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Invited Review

Biomarkers in Cancer: an Overview

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ABSTRACT

Traditional tumour markers such as carcinoembryonic antigen (CEA) that have been used for screening gastrointestinal neoplasia for many years are not specific. However, these markers are useful after diagnosis to monitor progress of the disease and recurrence. New biomarkers are constantly being developed to identify individuals with risk of cancer for early detection, to determine prognosis, to detect recurrence, to predict drug responses and to monitor response to treatment. There are several issues involved in the discovery of biomarkers and their development for clinical applications. This article provides a basic overview of the classes of biomarkers, the current status of molecular profiling and discusses the opportunities as well as challenges ahead to improve biomarker development.

Keywords: Biomarkers, risk, prognosis, recurrence, response to treatment

INTRODUCTION

According to the National Institute of Health USA Biomarker Working Group, a biomarker is defined as a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.[1] Hence, a biomarker is used not only to associate it with an increased risk of disease but also to monitor progress of disease as well as response to drug treatment.

For the sake of simplicity, we can group biomarkers in cancer under three major categories, namely, proteins, DNA or RNA-based biomarkers. Examples of these biomarkers are shown in Table 1.

These biomarkers can be used for risk assessment, screening, differential diagnosis, prognosis, treatment selection and for monitoring the course of the disease.[1,2] Traditional means of prognostication such as using TNM staging has been used in the last 50 years. Prognostically, stage I is better than stage II and stage II is better than stage III. Recent developments in expression profiling by microarrays have provided evidence that molecular markers can further improve prognistication.[3] Another important application of biomarkers is that it addresses the question of how to treat the cancer as well as influence the decision as to which drugs to use. Of course, it must not be forgotten that diagnostic imaging such as X-rays, computerised tomography (CT) scans, magnetic resonance imaging (MRI) and positron emission tomography (PET) also play an important role in diagnosis and monitoring

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Table 1. Categories and examples of biomarkers

Category	Examples
Protein	Secreted antigens: carcino-embryonic antigen (CEA) , prostate- specific antigen (PSA) Receptors: oestrogen receptor (ER), human epidermal growth factor-2 (HER-2)
DNA	Gene mutations, single nucleotide polymorphisms (SNPs), chromosomal translocations (*bcr-abl*), increased DNA copy number, microsatellite instability, epigenetic alterations
RNA	RNA expression profiling by microarrays

disease. Similarly, the use of pap smears and mammograms are still routinely used to assist in the detection of cervical and breast cancers, respectively.

RISK ASSESSMENT AND SCREENING MARKERS

Biomarkers for risk assessment and screening must be accurate and specific. Screening tests must be specific and not yield false positive and false negative results. However, there are some biomarkers that are not feasible for screening, for example, serum carcinooembryonic antigen (CEA) is used as a tumour marker for gastrointestinal cancers but is not specific. The sensitivity and specificity of serum CEA (>5 ng/ml) in colorectal cancer has been reported to be 39% and 90%, respectively.[4] It can be increased in breast cancer and in some benign conditions, hence, it is not the definitive test. However, once colon cancer is diagnosed by other invasive surgical procedures and treated, increased CEA is strongly suggestive of recurrence.[4, 5] This is also true for alpha-foetoprotein (AFP), whereby, the sensitivity and specificity of AFP was found to be 64% and 91%, respectively.[6] Prostate-specific antigen (PSA) has made an impact on detection of prostate cancer. The sensitivity and specificity of serum PSA has been reported to be 44% and 94%, respectively.[7] Although PSA level increases in testicular cancer following diagnosis, PSA can, however, be elevated in both benign and malignant prostatic diseases.[8] Thus, caution should be taken in interpretation of increased serum PSA test results.

GENETIC TESTS IN CANCER

There are a number of gene mutations that have been identified to be associated with cancer risks. The classical examples are the *BRCA1* and *BRCA2* genes for hereditary ovarian and breast cancer and *APC* for familial adenomatous polyposis.[2] Perhaps, genetic testing has the potential to reduce mortality, for example, there could be chemo-preventive measures to take, a person could perhaps have a higher motivation to change his/her diet or lifestyle such as 'quit' smoking. But do these measures really reduce the risk? If tested positive, the psychological impact could be greater, the individual would have to make behavioral changes, be more stressed out and the social consequences have to be borne by the individual as well as family members. If the genetic testing result is negative, it does not

mean that the individual is not going to get cancer; the risk is just the same as the general population. We have to bear in mind that fewer than 10 % of cancers can be traced to Mendelian inheritance. It can be seen that genetic testing has tremendous implications and therefore, proper genetic counselling must be put into place before testing is performed.

