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Mini-review

Molecular markers for cancer prognosis and treatment: Have we struck gold?

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ABSTRACT

The last decade has witnessed an emerging role for molecular or biochemical markers indicating a specific cellular mechanism or tissue function, often called 'biomarkers'. Biomarkers such as altered DNA, proteins and inflammatory cytokines are critical in cancer research and strategizing treatment in the clinic. In this review we look at the application of biological indicators to cancer research and highlight their roles in cancer detection and treatment. With technological advances in gene expression, genomic and proteomic analysis, biomarker discovery is expanding fast. We focus on some of the predominantly used markers in different types of malignancies, their advantages, and their limitations. Finally we conclude by looking at the future of biomarkers, their utility in the tumorigenic studies, and the progress towards personalized treatment strategies.

1. Introduction

Cancer is a multistage process that often involves alterations and/or defects in major cellular pathways including DNA damage response (DDR), proliferation, senescence, angiogenesis and usually accompanied by an extended chromosomal instability [1–3]. In general, all cancers have their unique characteristics based on the tissue from which they arise. For example, glioblastomas often overexpress growth factor receptors on their cell membrane [4]. Abnormal mitoses and chromosomal abnormalities are also observed in cancer. It is this principle that we utilize in order to differentiate a malignant tissue over a healthy one for the purpose of diagnosis and perhaps even prognosis [2,5,6]. However, cancer continues to be a major cause of mortality despite decades of efforts. Part of the problem lies in the late detection of the disease. Therefore, it is imperative that we come up with new methods of detection and prognosis. An avenue that is currently being actively pursued in clinic as well as translational research is looking at various molecular and biological markers often called 'biomarkers'.

A biological marker is a characteristic that is objectively measured and evaluated in biological samples as an indicator of conditions like normal biological processes, pathogenic states, or pharmacologic responses to a therapy by a variety of techniques. Biological markers i.e. biomarkers may be utilized to study efficacy and evaluate safety, detect disease conditions, and monitor health status. Molecular markers can be further classified as diagnostic, prognostic, and predictive [7]. Diagnostic biomarkers can help in disease diagnosis. Prognostic markers that are utilized extensively by clinicians can be correlated with an endpoint regardless of therapy. On the other hand, predictive biological indicators predict outcome to a specific therapy. One molecular indicator can fall into all three classifications. For instance, estrogen receptor (ER) in breast cancer can be used as a diagnostic biological marker when it is used to define the potential site of the primary tumor in a metastatic cancer. The same biological marker can be prognostic when it is used as surrogate to conclude that breast cancer with high expression of ER usually have a better long-term survival. Finally, since it predicts response to hormonal treatment, it is a predictive marker. It should be noted that diagnostic, prognostic, or predictive properties of a molecular marker is best evaluated in a large randomized clinical trial with a control group. This is due to the confounding effects present in most single-arm treatment trials for the determination of biomarkers. Cancer biological markers, employed across the entire healthcare spectrum (from the cancer biological research laboratory to patient monitoring in the clinic), have contributed greatly to our current understanding of the heterogeneous nature of specific cancers, thus, leading to improvements in treatment outcomes [8]. Biological indicator based diagnostics have applications for establishing disease

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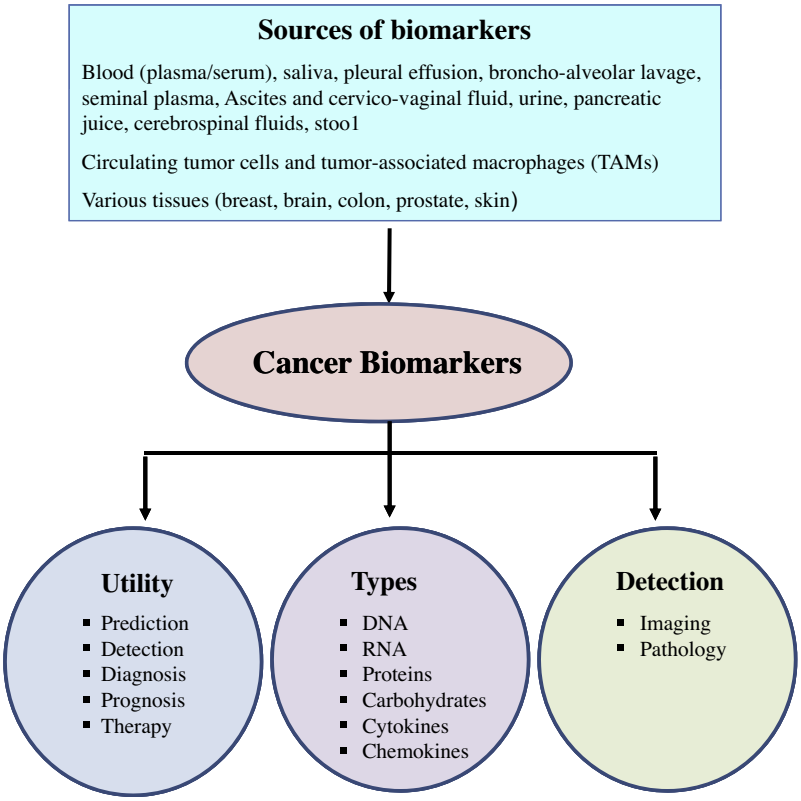

Fig. 1. Overview of cancer biomarkers: sources, types, and potential applications.

predisposition, early detection, cancer staging, therapy selection, identifying whether or not a cancer is metastatic, therapy monitoring, assessing prognosis, and advances in the adjuvant setting (Fig. 1).

