CANCER BIOMARKER DEVELOPMENT FROM BASIC SCIENCE TO CLINICAL PRACTICE

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ABSTRACT

The amount of published literature on biomarkers has exponentially increased over the last two decades. Cancer biomarkers are molecules that are either part of tumour cells or secreted by tumour cells. Biomarkers can be used for diagnosing cancer (tumour versus normal and differentiation of subtypes), prognosticating patients (progression free survival and overall survival) and predicting response to therapy. However, very few biomarkers are currently used in clinical practice compared to the unprecedented discovery rate. Some of the examples are: carcino-embryonic antigen (CEA) for colon cancer; prostate specific antigen (PSA) for prostate; and estrogen receptor (ER), progesterone receptor (PR) and HER2 for breast cancer.

Cancer biomarkers passes through a series of phases before they are used in clinical practice. First phase in biomarker development is identification of biomarkers which involve discovery, demonstration and qualification. This is followed by validation phase, which includes verification, prioritisation and initial validation. More large-scale and outcome-oriented validation studies expedite the clinical translation of biomarkers by providing a strong 'evidence base'. The final phase in biomarker development is the routine clinical use of biomarker.

In summary, careful identification of biomarkers and then validation in well-designed retrospective and prospective studies is a systematic strategy for developing clinically useful biomarkers.

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INTRODUCTION

According to the Biomarkers Definitions Working Group[1], 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". Tumour markers are molecules produced by cancer cells and either endogenously present in the cellular compartments of cancer cells or are secreted from the cells. They are measured in blood, urine, stool, body fluids (e.g. pancreatic cyst fluid) or tissues of patient with cancer. Tumour markers are often proteins but genetic changes (gene mutations) and changes in gene expression patterns are also used as tumour biomarkers. The alterations in the tumour biomarkers help in the categorisation of patients into distinct groups[2].

Biomarkers are used in the clinical management of patients with tumours and can broadly be classified as: susceptibility biomarkers that help in the identification of individuals at risk of developing cancer; screening biomarkers that help in the early detection of cancer in the general or at risk populations of developing overt disease (i.e. detects subclinical disease); diagnostic biomarkers that help in the diagnosis of patients with the disease; prognostic biomarkers that help to predict the course of disease (e.g. survival); predictive biomarkers that help to predict response to therapy (e.g. a drug or surgical intervention) or monitor the efficacy of a therapy[1,3-5]; and pharmacogenomics biomarkers which are "measurable DNA and/or RNA characteristics that are indicators of normal biologic processes, pathogenic processes and/or a response to therapeutic or other interventions"[6]. Some of these markers are already used in routine clinical practice[7-10]. Perhaps the earliest markers to help in the clinical diagnosis were carcinoembryonic antigen (CEA) in colon carcinomas[11] and prostate specific antigen (PSA)[12] in prostate cancer.

BIOMARKER DEVELOPMENT

Biomarker development passes through several phases of development from discovery to clinical practice[13]. It involves a series of identification and validation steps before clinical application (Figure 1).

1) Identification of biomarkers:

The first phase in biomarker development is the identification of suitable candidate biomarkers[14]. The purpose of the identification phase of biomarker development is to identify potential candidates with high sensitivity for detection. The emphasis therefore is to establish the association between biomarker expression and the tumour of interest. Biomarker identification thus uses a significant amount of resources, cost and utilizes modern technology. Identification passes through the following stages.

a) Discovery: Broadly two approaches can be used for biomarker identification. The first approach is to identify biomarkers based on current knowledge of pathophysiology of the disease through 'deductive reasoning'. The second approach is using molecular profiling techniques to identify candidates based on the differential expression between tumour and normal tissue[13]. Differentially expressed genes between PDAC and normal or reactive pancreatic duct are identified through high throughput genomic and proteomic studies[15,16].

b) Demonstration: High throughput technologies generate a list of potential biomarkers but based on different statistical models the biomarker list is further refined and selected biomarkers are demonstrated by molecular techniques. In carcinoma breast, the differential expression of genes is demonstrated by DNA microarray and polymerase chain reaction[17,18], whereas, differentially expressed proteins are demonstrated by gel electrophoresis and mass spectrometry[19-21].

c) Qualification: The purpose of the qualification is to confirm the differential expression of biomarkers using alternative techniques such as western blot and immunohistochemistry[19-22].

2) Validation of biomarkers:

After identification, the next phase is validation of biomarkers which is an important pre-requisite for clinical translation. 'Omics' technologies allow identification of promising biomarkers but these biomarkers require verification, prioritization and validation before they are used in clinical practice[23].

a) Verification: Biomarker verification is carried out to test whether the candidate biomarker has sufficient potential for future validation studies. Pilot studies in a relatively small sample size are used to investigate biomarker expression in both tumour and normal samples from a variety of patients[24,25]. Verification begins to assess the specificity of biomarkers but still focuses on optimum sensitivity[13]. This helps the researchers to select more specific candidates that are highly expressed in tumour for which they can invest their time, energy and money.