CLASSIFIERS FOR TREATMENT SELECTION

There are now a number of protein biomarkers that have been useful in guiding the choice of drugs, particularly useful for molecularly-targeted drugs. Some examples of USA Food and Drug Adminstration (FDA) approved cancer biomarkers are as follows: (1) Oestrogen receptor (ER) positivity is a predictive marker for choosing hormonal treatment such as with tamoxifen or aromatase inhibitors for breast cancer.[9] (2) Human epidermal growth factor receptor (HER)-2 positivity is used as a predictive biomarker for treatment of breast cancer with a drug called trastuzumab or Herceptin.[10] Another example of a drug selection biomarker is the epidermal growth factor receptor which is used for selection therapy with an antibody that targets the Epidermal Growth Factor Receptor (EGFR).[11]

Data has shown that the response rate towards a selective oestrogen receptor modifier, namely, tamoxifen is only up to 70 % of ER^+ cases. Thus, even if the tumour is positive for ER^+, there is still a chance that the drug may not be effective. In addition, it has been shown that >40 % of responsive tumours will eventually develop resistance.[12] Why is that so? Perhaps, there are variants of the oestrogen receptor that can outdo tamoxifen, thus, accounting for the non-effectiveness of the drug. In the case of drug resistance, perhaps, the tumour can utilise another signal transduction pathway. What this means is that the use of ER positivity by the standard immuno-histochemistry method does not always confirm that the hormonal treatment will work. Hence, new predictive biomarkers will be required to identify patients who may be resistant to endocrine agents such as tamoxifen. A recent report showed that RNA expression profiling by microarrays revealed that HRPAP20 and TIMELESS may be promising markers of tamoxifen resistance in women with ERα-positive breast tumors. [13]

The value of HER2 positivity as a predictive factor for trastuzumab has been reported.[14,15] HER2 is a member of the EGF receptor family and is over-expressed on malignant cells. Immuno-histochemistry is used routinely to show that a breast tumour tissue that is positive for HER2. In addition, we can also perform fluorescence *in situ* hybridisation (FISH) to measure the over-amplification of the HER2 gene.

In addition to monoclonal antibodies directed at receptors on cells, small molecule drugs are also useful therapeutic drugs. Perhaps, the most famous of the small molecule drugs is imatinib. This drug which is well-known as Gleevec, targets the tyrosine kinase enzyme which is encoded by the *bcr-abl* gene translocation, and is useful for treatment of chronic myeloid leukemia.[16] Gleevec can also be used to treat other cancers such as gastrointestinal stromal tumours, whereby, c-kit or platelet-derived growth factor receptor A (PDGFRA) is the target molecule. Thus, it appears that though biomarkers are important in guiding oncologists to the appropriate treatment regimen, problems of drug resistance do occur.

DNA BIOMARKERS

Increased DNA copy number of HER2 and *bcr-abl* gene translocation have clinical benefits. As mentioned above, over-amplification of HER2 and presence of *bcr-abl* gene translocation provides the guide for choice of trastuzumab and imatinib for treatment of breast cancer and chronic myeloid leukemia, respectively. Single nucleotide polymorphisms (SNPs), microsatellite instability and epigenetics are not currently used in routine diagnostics and medicine. It is generally more for research purposes. An example of a possible application of microsatellite instability tests for prognostication is as reported by Hawkins *et al.* [17] whereby a distinct and aggressive subset of sporadic colorectal carcinoma was found in microsatellite stable diploid carcinoma. In recent years, a new approach in DNA biomarker development is in the field of epigenetics. Epigenetics which is a study on chromatin and DNA modifications without changes in the underlying DNA sequence is a mechanism, whereby inactivation of gene expression occurs and this has been proposed to cause carcinogenesis. The advances in the use of epigenetics in cancer screening and therapy has been recently reviewed elsewhere.[18] At least 400 genes that are aberrantly methylated in cancers have been described.[19]

