those that can be assessed with radiological techniques *viz.*, mammograms *etc.*, and circulating levels of tumour specific (related) antigens for example, prostate-specific antigen (PSA). With the availability of complete human genome sequence, and advancement in key technologies such as high-throughput DNA sequencing, microarrays, and mass spectrometry, the plethora of potentially informative cancer biomarkers has expanded dramatically to include the sequence and expression levels of DNA, RNA, and protein as well as metabolites[10]. Advances in imaging technologies open up the possibility that pertinent molecular biomarkers (*e.g.*, those marking response to therapy) can be monitored in cancer patients non invasively.

Genetic and genome based approaches have played significant role in the diagnosis and prognosis of cancer. Alterations in the DNA content (hyper- and hypo- diploidy) arising on account of genomic instability during dysregulated proliferation has been extensively used, but has its limitations. Structural anomalies in chromosomes on the other hand are not a

universal phenomenon. Molecular genetic techniques providing information about the specific and subtle genetic changes have been quite useful in the identification of certain tumours. With the introduction of several comparative genetic analysis techniques, the detection of tumours by analyzing subtle changes in the genetic composition has become more feasible. Analysis of global gene expression changes provided by the micro-array technology has revolutionized the genome based approaches for studying biomarkers at large and cancer in particular. These techniques have in fact been quite successful in clearly dissecting subtle changes between different stages of tumours as well as resolving similar tumour types, which were otherwise not easily discernable.

Cytogenetic and cytokinetic markers

Structural and numerical aberrations in the chromosomes are classical markers of cancer as the association between chromosomal aberrations and neoplastic transformation has been well established. While deviations from diploid chromosome number leading both to hyper- and hypo-diploidy as well as aneuploidy have been noted in malignant tumours[11], sister chromatid exchanges and translocations give rise to structural aberrations that can be easily scored using various banding techniques. Further, double minutes and homogenously stained regions (indicative of gene amplification) are also often observed in malignant cells that can serve as markers[12]. Although, the ploidy changes complement the clinico-pathological findings, a weak association between ploidy, histological and clinical staging has been noted in many tumours[13]. Somatic mutations (in reporter genes, oncogenes and tumour suppressor genes) are promising biomarkers for cancer risk as these can capture genetic events that are associated with malignant transformation[14]. There is growing evidence that specific polymorphism in certain genes are associated with cancer risk[15,16].

Among other genome based biomarkers, identification of neoplasm from the level of lesion specific transcriptomes (mRNA of cytokeratin-19, EGFR, MUC 1, *etc*.) in the blood has been successfully employed in certain epithelial tumours[17,18]. Recently a novel transcriptome marker based on the levels of exon-3 deleted variant isoform of proghrelin (ghrelin is a growth factor involved in prostate cancer cell proliferation) has been developed that aims at reducing the false positives in prostrate and other endocrine cancers[19].

Enhanced cell proliferation is one of the most important hallmarks of cancer, which is easy to identify using a number of histological, biochemical and flow cytometric analysis. Although subjective in nature, histological assessment based on evaluating the number of mitotic cells present within a given sample, is still used as a routine clinical test and even for grading in certain tumours like breast cancer. Flow cytometric analysis of DNA content, which is automated, objective and rapid allowing large number of cells (and samples) to be measured, has been extensively used for the assessment of proliferation status. This complements histological analysis in most cases, besides allowing analysis of clonal and spatial heterogeneity, two important hallmarks of highly malignant tumours[11]. Identification of S-phase cells (unequivocal marker of proliferation) and analysis of a number of other antigenic determinants of proliferation (PCNA, Ki67 NOR, *etc*.) studied using a variety of cell biology techniques have also been used as complementary markers. Information provided by gene expression analysis has a distinct advantage over other assessments of proliferation (*viz.*, more quantitative, objective, and automated) and could form a component of genomic-based clinical diagnostics of cancer. Proteins encoded by the minichromosome maintenance (*MCM*) genes have also been proposed as useful markers of proliferation; with high levels of gene expression indicating poor prognosis[20]. All these genes are cell-cycle regulated and are found among the genes associated with proliferation in tumors[21].

Genetic biomarkers

Cancer is a genetic disease initiated by alterations in genes, such as oncogenes and tumour suppressors that regulate cell proliferation, survival, and other homeostatic functions. Gain/ loss of gene function is predominantly responsible for oncogenic transformation. Several proto-oncogenes get converted into oncogenes with as little as a point mutation on a chromosome, thereby altering the amount of its product *i.e.*, protein. Several non-random mutations, and translocations/ rearrangement within the regulatory region of the gene are also known to be associated with particular types of malignancy. For example, the "Philadelphia chromosome" is associated with chronic myelogenous leukaemia due to a translocation between chromosomes 9 and 22. Other examples occur in Burkitt's lymphoma and in follicular B-cell lymphomas. These translocations serve as highly specific tumour markers for unique clinical diagnosis.

Extensive allotyping of breast cancer for gene deletions of loci on multiple chromosomes has been reported[22]. Deletion of genomic material is important, because the lost segment of DNA may contain certain tumour suppressor activity. Gene deletions are discovered by polymerase chain reaction (PCR) using microsatellite probes to various chromosomes and sites. Tumour-suppressor genes are thought to play a role in specific tumour *e.g.*, in breast tumour p53, Rb, DCC, Brush-1, BRCA-1, BRCA-2. In addition, allelic losses with more or less significant breast carcinoma associations on virtually all chromosomes have been reported[23]. Unfortunately, unlike these well defined markers, random chromosomal abnormalities that are not associated with a particular morphological change give rise to clinical cancer[24].

Loss of heterozygosity as well as mutations within several protooncogenes can lead to microsatellite instability (MSI)[25]. Although detection of microsatellite instability/alterations in pathologic tissue samples require a comparison with normal tissue but it presents a valuable tool for early detection, occasionally at preneoplastic stage[26]. MSI can also be used for prognosis and evaluation for chemotherapeutic response[25].

Adenomatous polyposis coli (APC) gene: The *APC* gene normally responsible for suppressing cancer, is deactivated in many tumours, the altered gene has been found in 92 per cent of patients diagnosed with oesophageal adenocarcinoma, and in 50 per cent of patients with squamous cell carcinoma of the oesophagus[27]. Mutations in the *APC* gene occur in 60 per cent of patients with colorectal carcinoma and are thought to be the earliest genetic abnormalities in the progression of colorectal carcinoma[28]. Most of these mutations cause the production of an *APC* protein that is abnormally short and nonfunctional. This short protein cannot suppress the cellular overgrowth that leads to the formation of polyps, which could become cancerous[28]. Somatic mutation of the APC gene has been identified in sporadic colorectal cancer as well as in some cancer of the stomach, pancreas, thyroid, ovary and other primary sites[28]. Hence, hypermethylated *APC* gene is being utilized as a biomarker to determine the stage of oesophageal cancer, detect recurrent disease, and monitor disease progression or treatment response[27]. A high level of hypermethylated *APC* gene in the bloodstream is generally associated with poor survival; conversely, the prognosis improves dramatically for patients with low or undetectable blood levels of the altered gene[29]. APC expression is also induced during pregnancy and lactation in the mouse mammary gland, and APC deficiency results in defective lobule-alveolar development, therefore should be used with caution. PCR-based tests are widely used to detect these mutations.