Unfortunately, full adoption of cancer biological indicators in the clinic has to date been slow, and only a limited number are currently in routine use. Presently, a number of biological markers are being utilized in clinical trials. ER and progesterone receptor (PR) status are used to select patients for endocrine therapy, human epidermal growth receptor 2 (Her2) for treatment using Trastuzumab (Herceptin) [9,10]. Similarly, human chorionic gonadotrophin (HCG) is used to diagnose, stage, and monitor testicular cancer [11]. Prostate-specific antigen can aid in screening and diagnosing prostate cancer [12–14]. Alfa-fetoprotein is used as a biomarker in germ-cell hepatoma, lactate dehydrogenase in germ cell, carcinoembryonic antigen (CEA) in colon cancer, and thyroglobulin or calcitonin for thyroid cancer monitoring [15–17]. Cancer antigens (CAs) like CA19-9 are used in pancreatic cancer, CA15-3 in breast and CA125 in ovarian cancer [18–20]. Several major programs have been organized to facilitate the validation and assessment of cancer molecular markers alongside the established "standards of care" for cancer diagnosis and treatment. These programs are likely to be vital to increasing the rate of cancer biomarker adoption in the clinic setting. Further, the regulatory environment is progressing as more cancer biomarker products gain approval. Thus, the future of biological indicators in cancer therapeutics is promising.

2. Biomarker development: from bench to clinic

Molecular changes in tumors can be picked up utilizing various screening technologies like micro-RNA expression profiling, gene expression profiling, multiplex polymerase chain reaction (PCR), DNA micro-arrays, DNA sequencing, mass-spectrometry, genome hybridization, proteomic profiling, molecular imaging, assessing tumor specific antibodies and circulating tumor cells. Mutations can be screened using DNA sequencing, mass spectrometry based genotyping or mutation specific PCR [7]. Some mutations may be in DNA copy number which can be assayed using comparative genome hybridization to DNA micro arrays.

Another technique that has had recent success is proteomic or phospho-proteomic profiling based on mass spectrometry [21,22]. Proteomics is the large scale study of protein structure and functions [23]. A thorough understanding of the proteome, the structure and function of each protein and the complexities of protein–protein interactions, is critical for developing the most effective diagnostic techniques and therapy. Proteomics is used in studying specific protein biomarkers to diagnose disease. A number of techniques allow for the testing of proteins produced or hyper-regulated during a particular disease, which helps to diagnose the disease quickly [24]. Techniques include western blot, immunohistochemical staining, enzyme linked immunosorbent assay or mass spectrometry. Phosphoproteomics is a branch of proteomics that identifies, catalogs, and characterizes proteins containing a phosphate group as a post-translational modification [25]. Phosphorylation is a key reversible modification that regulates cell signaling networks by regulating protein function, subcellular localization, complex formation and, protein degradation. Phosphoproteomics tells us what protein or pathway might be activated because a change in phosphorylation status almost always reflects a change in protein activity. It also indicates what proteins might be potential drug targets. One of its biggest advantages is the rapid analysis of entire phosphorylation based signaling networks. Since the inception of phosphoproteomics, cancer research has focused on changes to the phosphoproteome during

tumor development since increasing amounts of data suggest that distinctive phosphoproteins exist in various tumors and that phosphorylation profiling could be used to fingerprint cancers from different origins [26–28]. The kinks in the process have to be sorted before the technique is embraced widely. Isolation methods such as anti-phosphotyrosine antibodies do not distinguish between direct and indirect interactions. Some relevant proteins will likely be missed since no extraction condition is all encompassing. It is possible that proteins with low stoichiometry of phosphorylation, in very low abundance, or phosphorylated as a target for rapid degradation will be lost. We must proceed with caution before fully utilizing this process in clinic.

Metabolomic profiling based on mass spectrometry is another promising technology being utilized for tumor characterization [29–32]. Specifically, metabolomics is the systematic study of the unique chemical fingerprints that specific cellular processes leave behind [33]. The process involves the study of their small-molecule metabolite profile. The metabolome represents the collection of all metabolites in the subject of interest i.e. a biological cell, tissue, organ or organism, which are the end products of cellular processes. Even though messenger RNA (mRNA) gene expression data and proteomic analyses fail to clearly depict the events at the cellular level, metabolic profiling can give an instantaneous snapshot of the physiology of that cell. In general, one of the challenges of systems biology and functional genomics is to integrate proteomic, transcriptomic, and metabolomic information to give a more complete picture of living organisms. All these technologies are based on assessing changes in the content or sequence of DNA, the mRNA or microRNA produced via transcription, protein products of translation, or the metabolic end products. On the other hand, some of the non-invasive strategies for molecular profiling of cancer include analyzing circulating tumor cells or DNA in plasma, molecular imaging, and assessing tumor specific antibodies. Metabolic biomarkers include molecules such as alanine, lipids, poly-unsaturated fatty acids, choline containing metabolites, glycine, lactate, Myo-inositol, nucleotides, and Taurine. These molecules are critical for various processes such as osmoregulation, glycogen metabolism, and hypoxia. One limitation that needs to be considered is that the number of transcripts present in the transcriptome is far greater than the existing metabolites in a human tissue. Therefore, a given metabolite pattern can reflect several genomic changes and thus needs to be approached with caution. We refer the readers to Griffin et al. for a comprehensive review on metabolic markers of cancer [34].