A substantial amount of data has been generated in the area of gene mutations in cancer. For example, Conlin *et al.* have attempted to associate prognosis with K-ras, *p53* and *APC* mutations. Poorer survival was associated with K-ras mutations but not with *p53* and *APC* mutations.[20] The analysis of a wide number of gene mutations is complex and is further complicated because mutations in some genes do not always generate the same phenotype outcomes. Hence, other than the classical *brca -1, brca -2, APC*, many of the gene mutations are not used in clinical diagnostics.[2]

RNA EXPRESSION PROFILING

In the last 10 years, there has been a number of publications in this area and most of the RNA biomarkers are currently undergoing clinical evaluation. Many of these reports have been published in the area of breast cancer. Instead of testing one gene only, we now can test an array of thousands of genes and the data obtained is generated by colour-coded clustered image maps. With complex data analysis, breast cancer was classified into at least 5 subtypes, namely, luminal subtype A or B or C or ERBB2$^+$ normal breast or basal subtype.[21]

Traditional methods with immuno-histochemistry, generally, classify breast tumours as ER$^+$PR$^+$HER$^+$ or negative tumours. Now, we can further classify them into additional subtypes. In the ER+ tumours, they can be classified as luminal subtype A or B or C. Note that there is the ERBB2$^+$ subtype, normal breast-like and basal subtypes. It was found that the basal subtype can be ER$^-$HER2$^-$. The study also provided clues on the relationship between subtypes and survival rates. Basal type and ERBB2$^+$ tumours were associated with the shortest survival rates. With the ER$^+$ tumours, luminal subtype B and C had poorer survival rates compared to subtype A. Thus, it is suggested that this method reveals that luminal subtypes B and C might represent a clinically distinct group with a different and worse disease.[22, 23]

Besides molecular classification of cancers, the use of expression profile for predicting survival and response to drugs have been reported and some of these examples are as

shown. Reports have been published not only on breast cancers, but also for many others such as brain,[24] colon,[25] ovarian,[26] head and neck,[27] oral cancers,[28] and leukemias.[29] It should be pointed out that RNA expression profiles may not be thought to be valid 'disease biomarkers' in the traditional way, but can now be categorised as predictive classifiers for treatment selection as exemplified in breast cancer treatment.

CURRENT STATUS OF RNA EXPRESSION PROFILING AND TISSUE MICROARRAYING IN BREAST CANCER

To date, five different gene sets are available for breast cancer to predict outcome for breast cancer patients. The five gene sets are as follows:

1. Intrinsic subtype model (luminal A, luminal B, basal-like, HER2-positive and estrogen-receptor-negative [$HER2^+$ and ER^-], and normal breast-like) developed by Perou and colleagues.[21, 22]
2. Seventy gene signature for good-versus-poor outcome model developed by van't Veer [23] and van de Vijver *et al.* [30]
3. Recurrence-score model developed by Paik *et al.*[31]
4. Two-gene-ratio model (the ratio of the levels of expression of homeobox 13 [HOXB13] and interleukin 17B receptor [IL17BR])[32]
5. Wound-response model developed by Chang *et al.*[33]

Of the 5 listed, two are commercially available, for example, Oncotype DX (the 70 gene predictor) and Mammaprint (21 gene predictor). Mammaprint has been recently approved by US FDA in April 2007. The results of these studies indicate the following:

1. In ER^+ tumours, there are two types; one that comes from luminal epithelial cells and this type often respond to hormonal treatments.
2. The other subtype, first called luminal type B and C often need other chemotherapeutic drugs.
3. The remaining ones are ER^- and they are divided into $HER2^+$ and the basal-like breast cancer that are negative for ER, PR (progesterone receptor) and HER2, also called the triple negative.

Recent progress has also been made in image-based quantitative pathology, whereby, not only tissue morphology but also molecular interactions can be made via calculating the ratios between markers.[34, 35] Using this technique, subgroups of cancers were defined with a number of biomarkers such as HER-2 and β-catenin.