Epigenetic biomarkers

In cancer cells, genes and their functional products are either modified by mutations, or through epigenetic modifications to chromosomes that alter gene-expression patterns. Epigenetic modifications can occur directly through DNA methylation of genes or indirectly by methylation, acetylation, or phosphorylation of histones and other proteins around which DNA is wound to form chromatin[30]. DNA methylation at the cytosine residue is the main epigenetic modification in humans which occurs in the context of 5'-CpG-3' dinucleotides[31]. In recent years it has become apparent that epigenetic events are potentially responsible for cancer initiation and progression as genetic abnormalities, with DNA hypo- and hyper-methylation promoting cancer development. Several studies have shown that the activity and DNA methyltransferases (DNMTs), which add methyl groups to DNA at cytosine residues, are altered in tumour cells and associated with several developmental abnormalities[32]. Genomic hypomethylation may lead to both genomic instability and stronger gene expression[33]. On the other hand, local promoter CpG island hypermethylation induces the functional silencing of tumour suppressor genes, mimicking their genetic mutations. Many genes involved in the process of carcinogenesis are the common targets for silencing, which includes cell-cycle control and apoptosis genes *viz. p14, p15, p16, Rb, DAPK*; DNA repair genes *MGMT, hMLH1;* adhesion and metastasis genes *CDH1, CDH13*; biotransformation genes *GSTP1* and signal transduction genes *RARβ, APC*[34]. Since, methylation pattern of many genes are altered in cancer, type specific panels for methylation of different genes have been suggested, *e.g.*, *GSTP1, RARβ, TIG1* and APC for prostate carcinoma; *p16, RASSF1A, FHIT*, H-cadherin and *RARβ* for non small cell lung cancer[34]; *VHL, p16, p14, APC, RASSF1A* and *Timp3* for kidney cancer, *etc*[34].

Hypermethylation markers may be used for the detection of both primary and metastatic or recurrent cancer cases. For example, hypermethylation of p16 promoter in the circulating serum DNA correlate well with recurrent colorectal cancer[36]. Aberrant methylation of the *p16Ink4* and *MGMT* promoters can be detected in DNA from the sputum of patients with squamous

cell carcinoma nearly 3 yr before clinical diagnosis while, methylation of *p16Ink4*, *RASSF1A*, or *PAX5-beta* genes appears to be associated with a 15-fold increase in the relative risk for lung cancer. Therefore, it has been suggested that alterations in methylation patterns of groups of genes in sputum samples may be an effective, non invasive approach for identifying smokers at risk of developing lung cancer[37]. Methylation of the O6-methylguanine-DNA methyltransferase (*MGMT*) gene, which encodes a DNA-repair enzyme has been shown to inhibit the killing of tumour cells by alkylating agents and methylation of the *MGMT* promoter of malignant glioma appears to be a useful predictor of the responsiveness to alkylating agents as patients with silencing of this gene seem to respond better to therapy[38]. Although, research in epigenetics has led to improved survival of patients with certain forms of lymphoma and leukaemias through the use of drugs that alter DNA methylation and histone acetylation, proposed methylation markers need further optimization and large scale clinical trial for further validation. The development of therapeutics that reverse epigenetic alterations in cancer cells, along with prognostic and diagnostic assays based on gene-methylation patterns, are promising new avenues for future improvements in patient care.

Cells as biomarker

In advanced stages of tumours, cells starts appearing in bloodstream where it can be easily monitored. Advanced clinical practice in certain malignancy have effectively used tumour and immune cells where it served as a good biomarker of prognosis, while its utility in other cancers are under evaluation at the present time.

Circulating tumour cells (CTCs): It is simple yet powerful biomarker in the field of oncology. The presence of CTCs has been shown to predict survival in patients with metastatic breast cancer at multiple time points throughout the course of therapy[39]. CTCs provide an early, reliable indication of disease progression and survival for patients on systemic therapy for metastatic breast cancer. Elevated CTCs at any time during therapy is a harbinger of progression, while elimination of CTCs indicates effectiveness of the therapy[40]. Selection of appropriate therapeutic regime can also be guided by assessment of the presence of the therapeutic target on the CTCs and, in contrast to CTC scans, the effect of therapy can be measured after the first cycle of therapy. CTCs are not only potential surrogate endpoint in oncology clinical trials but may guide the selection of patients into the trials[41].

CTCs have been shown to be superior to standard tumour markers (*e.g.,* Ca27-29) in predicting prognosis. Furthermore, the efficacy or benefit to systemic therapy can also be predicted by the level of CTCs as early as 3-4 wk after initiation of therapy. Patients with persistent CTCs (≥ 5) demonstrate lack of response to treatment or progressive disease at the time of restaging by standard imaging modalities, while objective remission have been reported in patients with < 5 CTCs. Available evidences clearly suggest that CTCs can be used as an early predictor of treatment efficacy and extremely useful in sparing patients from futile therapy early in the course of their treatment[42].

T-regulatory cells ($CD4^+$, $CD25^+$ and $Foxp3^+$): A number of mechanisms contribute to the capacity of the immune system to discriminate self from non-self, facilitating the maintenance of immunological tolerance to self-antigens and the induction of protective immunity to foreign antigens. It is becoming increasingly clear that regulatory T cells (T-regs) are equally important in inducing and maintaining peripheral self-tolerance and thus preventing immune pathologies[43,44]. These are subpopulations of CD4 cells, characterized by high CD25 expression along with Fox P3. They are thought to play a specialized role in controlling both innate and acquired immune responses[45]. Furthermore, studies in cancer patients suggests that increased T-regs activity may be associated with poor immune responses to tumour antigens and contribute to immune dysfunction resulting in tumour growth[46,47]. High numbers of T-regs have been found in lung, pancreatic, breast, liver and skin cancer patients, either in the blood or in the tumour itself[46,48-51]. However, levels of T-regs are also elevated in certain infectious diseases[52]. T-regs are able to inhibit proliferation and IFN-γ production by $CD4^+$ and $CD8^+$ T cells, as well as natural killer (NK) cell-mediated cytotoxicity. Studies on ovarian carcinoma patients have demonstrated that the presence of T-regs, which suppress tumour-specific T-cell immunity inversely correlate with survival[53]. Recent studies in Ehrlich ascites tumour bearing mice have also shown that higher numbers of T-regulatory cells are associated with tumour progression. Further, the T-regs levels were found to be significantly lower in animals that showed complete response (tumour regression and cure) to a given treatment (a combination of radiation and the glycolytic inhibitor 2-deoxy-D-glucose;2-DG), while an increase was found in those animals that did

not respond to the treatment suggesting a relationship between T regulatory cells and therapeutic response[54]. Therefore, it appears that although T-regs may serve as a surrogate immune marker of cancer progression (and perhaps prognosis), it seems to be more useful as a predictor of response to therapies.