b) Prioritization of candidates: Prioritization of candidate biomarkers from a list of potential biomarkers is very important for further clinical validation studies due to cost and limited clinical resources[14]. The role of the candidate biomarker in tumour biology greatly facilitate this selection process as markers involved in the progression of tumour will prove potentially more useful in clinical practice[8,26].

c) Validation of selected candidates in large-scale studies: Biomarkers achieving a suitably good combination of sensitivity and specificity from pilot studies in the verification and prioritization phases may be selected for further validation in large-scale studies. The further validation processes consist of three phases: analytical validation ensures the intra- and inter-laboratory reproducibility of the assay e.g. Immunohistochemistry (IHC) achieving similar expression patterns; clinical validation ensures the diagnostic sensitivity and specificity of the biomarker is consistent for the outcome (e.g. differentiation between benign and malignant disease); and clinical utility of the biomarker assesses whether it improves the diagnostic management of patients[27].

Early validation studies are carried out on archival pathology specimens (tumour and normal). These samples are retrospectively identified and used to observe the expression and clinical utility of candidate biomarkers. These retrospective studies typically overestimate the actual sensitivity and specificity of biomarkers. Most of the reported literature on biomarker studies uses archival pathology samples[28,29]. However, the clinical utility of biomarkers can be more clearly assessed in a prospectively designed study. But validation on archival samples is a pre-requisite before biomarker investigation in prospective clinical studies. Prospective clinical studies and biomarker trials lead to the qualification of biomarker for clinical use. Finally, biomarkers are used in clinical practice for the intended clinical use and they are monitored for their effectiveness. Figure 1 shows the path that a biomarker is likely to take from discovery to clinical use.

Biomarker validation is therefore an important but expensive and lengthy process and depends on the type of samples used for assessing the clinical utility. Successful implementation of biomarkers in clinical practice requires robust evidence from independent validation studies. A single study is unlikely to provide sufficient evidence for adoption of a biomarker in clinical practice. In a study by Ioannidis et al[30] the magnitude of the effect size of proposed biomarkers in highly cited papers was examined. It was found that primary studies often report a larger effect size compared to the subsequent meta-analysis assessing the same associations[30]. Therefore, clinical evidence from biomarker studies should be interpreted carefully and healthy scepticism is suggested[30]. More large-scale and outcome-oriented validation

studies expedite the clinical translation of biomarkers by providing a strong 'evidence base'.

IMMUNOHISTOCHEMESTRY

Immunohistochemistry (IHC) is a tissue based method that allows the visualisation of specific antigens in tissues and cellular compartments based on antigen-antibody reaction using microscopy[7]. IHC remains an important diagnostic tool even in the era of genomics and high throughput molecular diagnostics. IHC was first introduced in the 1960s and since then the amount of literature has increased exponentially. IHC has been used both in research and clinical settings. IHC characterises the expression of genes at the protein level and allows the observation and localization of protein expression simultaneously in tissue and cellular compartments[31,32].