ISSUES IN RNA EXPRESSION PROFILING

Can we use molecular profiling in making clinical decisions? There are number of issues to consider, namely, the readiness of the technology, the robustness and reproducibility of the test, the length of sample preparation time and the feasibility of sample storage condition. The question of feasibility arises when we need frozen tissues rather than paraffin embedded tissues for RNA work. The application of RNA expression profiling has been limited to snap-frozen or fresh tissues. However, collection of frozen samples is not the routine

practice unlike the use of formalin fixation followed by paraffin embedment. Formalin-fixed paraffin-embedded archived tissues provide a valuable source of material for molecular analysis. Unfortunately, formalin is a cross-linking agent that induces fragmentation and chemical modification of DNA or RNA and impairs quantification of gene expression. Success rates of only 60-80 % for DNA amplification has been reported.[36] Hence, these are issues that need to be addressed before embarking on RNA expression studies.

Perhaps, another issue that we need to consider is the question of the biological significance of measuring level of RNA expression which arises because not all RNA transcripts are translated into protein expression. The answer can only be obtained through further investigations. Once the relevant transcripts are identified, antibodies can be made to new target molecules and protein profiling can then be performed.

OPPORTUNITIES IN RNA EXPRESSION PROFILING

Though we have seen a number of advances in expression profiling, there are still a few problems. The current gene expression test does not cover for 50 % of HER2 positive patients that do not respond to the targeted drug. Therapeutic strategies for the group of ER^+ patients that fail to respond to antioestrogen treatment as well as the triple negative patients need to be investigated. Whilst this manuscript was in preparation, gene expression profiling and histopathological characterisation of triple-negative breast carcinoma was reported.[13] With availability of these markers, further work to determine the predictive markers for treating this group of patients can now be carried out.

CHALLENGES AHEAD

Cancer is a heterogenous disease and a cure for cancer will require understanding the biology of cancer. Perhaps, revisiting the massive data that has come out of gene expression profiling can give us an insight into the biology of these subtypes of breast cancer. We need to get good correlations between molecular profiling with the histopathological and clinal data; data needs to be updated, recorded properly, patient follow up has to be done and patient confidentially must be maintained. We also have to make sure that samples are properly collected and stored. Alterations that occur *ex vivo* will not accurately reflect the events that occur in the primary tumour. Lastly, we have to ensure that data is accurate and reproducible.

Besides, gene expression profiling, data with epigenetics is also emerging. Microarrays is not the only approach as other technologies such as SAGE and bead-based methods are also available. In addition, the proteomic approach with SELDI TOF and mass spectrometry contributes to the search for biomarkers.[5]

To summarise, examples of protein, RNA and DNA biomarkers mostly for prognosis and treatment of cancers with molecular-targeted drugs have been presented in this review. We are still at the beginning of cancer biomarker research. The traditional use of TNM staging and grading to predict survival and treatment selection is still useful. We should not do away with the traditional approach even though there is immense interest in the new DNA-and RNA-based biomarkers. There is still an immense amount to learn about the biology, mechanisms and pathogenesis of the disease through RNA expression profiling.

The data is large and complex but the scientific outcomes have been good. As for clinical use of gene expression profiling, it will be hard to adopt it in routine diagnostic tests unless clinical benefits can be demonstrated. These tests are expensive, running into thousands of dollars per sample, the data generated is so complex and difficult to analyse, and the laboratory setup may not be ready to meet with the demands of the technology. It will be useful to be able to cut down to a small set of genes, have the technology automated, make sure that data is reproducible and easy to interpret and cost is reduced, and definitely it must be of clinical benefit! The ultimate drive will be when molecular tests are found to be critical for clinical management of the patients; these newly-developed tests will have to be performed because it is not ethical not to perform these tests if they are of clinical benefit. Thus, for routine diagnostics, the relevant gene sets will need to be reduced for various subtypes of cancers. There is no doubt that exciting new discoveries will take place in the near future towards the development of non-invasive, accurate and sensitive tests for prognostication and prediction of drug responses.

CONCLUSION

In conclusion, it is important to emphasise that establishing a biomarker as a valid surrogate for measuring disease progression and clinical benefit is a lot more difficult than establishing a classifier that has clinical benefit for treatment selection. The use of the term 'biomarker' should be used carefully. Current protein biomakers and well-established DNA markers should continue to be used in routine diagnostics and the extensive molecular tests, for example, RNA expression profiling, need to be further improved and validated.

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