Regulatory T cells (T-regs) are primarily and specifically identified by the experiments of (transcription factor, FoxP3) using anti FoxP3 antibodies. The presence of FoxP3+ cells within tumours has been shown to predict the prognosis, invasiveness, and metastatic ability of some tumours by modulating the ability of the immune system to target tumour cells[55]. Depletion of regulatory T cells from tumours could lead to the rejection of both early- and late-stage tumours by the host immune system. These findings suggest that the widespread use of T-regs, and more importantly its intracellular marker FoxP3, should be explored as a biomarker for human tumours to enabling better decisions in patient care, as well as prepare the field for novel therapeutic agents directed at the elimination of regulatory T cells within tumours or in the peripheral blood.

Cancer stem cells (CSCs): It has long been recognized that subpopulations of cancer cells exist within the tumours that resemble the developmental hierarchy of the normal tissue from which the tumour arose. In recent years, the cancer stem cell model of tumourigenesis has received increasing attention. This model postulates that tumours are driven and maintained by a minority 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[56]. The former population has been termed cancer stem cells (CSCs), tumourigenic cancer cells, or tumour-initiating cells, by various investigators, to indicate that only these can give rise to new tumours when transplanted into immuno-deficient animals[57].

Evidence for the existence of CSCs initially came from studies of acute myelogenous leukemia (AML). Presence of CSCs have now been demonstrated in many solid tumours, including glioblastoma, medulloblastoma, breast cancer, melanoma, and prostate cancer[58]. The existence of CSCs has profound implications for cancer biology and therapy because it is likely that eradication of CSCs is the critical determinant in achieving cure. It has been proposed that CSCs may be particularly resistant to chemotherapy and radiation therapy as has been shown in a study with glioblastoma[59]. $CD133^+$ cells were earlier suggested as the tumourigenic population in primary glioblastoma multiforme specimens[60]; while more recent studies have shown that these are indeed more radioresistant compared with $CD133^-$ tumour cells, as their fraction increase after irradiation which appears to be mainly responsible for the tumour regrowth[59]. Therefore, it appears that identifying and characterizing CSCs for every possible tumour is of paramount importance and will likely lead to new therapeutic avenues. Also, work on radiosensitizers should begin to focus on preferentially affecting CSCs compared with normal tissues and normal tissue stem cells.

Viral biomarkers

Among viral induced cancers, hepatocellular carcinoma (HCC) is one of the most common cancers worldwide and a leading cause of death in developing countries, where nearly 80 per cent of the cases are reported[61]. Risk factors include chronic hepatitis infections mainly due to the endemic hepatitis B virus (HBV) infection, whereas association of hepatitis C virus (HCV) infection is also reported in a small fraction (12 - 17%) of the HCC cases[61]. Beside immuno-inflammatory reactions, HBV can also promote carcinogenesis through genetic instability generated by its common integration in host DNA[62]. A number of different types of biomarkers have been used to understand the aetiology and progression of HCC. Perhaps, the most well known are the serum/plasma markers of HBV or HCV infection[62]. These markers include analysis of viral DNA or proteins or antibodies produced against the viral proteins. HBV surface antigen (HBsAg) is most frequently used to determine chronic infection with high or low viral replication, while HBeAg is a measure of chronic infection with high viral replication[63]. The other major classes of biomarkers used in studies of HCC are analysis of antibodies including measurement of anti-HBV core antigen, anti-HBe antigen and anti-HBsAg[61,63].

Cervical cancer is the second most common cancer and predominant gynaecological cancer in women, causing most cancer related deaths world over[64]. There are several factors which contribute to high incidence of this disease are early age of marriage, multiple sexual partners, multiple pregnancies, poor genital hygiene, smoking and use of oral contraceptives. But the most predominant aetiological factor for cervical cancer is persistent infection of certain high-risk types of human papillomaviruses (HR-HPVs), while low risk types are associated with benign cervical lesions and genital warts[65]. HPV has also been detected in a significant

proportion of oral, oesophageal, anal, vaginal, vulvar, and penile cancer and in a small percentage of lung, laryngeal, and stomach cancer, as has been shown in some parts of the world[66]. HPV viral load, a measure of the amount of viral DNA in biopsy specimens, alone or in conjunction with well characterized HPV serologic assays, has been suggested to delineate the role of HPV among oral and oropharyngeal cases[66], while antibodies generated in the subjects against HPV E6 and E7 serve as markers of an invasive HPV-associated malignancy[67]. Viral load assessment can also be exploited to distinguish clinically relevant HPV infections in the cervix[64]. Prophylactic immunization of women who are negative for the HPV16 L1, E6 and E7 oncoprotein markers, has been reported to eliminate their risk for HPV16-related cervical intraepithelial neoplasia[67]. Recently, the two new HPV vaccines "Gardasil" and "Cervarix" have been shown to be highly immunogenic and effective in preventing infection with high-risk HPV types 16 and 18, the two most common oncogenic types associated with this disease[65]. Papillomaviruses were first identified, cloned and sequenced from cervical tumour specimens and subsequently established as important causative agents for development of cervical cancer[66].

Epstein-Barr virus (EBV) was the first human virus to be directly implicated in carcinogenesis. It infects more than 90 per cent of the world's population, out of which a small proportion develop tumours[68]. Although herpesviruses are ubiquitous in nature, interestingly humans serve as the only natural host for EBV and upon infection; the individual remains a lifelong carrier of the virus[68]. The vast majority of the world's population exhibits antibodies to EBV and the infection usually occurs early in childhood[68]. EBV has been implicated in the pathogenesis of Burkitt's lymphoma, Hodgkin's disease, non-Hodgkin's lymphoma, nasopharyngeal carcinoma, and lymphomas, as well as leiomyosarcomas arising in immuno-compromised individuals[68]. EBV infection of B lymphocytes is thought to occur in the oropharyngeal lymphoid organs, and in normal carriers, while the virus persists in circulating memory B cells. EBV is a herpesvirus with a 184-kbp long, double-stranded DNA genome that encodes more than 85 genes[68]. The EBV genome is maintained in B cells, either as a multicopy circular episome in the host cell or by integrating the viral DNA into the host genome. The virus thus ensures transmission to cell progeny when B lymphocytes replicate. EBV DNA in the peripheral blood predicted a high risk of distant metastases[69]. Detection and quantification of plasma EBV DNA serves as a useful molecular marker for diagnosis, monitoring, and prediction of relapse in patients with nasopharyngeal carcinoma and Hodgkin's lymphoma[69,70].

Thus viral biomarkers seem to have potential application in the diagnosis (particularly staging), prognosis and predicting as well as monitoring response to therapy.

Cancer antigens (biomolecules) based biomarkers

The cancer proteome contains information on perhaps every biological process that takes place in cancer cells, cancer tissue microenvironment, and cancer cell-host interaction. Cancer cells release many proteins and other macromolecules into the extra-cellular fluid through secretion that can also serve as biomarkers. Some of these products can end up in the bloodstream and hence serve as potential serum biomarkers. Some important cancer antigens that serve as diagnostic and prognostic biomarkers of cancer are summarized in the Table.