Recent technological advances allow for more rapid identification of biomarkers. Multi gene arrays, like Oncotype DX, can provide a far more detailed picture of an individual cancer by looking at multiple markers rather than only a few [35]. The Oncotype DX breast cancer test measures the expression of 21 genes in a sample of tumor tissue. The test generates a recurrence score that provides information about the likelihood of cancer recurrence and the likelihood of chemotherapy benefit in women with early-stage, estrogen receptor-positive breast cancer. Similar tests are also available for colon cancer. These tests are a step towards personalized cancer therapy.

Although a number of biological markers are currently available, they are limited in their efficacy. Table 1 summarizes the molecular markers, their advantages, as well as their limitations. In spite of all the biomarkers that are proposed in research journals every year, potential predictive or prognostic biological indicators are often elevated or detected at a late stage and thus are not feasible. Thus, timing is very critical for the optimum biomarker depending on its purpose. In addition, biological markers can be expensive to analyze and human error in analysis should be considered as well. While normal range is difficult to establish, biomarkers can still aid in objective assessment of the disease (Fig. 1).

3. Sources of biomarkers

Fluid based assays can be repeatedly performed if necessary, are convenient, minimally invasive, and cost-effective. They can be used to detect tumors all over the body like circulating tumor cells and macromolecules originating from tumor cells (DNA, proteins, RNA, microRNA, lipids). Blood (plasma), urine or saliva can be great sources of biomarkers (Fig. 1) [36–38]. Peripheral blood cells of breast, skin, and lung cancer patients have unique mRNA expression which can also be used for diagnosis [39–41]. Circulating tumor cell (CTC) detection utilizes the epithelial cell surface epitope EpCAM, the caveat being that although it is an excellent marker for epithelial cells, it is not expressed on all cancer cells [42,43]. In addition, guidelines from the American Society of Clinical Oncology currently do not recommend using (CTCs) for making clinical decisions because of a lack of evidence for an established, solid methodology for accurate detection of small number of CTCs (like in primary breast cancer) and with a proven clinical relevance for therapy [42]. Other advances include use of gene mutations (e.g. p53 in urine of bladder cancer patients and Ras mutations in stool of colorectal cancer patients), antibodies and filtrations based on cell size or density [44,45]. Urine analysis is used in prostate cancer detection while saliva has been successfully used in breast and oral cancer detection [46]. Biomarkers can be found in cerebrospinal fluid in brain cancer while nipple aspirate fluid, breast cyst fluid or ductal lavage can all be analyzed in breast cancer [47,48]. Cervicovaginal fluid can help in diagnosing cervical and endometrial cancer while pleural effusion or broncho-alveolar lavage can be analyzed in lung cancer [49,50]. Ascites fluid can help in diagnosing ovarian cancer and seminal plasma for prostate or testicular cancer [11,18,51]. The utility of pancreatic juice as a source of biomarker in pancreatic cancer needs further validation [52].

4. Epigenetics and molecular markers of cancer

In general, both genotoxic and non-genotoxic mechanisms are known to be implicated in malignant transformation with genotoxic mechanisms involving changes in genomic DNA sequences (leading to mutations) while non-genotoxic mechanisms modulating gene expression directly [1]. As such, the epigenetic pathway (involving changes in DNA methylation patterns and histone modifications) is considered to be a non-genotoxic mechanism capable of modulating gene expression and thus promoting malignant transformation. Thus, it is of paramount importance to be able to determine such epigenetic modifications in a way that we can expand on cancer biological marker development with clinical relevance [1]. In fact, epigenetic molecular marker development has been one of the hottest areas in cancer research because of their ability to contribute to cancer diagnosis and/or prognosis. More specifically, DNA methylation biomarkers hold a great deal of promise due to their high sensitivity and specificity. In addition, DNA methylation biological indicators have been beneficial because of their: (1) chemical stability, (2) easy extraction from a variety of samples, (3) comparability to absolute reference points (methylated vs unmethylated) and (4) persistence throughout the duration of tumor growth and thus allowing for consistent methylation signals. Thus, changes in DNA methylation patterns could potentially serve as biomarkers with clinical significance for various cancer types. These are discussed in detail below:

4.1. Promoter DNA hypomethylation

This particular modification has been observed for a number of genes including: *H-ras* (prostate and thyroid cancers), cancer-testis antigen gene (CAGE) (prostate, breast, lung and laryngeal cancers),

Table 1
Most important biomarkers for cancer prognosis and therapy based on current knowledge.