Figure 1: Pathway of biomarker development from discovery to clinical use

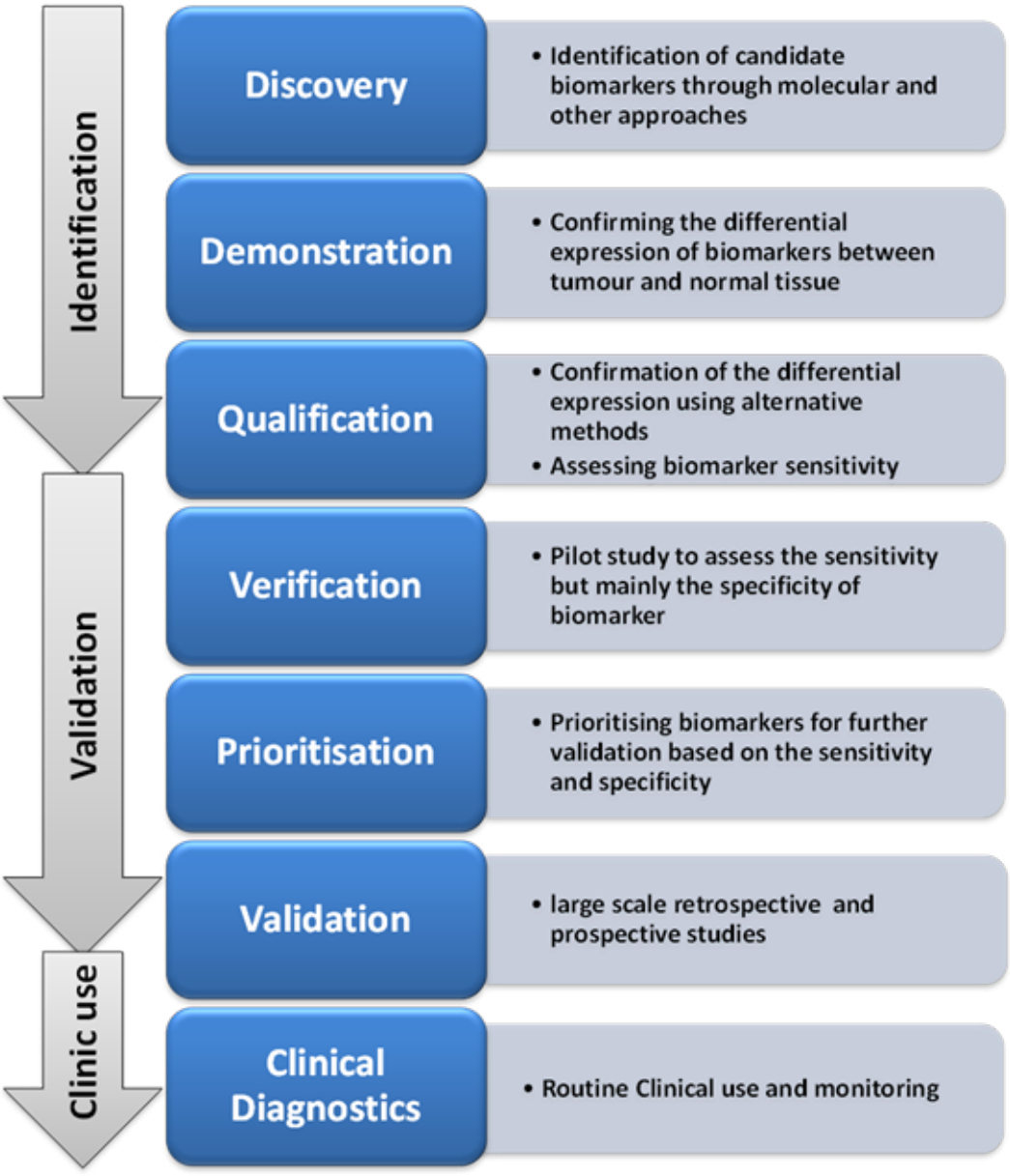

Figure Legend: The sequential stages from discovery to clinical diagnostics are shown in the middle vertical block. Furthermore, each stage is further elaborated in the right side vertical block. Adapted from a model based on Lee et al 2007[4].

It is a routine technique used in diagnostic pathology and is relatively inexpensive with widespread expertise in the technique. Therefore, biomarkers identified and validated by IHC have an enormous potential for clinical translation. In surgical pathology, a range of biomarkers in clinical use is assessed by IHC[7,8,33].

Biomarkers identified by IHC have the advantage of defining the role of markers in the tissue context. They give insight into the expression of markers in specific cell types of tissue (malignant cells, stroma and adjacent normal cells or other cell types) and the distribution of the marker in subcellular compartments (nuclear, membranous or cytoplasmic). Biomarker expression in a specific cell type (e.g. epithelial cells) or subcellular compartment (e.g. cytoplasm) might then be associated with tumour diagnosis. Biomarker expression in tumour can also be associated with the clinical follow-up (e.g. survival of patient) or the clinicopathologic characteristics (e.g. lymph node invasion) of the patient[34,35].

Over the last few decades many IHC biomarkers have been investigated for improving the diagnosis and prognosis of tumours. The diagnostic IHC biomarkers help in the diagnosis and sub-classification of tumours. IHC biomarker c-kit helps in the diagnosis of gastrointestinal stromal tumours (GISTs)[9] and p63 helps to detect the presence of basal cells which indicate normal prostate gland[10,36]. Furthermore, nuclear immunostaining of ki-67 as a proliferation marker[37], chromogranin A, CD56 and synaptophysin for the diagnosis of neuroendocrine tumours[38,39] and the use of E-Cadherin in the differentiation of ductal and lobular carcinomas of the breast[40] are used in clinical practice.

An ideal diagnostic IHC biomarker should be 100% sensitive and specific which is almost never achieved as sensitivity increases at the expense of specificity and vice versa. The panel of biomarkers are thus becoming more relevant. These include CK20, P53, CK5/6, CD138, and Her2/Neu in the diagnosis of urothelial carcinoma in situ[41]; a panel of napsin-A, thyroid transcription factor 1, Cytokeratin 5, and P63 in differentiating adenocarcinoma from squamous cell carcinoma of the lung[42,43] and a panel of S100P and XIAP in the differentiation of pancreatic cancer from non-neoplastic pancreatic tissue[44,45].

In addition, IHC biomarkers are used for predicting the survival of patients, predicting the response to specific therapies and subsequent stratification of patients for different treatment options. Estrogen receptor (ER), progesterone receptor (PR) and HER-2/neu are used for the management of patients with breast cancer[8,46-48]. Panel of biomarkers are also used for prognostic and predictive purposes e.g. IHC4 (a panel of ER, PR, HER2 and Ki-67) is an assay which estimates recurrence risk for early stage breast cancer patients[49].

CONCLUSION

Careful identification of biomarkers and then validation in well-designed retrospective and prospective studies is a systematic strategy for developing clinically useful biomarkers. Immunohistochemistry biomarkers are useful tools that could potentially be translated to clinical practice if suitable biomarkers are identified and validated in independent cohorts.

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CONTRIBUTORS

AA conceived the idea, drafted the manuscript and gave final approval. ZA and YMY did literature review, drafted and critically revised the manuscript. KAO critically revised the manuscript and supervised the study. All authors contributed significantly to the submitted manuscript.