Prostate specific antigen (PSA), a 33 kDa serine protease belonging to the family of "Kallikrein genes" and produced by both normal as well as neoplastic prostate epithelial cells is the most widely studied biomarker in prostate cancer. Among all kallikreins, hK2 and hK3 expression is highly restricted to the prostate in males and are therefore useful as biomarkers[71]. Being a protease, it appears to be involved in the initiation and growth of prostate cancer by abnormal release of growth factors or proteolysis of growth factor binding proteins. It may also have a role in invasion and metastases through the degradation of collagen and laminin. PSA was first identified by in 1971 while attempting to find a substance in seminal fluid that would aid in the investigation of forensic cases. PSA was first measured quantitatively in the blood by Papsidero and colleagues in 1980, who also reported its clinical use as a marker of prostate cancer[72]. PSA is present in small quantities in the serum of normal men, and is often elevated in the presence of prostate cancer and other prostate disorders. However, prostate cancer can also be present in the complete absence of an elevated PSA level[73]. PSA expression is androgen dependent and therefore less sensitive in older population. Obesity has been reported to reduce serum PSA levels[74], while increase has been found in prostate infection, irritation, benign prostatic hyperplasia (BPH), and ejaculation[75]. Limitations of PSA as a biomarker for monitoring

Table. Cancer biomarkers for diagnosis and prognosis of the disease

Biomarker	Tumour	Application	Sample type/ Method of detection
Cancer antigen (biomolecules) based biomarkers:			
Prostate specific antigen (PSA)	Prostate cancer	Diagnostic and prognostic	Serum/ Immunoassay
Alpha-foetoprotein (AFP)	Hepatocellular carcinomas (HCC)	Diagnostic and prognostic	Serum/ Immunoassay
Cancer antigen 125 (CA125)	Ovarian cancers Fallopian tube cancer	Diagnostic and prognostic	Serum/ Immunoassay
Cancer antigen 15-3 (CA15-3)	Breast cancer	Diagnostic and prognostic	Serum/ ELISA, Lymph node/ IHC, Bone marrow/ IHC
Cancer antigen 19-9 (CA 19-9)	Pancreatic cancer Bladder cancer	Diagnostic and prognostic	Serum/ ELISA Urine/ ELISA
BRCA-1, BRCA-2	Breast cancer	Diagnostic	Tumour samples/ RT-PCR
Carcinoembryonic antigen (CEA)	Colorectal cancer	Diagnostic and prognostic	Serum/ ELISA
Human chorionic gonadotrophin (hCG)	Germ cell tumours (ovarian and testicular)	Diagnostic	Serum/ ELISA
Thyroglobulin (Tg)	Papillary and follicular thyroid cancer	Diagnostic and prognostic	Serum/ ELISA or IHC with TPO Ab
Heat shock proteins (HSPs) Hsp27; Hsp70	Gastric, prostate carcinoma, osteosarcomas, uterine, cervical, and bladder carcinoma	Diagnostic and prognostic	Serum/ ELISA
TGFβ	Malignant tumours	Diagnostic and prognostic	Serum / ELISA
Metabolic biomarker:			
Glucose metabolism	All cancers, general	Daignostic, prognostic and therapeutic	Imaging/ FDG-PET scan
Genetic biomarkers:			
Genetic translocations *viz*. Philadelphia chromosome, Bcl2 and other gene translocation fusion products	AML, ALL, CML, MDS and Burkitt's lymphoma	Diagnostic	Bone marrow or peripheral blood/ FISH
APC gene	Adenocarcinoma, squamous cell carcinoma of the stomach, pancreas, thyroid and ovary	Diagnostic and prognostic	Blood, Tumour sample/ RFLP of chromosome 5q21-22, Methylation status of APC gene
Cells as biomarker:			
Circulating tumour cells (CTCs)	Metastatic breast cancer, *etc*.	Diagnostic and prognostic	Blood/ Immunocytometry
Cancer stem cells (CSCs)	AML, melanoma, brain tumour, breast cancer, prostate cancer	Diagnostic, prognostic and therapeutic	Tumour sample/ Immunocytometry

response to the therapy have been identified, as increase in serum level not correlating with tumour regression following radiotherapy has been reported in some instances[76]. A blood test to measure PSA is considered the most effective test currently available for the early detection of prostate cancer.

Alpha-foetoprotein (AFP) is the major serum foetal protein in mammals, which is actively produced and secreted during the foetal life by the liver hepatocyte. Major tumours that secrete AFP are endodermal sinus tumour (yolk sac carcinoma), neuroblastoma, hepatoblastoma, and hepatocellular carcinoma[77]. It is a well-known diagnostic biomarker used to follow the development of hepatocellular carcinomas (HCC) where its synthesis is frequently upregulated. Significant increase in the amount of serum AFP is usually detectable in patients with poorly differentiated and highly malignant tumours[77]. However, AFP test is

not sensitive or specific enough for early detection of the hepatocellular carcinoma and therefore by itself is not diagnostic, but could only be suggestive and useful as a prognostic marker. Since the levels of AFP may be elevated in serum from patients with other chronic liver disease; AFP is not useful for screening in patients suffering from cirrhosis or hepatitis C[78,79].

CA125: The CA 125 antigen is a membrane glycoprotein produced by tissues derived from coelomic epithelium that is expressed by most epithelial ovarian cancers[80]. CA125 was initially detected in 1983 using the monoclonal antibody designated OC125; hence the name CA125. CA125 is a powerful index of risk of ovarian and fallopian tube cancer in asymptomatic postmenopausal women. It is found in the serum of more than 80 per cent of the patients with epithelial ovarian tumours, with half life of 4 days. CA-125 antigen remains the only serum tumour marker routinely used in epithelial cancer of the ovary for patient prognosis, disease progression, and response to chemotherapy[80,81]. Normal values of CA125 in serum range from 0 to 35 U/ml. CA125 is also expressed by a number of tissues of both cancerous and noncancerous origin[82]. It may also be elevated in other malignant cancers, including those originating in the endometrium, fallopian tubes, lungs, breast and gastrointestinal tract[83]. Certain physiological conditions also modulate CA125 levels, as the levels are elevated slightly during menstruation[84] and more prominently during the first trimester of pregnancy. A decrease in CA125 level is generally associated with tumour response to therapy, whereas a rising level is suggestive of drug resistance[85]. Indeed, CA125 is an accurate marker to define relapse of ovarian cancer. CA125 has numerous applications in the design of clinical trials, from prognosis to follow up and is a tool that is complementary to standard criteria for disease measurement[86]. The key problems in using the CA125 test as a screening tool are its lack of sensitivity and its inability to detect early stage cancers.