Cancer	Biomarker	Advantages	Limitations	References
Prostate	Prostate-specific antigen (PSA)	• Easy to analyze in body fluid • FDA approved to monitor recurrence in patients who have a history of prostate cancer • Convenient • Minimally invasive • Low-cost	• Cannot distinguish between benign prostate conditions and cancer • Does not equate to saving lives due to differences in growth rate and aggressiveness of different tumors • False-positive results lead to additional medical procedures that have potential risks and significant financial costs and can create anxiety for the patient and his family • Most men with an elevated PSA test result turn out not to have cancer; only 25–35 percent of men who have a biopsy due to an elevated PSA level actually have prostate cancer • False-negative test results lead to failure in detecting the disease prior to significant progression	[12,134,135]
Breast	Estrogen receptor (ER)/ progesterone receptor (PR)/ human epidermal growth factor receptor (HER2)	• Convenient • Minimally invasive • Low-cost • Has had success in clinic to design treatment/predict response	• Lack of standardized method to determine positivity • Sampling and consistency concerns	[10,101,136–138]
	CA-15-3 or CA-27-29	• Serum based glycoproteins for monitoring breast cancer • Antibody-based immunochemical test • Ease of use	• Lack the specificity and sensitivity for use in early detection	[139]
	Cytokeratins	• Protein markers for prognosis • Serum based and thus easy to analyze	• Dependent on sample preparation and antibody	[140–144]
	Oncotype DX	• Multi-gene expression assay • Quantitative assessment of the likelihood of chemotherapy benefit and distant recurrence • High-throughput assay • Sensitive, specific, and reproducible	• Invasive-tumor tissue needs to be extracted from patient	[35,145]
Pancreatic	CA19-9	• Minimally invasive-can be tested using serum • Can be used to monitor response to therapy	• Not useful for early diagnosis-poor sensitivity and thus fails to detect the disease early • False positive-levels may also increase in inflammatory diseases of the pancreas like chronic pancreatitis • Not useful for mass screening	[20]
Lung	Carcino-embryonic antigen (CEA), Cytokeratin 19 fragment (CYFRA21-1), tumor M2 pyruvate kinase	• Can help predict response to therapy • Protein based biomarkers • Convenient • Minimally invasive • Low-cost	• False positive-levels may also increase in chronic, benign, or inflammatory diseases of the lungs • Not specific to lung cancer	[146,147]
	Mutations in the EGFR pathway	• Erlotinib, gefitinib, cetuximab, panitumumab have been used successfully to treat lung cancer harvesting mutations • Can help predict response and design therapy	• A number of mutations are possible	[105,146,148–152]
Colon	CEA	• Can be used to gauge response to therapy • Convenient • Minimally invasive since it can be detected with a simple blood test	• Not specific to colon cancer • False positives in inflammatory diseases • Both benign and malignant cancers can have a positive result • Elevated mostly in the late stage of cancer • Variability in testing	[153]
	Adenomatous polyposis coli (APC)	• Non-invasive • Convenient • Low-cost	• Not all patients have high mutant APC DNA in fluids	[154–156]
	Microsatellite instability (MSI)	• Minimally invasive • Convenient • Low-cost	• Can only be used in combination with other biomarkers to diagnose the disease • Samples vary widely in tissue quantity (e.g. fine needle biopsy vs. resection specimen), quality (e.g. fresh frozen vs. formalin-fixed for 72 h), and heterogeneity of tumor and non-tumor tissue • Variability in testing qualities • Difficult to determine clinically relevant threshold	[157,158]
	Oncotype DX	• Multi-gene expression assay • Quantitative assessment of the likelihood of chemotherapy benefit and distant recurrence • High-throughput assay • Sensitive, specific, and reproducible	• Invasive-tumor tissue needs to be extracted from patient	[159]
	K-Ras mutation	• Mutated in about 40% of patients	• Not consistent in predicting response to therapy	[157,158]

(*continued on next page*)

Table 1 (*continued*)

Cancer	Biomarker	Advantages	Limitations	References
		• Observed early in tumorigenesis • Mutations can help predict response to therapy • Is currently used in diagnosis and treatment		
Head and neck	HPV	• HPV16 integration is a significant marker of favorable prognosis and response to therapy for head and neck cancer • Offers diagnostic, prognostic, and therapeutic opportunities • High sensitivity and can pinpoint location of cancer	• Geographic variability in patient status • Poor specificity	[160]
	EGFR	• EGFR-amplification and overexpression is a poor-prognostic indicator • Can be used to design therapy	• Variability in testing	[161]
Leukemia	Philadelphia chromosome	• Excellent marker for chronic myeloid leukemia (CML) • Unique and consistent phenotype		[162]

X-inactive specific transcript (*XIST*) (prostate cancer), *Erythropoietin* (EPO) (prostate and breast cancers), *Maspin* (ovarian, pancreatic and lung cancers), *γ-Synulcein* (ovarian and breast cancers), *c-myc* (breast and lung cancers), *Urokinase-type plasminogen activator* (breast cancer), *S100P* (pancreatic cancer) and *Melanoma-associated antigen A* (*MAGE-A*) (lung cancer) [53].