CA15-3: The CA15-3 protein is a member of the family of proteins known as mucins, whose normal function is cell protection and lubrication. It plays a role in reducing cell adhesion and is found throughout the body. Elevated levels of this antigen are found mainly in breast cancer where it appears to be involved in metastasis[87]. CA15-3 level is elevated in nearly 11 per cent of women with operable breast cancer, and 60 per cent of women with metastatic disease. Pre-operative concentration of CA15-3 is associated with worse prognosis than those with low concentrations[88]. CA15-3 appears to be a marker for individualizing therapy in patients with breast cancer, where patients with high CA 15-3 show good response to aggressive treatments[89]. Serum CA15-3 has been used as a surrogate marker of disease bulk to monitor metastatic breast cancer patients undergoing treatment and for the preclinical detection of tumour recurrence[89]. Elevated levels of CA-15-3 has also been found in patients with other cancers (lung, colorectal, ovarian, pancreatic) and hepatic dysfunction[43]. The upper limit of normal level of this marker is 25U/ml.

CA19-9 (cancer antigen 19-9) or GICA (gastrointestinal cancer antigen) is a glycolipid with unknown biological function, which was the first successful tumour marker used for serological diagnosis of pancreatic cancer[90,91]. The nomenclature derives from the monoclonal antibody clone 19-9 that was developed by Koprowski. The concentration of Ca 19-9 in serum has been shown to be a sensitive and specific marker for pancreatic cancer, while its elevated levels in urine have been found in bladder cancer[91]. The amount of Ca 19-9 in urine seems to reflect not only the presence of tumour(s) but also the existence of urothelial dysplasia or carcinoma *in situ*[92]. Owing to its high specificity, it plays an important role in the diagnosis, therapeutic monitoring and monitoring of the course of gastrointestinal carcinomas, in particular in the case of pancreatic carcinoma, hepatobiliary carcinoma (carcinoma of the liver, carcinoma of the bile ducts) and carcinoma of the stomach[93]. However, increased levels of CA 19-9 can also be found in patients with nonmalignant inflammatory diseases, such as cholecystitis and obstructive icterus, cholelithiasis, cholecystolithiasis, acute chlolangitis, toxic hepatitis and other liver diseases and therefore should be used with caution[92,93]. The normal blood levels of CA 19-9 are below 37 U/ml and with this reference level a false positive rate of 20 per cent has been reported in pancreatitis[92].

Carcinoembryonic antigen (CEA) is a 200 kDa glycoprotein, isolated first by Gold and Freeman in 1965 [94] using an antibody raised in rabbits by injecting an extract of human colonic carcinoma. Elevated levels are found in patients with colorectal, breast, lung, or pancreatic cancer[95], and also in smokers[96]. The first success in developing a blood test for CEA was in 1965, when the antigen was detected in the blood of some patients with colon cancer. Blood levels of CEA are also elevated in many other cancers such as those of the thyroid, pancreas, liver, stomach, prostate,

ovary, and bladder[96]. Post-operative normalization of serum CEA level has been reported to be a favourable prognostic indicator in lung cancer and the identification of abnormal pre- and post-operative serum CEA levels may be useful in the auxiliary cancer prognosis or post-operative surveillance of colorectal cancer patients[97].

Human chorionic gonadotrophin (HCG) is a hormone produced normally by the placenta, whose level is elevated in the blood of patients with certain types of testicular and ovarian cancers (germ cell tumours) and choriocarcinoma[98]. In addition, synthesis of free βhCG and its subunits by pelvic carcinomas such as those of the colon, urinary tract, prostate, uterus and vulvo-vagina has also been reported[98]. The presence of increased serum levels of hCG and its metabolites is generally considered to be a sign of a poor prognosis and it has been suggested that βhCG might directly modify the growth of the cancer, leading to a worse outcome. The clinical use of free βhCG as a tumour marker has been limited to a small number of patients owing to a short half life and rapid renal clearance[99]. An elevated blood level of hCG is also be found in the urine of pregnant women and therefore may not be useful as a marker under this condition.

Thyroglobulin (Tg) is a large glycoprotein stored in the follicular colloid of thyroid gland and acts as pro-hormone in the intra-thyroid synthesis of thyroxine (T4) and triiodothyronine (T3). This is another organ-specific tumour marker; associated mainly with patients harbouring differentiated thyroid cancer that arise from the follicle cells (*viz*., papillary and follicular thyroid cancer) frequently resulting in increased levels of thyroglobulin in the blood[100]. The thyroglobulin test is primarily used as a tumour marker to evaluate the effectiveness of treatment for thyroid cancer and to monitor recurrence[100]. Tg is generally measured in serum, but measurements can also be made in thyroid cyst fluids and other fluids/tissue obtained by fine needle biopsy of thyroid nodules[101]. Concomitant measurement of antithyroglobulin is essential as anti-Tg antibodies interfere with the Tg assay and render the assay for thyroglobulin in valid[102]. This important factor should be taken into consideration during the evaluation of patient's status. Further, this will not be a very useful marker in tumours which do not release a significant amount of thyroglobulin into the circulation. Although an undetectable level of serum Tg after thyroidectomy and ^{131}I ablation suggests that patients are free of disease, several studies have shown that a minority of patients, under the influence of TSH stimulation, have elevated serum Tg[103] levels and therefore interpretation needs to be made with caution.

Heat shock proteins (HSPs) expression is tailored for particular stress response, with accumulation of denatured proteins as the proximal signal for its induction[104]. Heat shock proteins (Hsps) are overexpressed in a wide range of human cancers and are implicated in tumour cell proliferation, differentiation, invasion, metastasis, death, and recognition by the immune system. At present it is unclear as to how the Hsps become overexpressed in cancer; one hypothesis is that the physio-pathological features of the tumour microenvironment (low glucose, *p*H, and oxygen) stimulate the Hsp induction[105,106].

Although Hsp levels are not informative at the diagnostic level, due to overexpression in a wide range of malignant cells and tissues, these are useful biomarkers for carcinogenesis in tissues and are suggestive of the degree of differentiation and the aggressiveness in certain types of cancers[107]. Further, the circulating levels of Hsp and anti-Hsp antibodies in cancer patients may be useful in tumour diagnosis. Several Hsp are implicated in the prognosis of specific cancers; most notably Hsp27, whose expression is associated with poor prognosis in gastric, liver, and prostate carcinoma, and osteosarcomas, while Hsp70 is correlated with poor prognosis in breast, endometrial, uterine cervical, and bladder carcinomas. Increased Hsp expression has also been found to predict the response to certain anticancer treatments. While Hsp27 is associated with a poor response to chemotherapy in leukaemia patients, Hsp70 expression predicts a better response to chemotherapy in osteosarcomas[104]. Implication of Hsp in tumour progression and response to therapy has led to its successful targeting in therapy by use of Hsps in anticancer vaccines, exploiting their ability to act as immunological adjuvant[107].

Oral fluid contains proteomic signatures that may serve as biomarkers for human diseases such as oral cancer. Therefore, it has been suggested that proteomic analysis of human oral fluid such as whole saliva holds promise as a non-invasive method to identify biomarkers for human oral cancer[108]. Most recently detection of five proteins in the saliva of cancer patients has been found to be useful markers of oral cancer with 90 per cent sensitivity and 83 per cent specificity for oral squamous cell carcinoma. These proteins include (*i*) calcium-binding protein MRP14 implicated in several types of

cancer; (*ii*) CD59 overexpressed on tumour cells that enables them to escape from complement-dependent and antibody-mediated immune responses; (*iii*) Profilin 1, a protein involved in several signaling pathways with cytoplasmic and nuclear ligands, generally secreted into tumour microenvironments during the early progressive stage of tumorigenesis; and (*iv*) catalase, a member of the enzymatic antioxidative system, whose level is elevated in many human tumours and involved in carcinogenesis and tumour progression[109]. However, long-term studies employing large number of oral cancer patients as well as subjects at high risk of developing oral cancer are needed to validate these potential biomarkers.