4.2. Promoter DNA hypermethylation

This modification has been associated with altered expression of critical genes associated with various cancers including: *Breast cancer susceptibility protein 2* (*BRCA2*: prostate, breast, pancreatic and ovarian cancers), *Breast cancer susceptibility protein 1* (*BRCA1*: ovarian, pancreatic and breast cancers), *Von Hippel–Lindau tumor suppressor* (*VHL*) and *p53* (breast cancer), *P16INK4a*, *H-cadherin*, *Death-associated protein kinase 1* (*DAPK1*), *MDM2*, *p53* (lung cancer), *p14ARF* (lung, esophageal and colorectal cancers), *hHML1* (colorectal cancer) and *RASSF1A* (lung and nasopharyngeal cancers) [53].

On the other hand, besides changes in DNA methylation patterns, the chromatin has also been shown to regulate transcriptional activity. To this end, any modifications in core histone proteins (e.g. H2A, H2B, H3 and H4) can have an impact in the activation and/or repression of transcription. Such modifications can include methylation, acetylation, deacetylation, phosphorylation, ubiquitination, etc. A number of these modifications have been observed in various cancers and contribute to their pathophysiology. Some of these may include the following: (1) Histone acetylation and/or deacetylation observed in breast, prostate, colon, testicular, renal and pancreatic cancers, (2) Histone demethylation observed in breast, prostate, colon, testicular and esophageal cancers, (3) Histone H3 lysine 27 tri-methylation observed in breast, ovarian, colon and pancreatic cancers, (4) Histone H3 lysine 9 and/or Histone H4 lysine 20 tri-methylations observed in breast, lung and hepatocellular cancers, (5) Histone H3 lysine 4 methylation observed in breast, ovarian, colorectal and hepatocellular cancers.

Overall, early detection is the key in improving survival among cancer patients. However, due to the limitations of the current biological markers, there has been an active pursuit for new markers with good sensitivity for early-stage disease and the ability to differentiate between the cancer itself and other benign conditions. For instance, although breast cancer is one of the most commonly diagnosed cancers among women in the United States, many of these patients end up being over- or under-treated for the disease because the lack of reliable biomarkers. In addition, survival rates of pancreatic cancer patients have not improved dramatically despite routine use of chemotherapy and radiotherapy implying an urgent need for novel therapeutic approaches. While the failures of targeted therapies in the clinic are discouraging, new therapeutic targets hold promise for the future management of the disease. Thus, efficacious molecular biological markers will be a step in the right direction. Moreover, markers to identify lung cancer patients who may benefit from targeted therapy have currently seen significant development. Translational research and a better understanding of the molecular basis of cancer has allowed for the identification of a number of potential molecular targets. With the development in genomics and proteomics, there has been great advance in the discovery and development of lung cancer indicators. In the case of colorectal cancer, it is often diagnosed at a late stage with concomitant poor prognosis as expected. Though prognosis is greatly improved with early detection, the invasive, generally unpleasant, and inconvenient nature of current diagnostic procedures limits their applicability. To this end, DNA methylation, histone modifications and proteomic profiling could all potentially serve as clinical biomarkers in all the above-mentioned cancer types [53,54]. Finally, progression of any cancer is accompanied by genetic alteration(s) which leads to altered protein structure and function. In the case of head and neck cancers, their association with human papilloma virus (HPV) has been solidified over the last few years [55–61]. Interestingly, these cancers exhibit a better prognosis and appear to respond better to chemo-radiation. Saliva or serum of head and neck cancer patients can be analyzed for: (1) *p53*, epidermal growth factor receptor (EGFR) and HPV status, (2) microsatellite alterations and perhaps even and (3) epigenetic modifications. Nevertheless, further research is warranted prior to implementation of these molecular markers in a clinical setting.

5. DNA repair and molecular markers of cancer

Cells are under constant assault from a variety of insults that threaten the integrity and fidelity of the genome. These attacks can lead to DNA lesions which can block genomic replication, transcription and cause mutations. One ubiquitous threat to the genome is from a class of compounds known as reactive oxygen species (ROS), which can indiscriminately react with biological molecules such as proteins, lipids, and DNA. This results in a variety of oxidation products which are a threat to genome stability.

More critical are the oxidatively induced clustered DNA lesions (OCDLs), defined as two or more oxidative lesions present within 10 bp of each other. ROS can be produced by both endogenous and exogenous sources [62]. Exposure of cells to exogenous environmental agents including ionizing radiation, light, chemicals, and metals as well as cellular metabolism including mitochondrial ATP generation can result in ROS production and inflammation [5,6]. However, ROS also serve a variety of critical cellular functions and optimal ROS levels are maintained by multiple cellular antioxidant defenses. Attempted simultaneous repair of these lesions can lead to formation of DSBs. The concept of clustered DNA damage has gained particular attention in recent years since these lesions are resistant to repair and can cause increased mutagenesis [63–65] and/or chromosomal instability [66–68]. In addition, high levels of OCDLs have been detected in a variety of tumors and human cancer patients [69,70] as well as *in vivo* as the result of tumor growth in mice [71,72]. Damage persisting for an extended period of time increases the probability of encountering a replication fork thus increasing the risk of replication errors, apoptosis, or DSB formation.

Cells have evolved intricate mechanisms, collectively termed the DNA-damage response (DDR), to detect DNA lesions, signal their presence, initiate cell cycle checkpoints, execute repair of DNA damage, and activate programmed cell death to avert or resolve any potential damage incurred. This is critical for maintaining genomic integrity and for preventing cancer development [73]. Several repair pathways are in place to repair the damaged DNA and avoid passing the damaged DNA onto the progeny cells. These repair pathways include base excision repair (BER), nucleotide excision repair (NER), double strand break (DSB) repair via homologous recombination (HR) or non-homologous end joining (NHEJ), mismatch repair (MMR), several sub-pathways (like B-, D-NHEJ), and an interplay between these traditionally outlined repair mechanisms [74–76]. As little as one unrepaired DSB can be lethal to the cell. Oxidative DNA lesions can be efficiently repaired by BER or NER. Increase in ROS levels beyond the cellular equilibrium can overwhelm the cell's DNA repair capacity and lead to the accumulation of oxidative DNA damage products including OCDLs, which are more difficult to repair than individual isolated DNA damage products. Taking the significant cross-talk between the repair processes into account, it is truly fascinating how a cell decides which path to follow. In addition to the DNA repair pathways, multiple checkpoints are also put in place throughout the cell cycle that detect DNA lesions and halt further progression of DNA replication and cell division. Checkpoints can arrest the cell either transiently or permanently (senescence), as well as activate specific DNA repair pathways in response to DNA damage. Deficiency in DDR often results in heightened sensitivity towards DNA-damaging agents which can be exploited for cancer therapy. Dysregulated DNA repair has frequently been implicated in tumorigenesis and deficiency in DNA repair genes is associated with high susceptibility to cancer. Since DNA damage and repair pathways can be targeted to improve tumor response to therapy, molecular markers for disease aggressiveness, prediction of treatment outcomes, and tracking disease progression are critical. This information can open up the possibility of pursuing either more aggressive or less aggressive treatment strategies involving inflammation and oxidative stress [77].

One of the first responders to DSB is ATM, a cell cycle regulator protein. The MRN complex, consisting of RAD51, MRE11, and NBS1 is responsible for recruiting ATM which then phosphorylates CHK2 and MDM2, halting cell cycle progression to allow for repair [78]. ATM has been suggested as a biomarker and prognostic factor for several different types of cancers including HNSCC and non-small cell lung cancer and specifically in lung cancer, ATM expression in tumor compared to normal tissue has been linked with worse overall survival [79]. Interestingly, in breast cancer, a high ATM gene expression level correlated with favorable prognosis [80]. These observations support the utility of analyzing ATM as a prognostic factor for cancer patients.

Other critical repair proteins are BRCA1 and BRCA2. BRCA1 has been reported to be involved in both HR and NHEJ-mediated DSB repair [81]. These proteins are critical for maintaining genomic stability by promoting efficient and precise repair of DSBs. BRCA2 is involved in regulating the function of RAD51 whereas BRCA1 has a broader role upstream of BRCA2, participating in various cellular processes in response to DNA damage, including modulation of cell death. The DNA repair defect associated with mutations in BRCA1 or BRCA2 can be exploited to develop new targeted therapeutic approaches for cancer occurring in mutation carriers. Incidence of breast cancer has been strongly associated with the BRCA2 Met/1915Thr homozygous polymorphic variants, whereas the heterozygous variant has been associated with significant reduction in breast cancer risk. Moreover, interaction between the BRCA2-Met1915Thr Thr/Thr and BRCA2-Met784Val Met/Met homozygous variants increased the risk for breast cancer. Thus, the Met1915Thr polymorphism in the BRCA2 gene may be a reliable independent marker of breast cancer [82]. Another pivotal BER protein, Poly(ADP-ribose) polymerase (PARP) has been implicated in a variety of cancers and inflammatory diseases since its activation induces cell death and promotes inflammatory responses [83]. PARP inhibitors are in clinical trials for cancer therapy for HR deficient cancers. Interestingly, disruption of PARP inhibits HR-dependent repair by suppressing expression of BRCA1 and RAD51 due to increased occupancy of the promoters by repressive E2F4/p130 complexes [84]. Thus, BRCA1, BRCA2, and Rad51 are potential biological markers for cancer prognosis and treatment [85].