Mitochondrial markers Mitochondria typically contain multiple haploid copies of their own genome (16.5 kb), including most components of transcription, translation, and protein assembly. mtDNA is present at 1000-10,000 copies/cell, and the vast majority of these copies are identical (homoplasmic) at birth. Several mutations in the mtDNA, particularly in the D-loop region have been recently found in breast, colon, oesophageal, endometrial, head and neck, liver, kidney, leukemia, lung, melanoma, oral, prostate, and thyroid cancer[110]. The majority of these somatic mutations are homoplasmic in nature, suggesting that the mutant mtDNA played an active role in tumour formation[111]. By virtue of their clonal nature and high copy numbers in cancer cells, mitochondrial mutations may provide a powerful molecular marker for noninvasive detection of cancer. It may also be useful in early detection, diagnosis, and prognosis of cancer outcome and/or in monitoring response to certain preventive and interventional modalities as well as therapies[112,113]. Mutated mtDNA has also been detected in the body fluids of cancer patients and indeed is much more abundant than the mutated nuclear p53 DNA[114]. Since the mitochondrial gene expression signatures of transformed cells have now been identified, development of mitochondrial functional proteomics is expected to identify new markers for early detection and risk assessment, as well as targets for therapeutic intervention[111]. Many advanced techniques are currently available or being newly developed for the studying the mitochondrial proteome *viz*. IP, DiGE, ICAT, SELDI, MALDITOF, Protein/antibody array, *etc.*, are expected to facilitate further this process.

Metabolic biomarker (glucose metabolism)

Enhanced glucose utilization is a prominent and fundamental change in many tumours irrespective of their histological origin and the nature of mutations, first observed by Warburg[115,116]. Mechanisms underlying this fundamental alterations in metabolism during carcinogenesis include mutations in the mitochondrial DNA resulting in functional impairment, oncogenic transformation linked upregulation of glycolysis, enhanced expression of metabolic enzymes and adaptation to the hypoxic tumour micro-milieu in case of solid tumours[117]. Based on these observations, a bioenergetic index of the cell (BEC index) has been suggested that could be used for classification and prognosis of cancers, besides predicting the response to therapy[118]. Positron emission tomography (PET), which allows non invasive and quantitative analysis of various biologic processes, uses a glucose analogue (2-deoxy-D-glucose) labelled with a positron emitter Fluorine 18; FDG that is partially metabolized and trapped as its phosphate (2-DG-6-P) in the tumour tissue thus localizing the tumour[119]. The extent of increase in glucose utilization measured by FDG-PET has been correlated with the degree of malignancy in some of the tumours[120]. Glucose utilization is also inversely correlated with treatment response in a number of tumours, while changes in tumour glucose utilization during the first weeks of chemotherapy are significantly correlated with patient outcome[121,122]. Therefore, glucose utilization appears to be a useful metabolic marker for diagnosis, prognosis and prediction of tumour response to a variety of therapies[123].

Therapeutic biomarkers

Cytotoxic chemotherapy and radiotherapy remain the most effective treatments for cancer; however, these can cause serious side effects as these do not often show adequate differential effect between tumour and normal cells. Advances in understanding the molecular basis of cancer made possible by the identification and functional analysis of tumour-specific genetic alterations have opened exciting new opportunities for the design of therapies that specifically target the molecular pathways involved in promoting tumour cell growth and circumvent death pathways like apoptosis.

In the past decade, there have been considerable improvements in the way that human tumours are characterized. Knowledge of cancer at the molecular level has therefore increased greatly, and has catalyzed a shift towards using targeted therapies for cancer. In principle, "targeted therapies" display greater selectivity for tumour cells, and indeed several such therapies have already shown promise in the clinic. These include small molecule drugs that inhibit the activity of protein

tyrosine kinases [*e.g.*, imatinib and erlotinib, targeting ABL and the epidermal growth factor receptor (EGFR), respectively] and neutralizing antibodies that inhibit trans-membrane signaling receptors (*e.g.*, trastuzumab, targeting HER2). Other targeted therapies include drugs that block the activity of molecules in the host microenvironment that support tumour growth (*e.g.*, the antibody bevacizumab, which targets a growth factor that stimulates tumour blood vessel growth). To date, many of these therapies have conferred only modest benefits on patient survival, but refinements in the mode of use of these drugs (*e.g.*, as combination therapies and with biomarker-guided patient selection) are expected to improve their efficacy. Development of clinical tools to identify which patients are most likely to benefit from particular targeted therapies will aid the individualization of molecular targeted therapies thereby enhancing the efficacy of therapy.

Glycolysis

Enhanced glucose dependency is one of the prominent characteristics of most malignant tumours and correlate well with resistance to radio- and chemotherapy[124,125]. Metabolic status linked alterations in cell signaling related to defense against oxidative stress, redox signaling and damage response pathways, particularly the downregulation of mitochondrial dependent apoptosis in tumour cells with enhanced glucose usage and Hexokinase II levels have been observed[126]. Recent observations in many human tumour cell lines with varying degrees of glycolysis (endogenous and induced) have shown an inverse relationship between the rate of glycolysis (glucose usage and lactate production) and manifestation of damage induced by radiation and chemotherapeutic drugs[127-129] similar to the correlations between FDG uptake and responses to therapy clinical responses. These observations have prompted the targeting of this phenotype (elevated glycolysis) as an attractive proposition for developing therapeutics and adjuvant in cancer therapy[130,131].

In vitro and *in vivo* studies with several murine and human tumour cells have indeed shown that glycolytic inhibitors like 2-deoxy-D-glucose, 3-bromo-pyruvate *etc*., are selectively cytotoxic to tumour cells, inducing both growth inhibition and cell death[132]. Unfortunately, therapies using pharmacological inhibitors of glycolysis as a primary therapeutic agent have not produced remarkable results in providing effective local tumour control, besides showing systemic toxicity, particularly to the central nervous system[133]. A more promising approach for improving cancer therapy exploiting the inherent differences in glucose metabolism between tumour and normal cells, employs 2-DG as a differential modifier of cellular responses to the widely used therapeutic agents such as radiation and/or cytotoxic drugs[134-137]. The rationale behind this approach is based on the bioenergetics of cellular damage response pathways including DNA repair, cell proliferation and cell death on the one hand and enhanced glucose dependency in tumour cells on the other. Several studies using *in vitro* and *in vivo* models of tumours have shown that 2-DG selectively sensitizes tumour cells to ionizing radiation, while reducing the damage to normal cells[138,139]. Since mechanisms underlying cellular responses to damage caused by many anticancer drugs are similar to radiation, it has been suggested that 2-DG has the potential to enhance the efficacy of chemotherapy[140,141]. Indeed, 2-DG has been found to enhance the damage caused by certain chemotherapeutic drugs *in vitro*[142-145] and *in vivo* [146,147].