53BP1 is a mediator that relays signals from DNA damage sensors and activates various effectors for the DNA repair and cell survival. Expression of 53BP1 has been correlated with tumor stage, lymphovascular invasion and poor clinical outcome in lung adenocarcinoma [86]. Not surprisingly, increased 53BP1 expression correlates with elevated drug resistance and thus may help predict the prognosis for lung adenocarcinoma and other cancers such as breast. BRCA1-mutated tumors are prone to genomic instability, mainly as a consequence of impaired HR. These germ-line mutations in BRCA1 result in predisposition to breast and ovarian cancer. 53BP1 has been reported to be essential for sustaining the growth arrest induced by BRCA1 deletion. Depletion of 53BP1 abrogates the ATM-dependent checkpoint response and G2 cell-cycle arrest triggered by the accumulation of DNA breaks in BRCA1-deleted cells. Additionally, reduced 53BP1 expression in subsets of sporadic triple-negative and BRCA-associated breast cancers has also been reported [87]. These findings suggest that 53BP1 can potentially be utilized as a predictive marker for response to agents that target DNA repair deficient cells, such as PARP inhibitors.

The PARP family of proteins is critical for DNA repair and is responsible for repairing single strand break (SSB) nicks in the DNA. These SSBs can be converted to DSB during DNA replication. Failure to repair the damage leads to cell death [88]. Activation of PARP is an immediate cellular response to metabolic, chemical, or radiation-induced SSB lesions. On detection of a SSB, PARP binds to the DNA and begins the synthesis of poly(ADP-ribose)chain (PAR) as a signal for the other DNA repairing enzymes and following successful repair, PAR chains are degraded via PAR glycohydrolase (PARG). PAR functions as a docking polymer for a variety of transcription factors, chromatin, DNA replication and repair proteins, e.g. NFκB, PAX 6, AP 2, histones, XRCC1, Ku, DNA polymerase, DNA-Pkcs, ligases, condensins, and DNA topoisomerases thus modulating gene regulation, chromatin structure, DNA replication and repair. PARylation acts as an early DDR post-translational modification that recruits repair proteins to the DNA damage site. With

the inhibition of PARP with novel drug inhibitors like Veliparib (ABT-888) and Olaparib (AZD2281) in combination of DNA damaging agents like radiation and chemotherapy drugs like temozolomide, the cell is flooded with DNA damage as a cumulative effect of various DNA repair deficiencies including BER and NHEJ [89,90]. This is a potential treatment strategy for cancer patients. Besides, the potential use of PAR and PARP as potential biomarkers for response was investigated in recent clinical trials [91,92]. Low-expression of several DNA repair proteins in triple negative breast cancer was associated with worse progression-free survival. The study showed that patients can be further stratified into recurrence risk categories using a panel of antibodies and, thus, PAR/PARP biological markers have prognostic value. Therapeutic regimens in oncology involve DNA-damaging agents and so these DNA repair molecular markers can be utilized to predict tumor response to therapy as well [92].

Another important family of proteins involved in DNA repair is the EGFR. EGFR plays an essential role in carcinogenesis by modulating a number of cellular processes, including cell proliferation and survival, differentiation, angiogenesis, and DNA damage response and repair [93–96]. More specifically, with regards to DNA damage response, EGFR has been shown to translocate to the nucleus and interact with DNA-PK to activate NHEJ-mediated DNA repair [89,97,98]. Activated EGFR can also increase Rad51 foci and expression levels to regulate HR-mediated repair [99]. Thus, the EGFR family of proteins has been targeted in cancer therapy [93–96]. Inhibition of EGFR blocks its nuclear translocation, interaction with DNA-PK, and subsequently downregulates DNA repair via both NHEJ and HR-mediated repair pathways [89,97,98]. EGFR overexpression or mutation has been observed in many different tumors, including head and neck, esophageal, lung, renal cell, prostate, bladder, cervical, ovarian, breast, glioblastoma, colorectal, and pancreatic [100,101]. For example, in approximately 80–100% of squamous cell carcinoma of the head and neck (HNSCC), aberrant expression and dysregulation of EGFR is seen which has been correlated with worse outcomes in both overall survival and locoregional recurrence [93,102,103]. EGFR expression in non-small cell lung cancer is associated with a poorer prognosis as well [104]. Additionally, EGFR mutations in lung cancer have been shown to correspond to response to tyrosine kinase inhibitors (TKIs) such as erlotinib. This has led to the design of new clinical trials which stratifies patients with EGFR mutations to potentially receive these inhibitors as first line therapy [105–107]. Finally, in colorectal carcinoma, EGFR expression has been associated with advanced stage but its impact on prognosis has not been clarified yet [108].

Another DSB repair protein that has been implicated in cancer detection and prognosis, the γ-H2AX, is a member of the H2A family of histones which modulate DNA structure, undergoes phosphorylation by DNA-PK, ATM, and ATR proximal to DNA DSBs [109–111]. Elevated levels of γ-H2AX are observed in many different types of cancers and thus may be used as a marker of treatment efficacy as well as tumor recurrence [111,112]. Indeed, γ-H2AX has been tested in clinical trials as a pharmacodynamic marker and has yielded promising results as a sensitive and accurate marker to gauge response to therapy [85,113,114]. Furthermore, hair follicles were shown to be effective surrogate tissue for of γ-H2AX analysis. This very promising approach is currently being investigated in phase I clinical trials. Ku80 (XRCC5) serves as a docking protein for DNA-Pkcs which signals other events for NHEJ-mediated DSB repair [115]. Ku80 has previously been linked to resistance to chemotherapy and radiation in head and neck cancer [116,117]. Recently, Ku80 overexpression was shown to be a predictor of locoregional failure and mortality after radiotherapy in head and neck cancer [118].