Clinical trials in patients with malignant brain tumours (glioblastoma multiforme) using a hypofractionted radiotherapy protocol combined with 2-DG have been very encouraging[134]. Excellent tolerance to the combined treatment, with minimal acute toxicity and late radiation effects as well as increase in survival and significant improvement in the quality of life has been reported[134, 135].

Mammalian target of rapamycin (mTOR)

This is an evolutionarily conserved serine-threonine protein kinase that belongs to the PIKK [phosphoinositide 3-kinase (PI3K)-related kinase] family, and plays an important role in regulating cell growth and proliferation[148]. Upon activation, mTOR increases the phosphorylation levels of its downstream targets that include p70S6K and 4EBP1, which leads to increased levels of translation, ribosome biogenesis, and reorganization of the actin cytoskeleton and inhibition of autophagy. As a result, mTOR activation promotes cell growth and proliferation, whereas mTOR inhibition stops cell growth and initiates catabolic processes, including autophagy[149].

The phosphatidylinositol-3-OH kinase (PI(3) K)–PTEN–mTOR signaling pathway is aberrantly activated in many tumours, leading to dysregulation of cell growth and proliferation[148]. Activation of this pathway can be assessed by biomarkers such as loss of PTEN mRNA or protein production in tumour tissue.

Biochemical inhibition of mTOR by rapamycin can be assessed by biomarkers such as the abundance of the phosphorylated form of the ribosomal protein S6, and its therapeutic effects on tumour cells can be assessed by the proliferation marker Ki-67[150].

A number of mTOR inhibitors have potent antiproliferative properties which make them useful for cancer chemotherapy, particularly of advanced solid tumours[151]. It has surprisingly been found that S6 40S ribosomal protein (otherwise known as S6) is a useful biomarker which is predictive of sensitivity of proliferative diseases to treatment with an mTOR inhibitor. In particular, it has been found that the phosphorylation state of S6 correlates well with sensitivity to mTOR inhibitors. mTOR inhibitors are more likely to show a significant antiproliferative effect when used to treat cancer cell lines showing higher levels of expression of phosphorylated S6. Moreover, the method may be used to select an appropriate dose of an mTOR inhibitor in order to individualize therapy for each patient[152].

Telomerase

Telomeres are tracts of repetitive DNA (TTAGGG/AATCCC for human telomeres) that protect chromosomes from degradation and loss of essential genes. Under normal circumstances, telomeres progressively shorten in most human cells with each cycle of cell division and the length in adult human tissues is approximately half that of the new born. Telomerase belongs to a class of enzymes known as reverse transcriptases that use RNA as a template for creating DNA and it contains both RNA and protein components. The enzyme ensures the maintenance of telomere and thereby protecting the cell from degradation and death[153]. Since telomerase is found in nearly 90 per cent of human cancers and is responsible for indefinite growth of cancer cells[154,155], it has been a target for anticancer therapeutics that turn-off telomerase and thereby inhibit tumour growth. The levels of telomerase are also elevated in stem cells allowing unlimited division necessary for the repair of damaged and worn out tissues. Most human tumours not only express telomerase but interestingly also have very short telomeres. Telomerase is one of the best markers for human cancer, associated with only malignant tumours and not the benign lesions making it a diagnostic marker as well as an ideal target for chemotherapy[156-158].

In normal cells, telomerase is sequestered in an area of the cell nucleus called the nucleolus, away from the chromosomes. The enzyme is released only when needed during cell division, and then returns quickly to the nucleolus thereafter. In cancer cells, however, telomerase is found throughout the cell, implying that the telomerase-shuttling system is impaired. Identification and manipulation of proteins normally involved in telomerase transfer could prove to be useful targets for anti-telomerase therapies[159]. Currently two clinical trials; one using a vaccine (GRNVAC1) and the other a lipidated drug (GRN163L) are underway to evaluate the efficacy of telomerase inhibitors[160].

p53

The *p53* gene is one of the tumour suppressor genes that normally prevent uncontrolled multiplication of abnormal cells and experimental findings from the last two decades have established a crucial role for wild-type p53 in intrinsic tumour suppression[161,162]. Upon stimulation (*e.g.*, by moderate levels of DNA damage), *p53* activates molecular processes that delays the cell cycle progression of proliferating cells and simultaneously stimulating DNA repair processes[163,164]. On the other hand, higher level of damage has been found to activate p53 mediated cell death pathway (typically apoptosis), a mechanism that is purported to be responsible for the prevention of carcinogenesis. During malignant transformation p53 or p53-pathway related molecules are disabled most often and a mutant form of p53 may not only negate the wild type *p53* function but plays additional role in tumour progression[165]. Nearly 50 per cent of all human tumours carry a mutated *p53* gene[165]. Clinical studies in patients with various types of cancer have shown that certain mutations in the *p53* gene are significant predictor of resistance to therapy[163].

Although p53 is not a typical cancer-specific antigen, its central role in the control of cell growth and apoptosis and frequent mutations in tumours make p53 a unique target for cancer therapy. Radiation and many of the anticancer drugs damage the DNA of cancer cells, triggering the action of the p53 leading to apoptosis. Hence, an intact wild type *p53* gene is essentially required to stimulate programmed cell death of a cancer cell in response to treatment. Investigations in several types of cancer have shown that the *p53* gene is a potentially useful biomarker for predicting prognosis and patient's response to therapy[165]. Experimental evidences show that either mutation in the gene or overexpression of the p53 protein can be used to predict many aspects of

prognosis and outcome of patients with various type of cancer.

Among the different approaches targeting p53, replacement gene therapies that have been explored extensively in recent years aims at restoration of p53 function in cancer cells by introduction of exogenous p53. Various protocols and vectors have been employed, including retroviruses, adenoviruses and vaccinia-derived vectors[166]. Recent studies have focused on adenoviral vectors, with Ad-p53, adenovirus serotype 5 carrying *wt p53* genes, as a model example. Although preliminary results were promising, recent clinical data failed to demonstrate anti-tumour activity in patients and some trials have indeed been discontinued[167]. New trials aim at a combination of gene transfer with chemotherapy or radiotherapy. In addition to the strategy of p53 reactivation in tumours, modulation of p53 activity in normal cells may protect them from the side effects of chemotherapy or radiotherapy. Several new compounds targeting p53 have entered clinical trials and therefore, p53-oriented therapy will be one of the major areas of intense investigations in the coming years. However, the approach of restoring of p53 function in tumour cells has been nearly questioned after a few contradictory results, which shows that prostate cancer cells are protected from ionizing radiation-induced DNA damage through activation of p53[168] and cells transformed with oncogenic tyrosine kinase BCR/ABL may actually benefit from activation of p53 upon DNA damage[165]. These observations have led to the reexamination of restoring p53 function in tumors as a therapeutic strategy.