Excision repair cross-complementation (ERCC) is a family of gene products responsible for NER-mediated repair. Large bulky DNA adducts in part created by chemotherapeutics as well as ionizing radiation are repaired via NER-mediated repair. There are multiple members of this family. Polymorphisms of ERCC1/ERCC2/ECRR4/ECRR5 have been associated with increased risk for development of several different cancers including lung, esophageal, endometrial, bladder, blood, and central nervous system [119–121,122 2005 #12313, 123,124]. NER capacity has been finally associated with resistance to platinum based therapy as well [125].

A number of other biological markers have been suggested. Undoubtedly inflammation markers hold a very important role in the pool of 'prognostic markers' since tumor microenvironment and inflammation have been associated for many years [126,127]. The infiltration of tumor cells and tumor-associated macrophages (TAMs) and other immune response cells like the white blood cells, cytokines like the tumor necrosis factor (TNF), interleukins IL-2 and IL-6 and chemokine ligands (CCLs) like CCL2, CCL18 and CXCL8 are considered as promising markers for tumor presence or growth in the organism [2,6,126,128]. Related to the above, very recently using proteomics, Wang et al. found that the combined use of chemokines CCL18 and chemokine CXC motif ligand 1 (CXCL1) can prove reliable biomarkers for ovarian cancer with a high sensitivity of 92% and a specificity of 97% [129]. Survivin, cytokeratin, and uroplakin levels can be analyzed in the urine or serum as markers of bladder cancer. CD44, *p53* and microsatellite alterations are also great markers of the disease. On the other hand, tyrosine kinase, signal transducer and activator of transcription (STAT) family of proteins, and breakpoint cluster region (BCR)-Abelson murine leukemia viral oncogene homolog (ABL) family of proteins have been instrumental biomarkers in improving leukemia treatment and survival rates. BCR-ABL is a fusion protein that is present in nearly all chronic myeloid leukemia (CML) cells (Philadelphia chromosome positive CML). It is inhibited by imatinib and it has had great success in improving the quality of life for patients suffering from leukemia. Unfortunately, mutations in the BCR-ABL region have been observed following treatment with ABL inhibitors and a 2nd generation drug is being utilized in that setting. Constitutively active STAT5/STAT3 signaling is also seen in a population of leukemia patients. Utility of other biomarkers warrants further validation.

Finally, a number of biomarkers have been developed to predict tumor radiosensitivity. These may include tumor specific oncogene alterations like EGFR over-expression, changes in tumor suppressor genes, and DNA repair genes. Both endogenous and exogenous tumor hypoxia markers like HIF1α, CA IX, osteopontin, partial pressure of oxygen, and VEGF can be used to predict radiotherapeutic response. We refer the reader to some excellent reviews of biomarkers for predicting response to radiotherapy with or without concurrent chemotherapy [130–133].

A number of challenges prevent the clinical translatability of scientific discoveries such as novel biomarkers. Funding opportunities, rules and regulations and availability of resources are limitations to consider. Issues with pharmacokinetics, pharmacodynamics and delivery mechanisms also prop up while translating bench work to the clinic floors. Thus, what may show promising results *in vitro* may not hold up *in vivo* (in humans). Further work is thus necessitated in this field.

6. Conclusions

One of the goals of molecular markers is to identify a few cancer cells in the ocean of normal cellular tissue. Since clinical samples are a heterogeneous mixture of normal and cancer cells, the sensitivity and specificity of the biomarker utilized to identify cancer cells are important factors to consider. Several factors need to be

considered before the initiation of biomarkers in clinic. In order to reveal the usefulness of potential biomarkers, they need to be studied thoroughly in a large set of clinical samples including other cancer types and other diseases conditions, especially inflammatory diseases. One specific biomarker cannot predict or monitor cancer, necessitating the combination of several good biomarkers with quantitative information, to be beneficial for therapy. Determination of sensitivity and specificity of the biomarker requires careful extensive testing and validation using a large number of tumor and normal cells. Perhaps because they fail to go through this rigorous testing, a large number of biomarkers that are published in thousands of research papers every year ultimately fail to develop into reliable markers and so are not used routinely in clinical settings. Most medical journals have established very specific criteria for the publication of papers claiming to have identified a novel biomarker and these criteria are designed to improve the rigorous testing and usefulness of findings. Clinical trials should be designed to incorporate assays to develop biological and correlative markers to overcome this issue. Gene expression and proteomic profiles of patient response prior to and after treatment will be invaluable. Combining molecular markers with procedures like imaging and histology helps to bring more reliability to detection, diagnosis and monitoring of cancer. Cooperation between molecular biologists and clinical researchers will aid in the development of novel and effective biomarkers. Although we have come a long way, further development of molecular markers are needed as we continue to improve in terms of cancer prognosis, diagnosis and treatment monitoring and to finally strike gold.

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