Tyrosine kinase

Tyrosine kinases are a class of enzymes that regulate multiple cellular processes by acting primarily as important transducers of extracellular signals influencing diverse functions such as cell growth, differentiation, migration, and apoptosis that contribute to tumour development and progression. Many human tumours display aberrant activation of tyrosine kinases caused by genetic alterations that could be related to the malignant transformation[169]. The erbB or HER family of transmembrane tyrosine kinase receptors, especially receptors erbB1 (or EGFR) and erbB2 (or Her2/neu), has been identified as an important therapeutic target in a number of cancers. Her2/neu, is overexpressed in nearly 30 per cent of patients with aggressive breast cancer, while EGFR is overexpressed in several solid tumours[170]. Therefore, targeting protein tyrosine kinases as a therapeutic strategy has been very attractive and results from the recent clinical studies are indeed quite encouraging. Current approaches include blocking kinase-substrate interaction, inhibiting the enzyme's adenosine triphosphate (ATP) binding site and blocking extracellular tyrosine kinase receptors on tumour cells. Several tyrosine kinase inhibitors (TKIs) (*viz.*, gefitinib and trastuzumab) have already been approved as anti-cancer agents.

Histone deacetylases (HDACs)

Acetylation of proteins orchestra the dynamic interplay between various processes like repair of DNA damage, cell cycle arrest and apoptosis determining the cellular response to radiation and various chemotherapeutic drugs. This acetylation is catalyzed by histone acetylases (HATs) that uses acetyl-CoA as substrate and the acetyl group is transferred to the € amino group of certain lysine side chains within histones N-terminal tails and other nuclear receptor proteins thereby regulating chromatin remodeling and gene expression[171]. Chromatin remodeling during the regulation of gene expression is orchestrated by a concerted action of HATs and HDACs that condenses and decondenses the chromatin structure by acetylating and deacetylating histones and other nuclear receptor proteins. Further, HDACs appear to be closely associated with oncogenesis by regulating the expression of certain tumour suppressor genes leading to excessive proliferation and tumourogenesis[172]. HDAC have recently been among some of the attractive targets for cancer therapeutics, and HDAC inhibitors with diversified structures have indeed shown promising anti-tumour activity (cell cycle arrest, cellular differentiation and apoptosis) both *in vitro*[173] and *in vivo*[174]. Many of the HDAC inhibitors are currently under clinical investigation in a number of haematological malignancies and solid tumours[175,176]. Further, HDAC inhibitors are also being investigated as adjuvant together with other anti-cancer therapeutics[177]. It appears therefore, that HDAC inhibitors with pleiotropic actions in modulating multiple genes, signaling pathways and biological features of malignancy are useful in the treatment of cancers with multiple oncogenic abnormalities targeting the protein acetylation involved in the regulation of cell signaling[178].

PIN1

It is well known that the functional status of many proteins is regulated by kinase mediated phosphorylation

and other post-translational modifications. Recently, regulation of proteins beyond phosphorylation has been unraveled, which is in the form *Cis* and *Trans* isomerization (a post-phosphorylation event) of phosphoserine/threonine - proline peptide bonds at selective sites catalyzed by peptidyl-prolyl isomerase (PPIase), Pin1[179,180]. These conformational changes can have profound effect on the function of proteins, modulating their activity, phosphorylation status, protein-protein interactions, subcellular localization and stability. Overexpression of Pin1 has been reported in human breast cancer cell lines and tissues, and its expression closely correlates with the level of cyclin D1 (important cyclin required for cell proliferation) in tumours[181]. Pin1 overexpression not only confers transforming properties on normal mammary epithelial cells, but also enhances transformed phenotypes of Neu/Ras-transformed mammary epithelial cells and implicated in mitotic regulation[182]. In contrast, inhibition of Pin1 suppresses the Neu- and Ras induced transformed phenotypes or induces tumour cells into mitotic arrest and apoptosis[182,183]. Pin1 opens a new target for the development of specific therapeutics and has received greater attention as phosphorylated p53 is among the known substrates of Pin1[184,185]. Inhibition of Pin 1 through various approaches, such as mutations, deletions or expression of antisense, induces mitotic arrest and apoptosis in tumour cell lines[186]. It appears that Pin1 can be used as a diagnostic marker for the detection of the cancer or to stage the disease, albeit in only certain types of cancers.

Recent studies have shown that treatment of cells with pin1 inhibitor juglone delays the growth of various tumour cell lines[187], suggesting that inhibition of pin1 can be used as an approach for inhibiting tumour growth. We have recently identified potential Pin1 sites on topoisomerase IIα (a vital nuclear enzyme and mitotic protein) and shown that the two proteins functionally interact with each other resulting in the activation of topo IIα[188,189]. Moreover, using inhibitors of topo IIα (etoposide) and Pin1 (Juglone), we have shown that the combination (etoposide and juglone) may improve the therapeutic potential[188,190]. Pin1 appears to be an attractive target for diagnosis and therapy. Further understanding on the role of Pin1 in tumourigenesis is required before its use as a target for developing antagonists ensuring specificity, selectivity and safety.

Conclusion

Discovery and clinical application of new biomarkers, is expected to play a significant role in reshaping life science research and life science industry, thereby profoundly influencing the detection and treatment of many diseases and cancer in particular. Clinical oncology is poised to enter a new era in which cancer detection, diagnosis, and treatment will be guided increasingly by the molecular attributes of the individual patient, acquired from several different sources *viz.*, tumour tissue, host cells/tissues that influence tumour behaviour and body fluids. The resultant panel of biomarkers will not only help the detection and diagnosis, but also answer fundamental questions about biologic behaviour of tumours, resistance to therapy and sensitivity to therapy facilitating individualization of therapy, besides identifying individuals predisposed to cancer. The future of cancer therapy lie in the use of biomarkers that offer the potential to identify and treat cancer years before it is either visible or symptomatic. Exploring the presence of such markers that does not require the tumour tissue to detect them, but are secreted by cancer cells into the blood stream will not only facilitate easy detection without even minimal surgical procedure, but will also be candidates for population based screening.

Contemporary as well as upcoming genomic and proteomic technologies are quite promising in identifying new biomarkers, which can significantly enhance the efficacy of cancer management by facilitating the individualization of therapy targeting the patient specific molecular lesions and also by providing tools for predicting/monitoring of therapeutic response. Although the current understanding of signaling pathways has identified specific targets for developing newer drugs and therapeutic strategies, a comprehensive understanding of how the complex signaling networks function in intact cell is still required, to evolve strategies based on the genetic alterations in individual cancers.

Future challenges in the biomarkers using genomic and proteomic diagnostic technology include the development of complex mathematical algorithms to handle simultaneous analysis of many parameters (perhaps up to thousand even) to aid the diagnosis instead of a single parameter. Further, issues regarding quality control methods and procedures also need to be developed for using these markers with reliability and reproducibility. A comprehensive understanding of the relevance of each biomarker will be very important to efficiently diagnose the disease and provide appropriate direction in the multiple therapeutic alternatives currently available that is likely to benefit the unfortunate patients.

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