Tumor Diagnostic Markers in Primary Liver Cancers

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Abstract

The primary liver cancers (hepatocellular carcinoma and cholangiocarcinoma) are frequently occurring malignancies amongst the most common cancer types throughout the world. For successful therapy, primary liver malignancies must be detected at early stages. Identification of novel markers for early diagnosis is critical for this goal. A tumor marker could help in diagnosis, early detection, staging, prognosis, and post-treatment follow-up. However, no currently accessible single marker works optimally in all situations due to a lack of sensitivity and specificity; performance characteristics are dependent on unique factors of each malignancy, such as prevalence, tumour heterogeneity, and treatment response. Technological advancements in molecular biology are opening new doors for the identification of tumour markers, resulting in a rapidly evolving area. Recognizing that the optimal tumour marker for malignancies has yet to be discovered, it is critical to study the qualities of what would be an excellent tumour marker.

Introduction

The light chain of immunoglobulin in the urine was the first cancer biomarker discovered, and it was found in 75 percent of myeloma patients in an 1848 study (Jones 1848). This marker's test is still used by physicians today, although with more modern quantification procedures. Between 1930 and 1960, scientists discovered a slew of enzymes, hormones, and other proteins whose concentrations were altered in cancer patients' bodily fluids. The discovery of alfa-fetoprotein (a serum biomarker for HCC) and carcinoembryonic antigen (CEA), as well as the introduction of immunological techniques like as the radioimmunoassay, helped in the contemporary era of monitoring malignant disease in the 1960s. Recently,

a combined group of AFP, CAE and CA19-9 biomarkers has been used to detect the hepatocellular carcinoma at its early stage (Edoo et al., 2019). Similarly, joint detection of CA19-9, AFP, CAE and CA125 was described by Li et al., 2015 in sera of cholangiocarcinoma and HCC patients with high rates of specificity and sensitivity (Li et al., 2015). The development of the ovarian epithelial carcinoma marker carbohydrate antigen 125 was made possible by hybridoma technology in the 1980s (CA 125). PSA [KLK3] (prostate-specific antigen) was identified in 1980 and is now considered one of the finest cancer markers. Every new phase of biomarker discovery appears to be linked to the introduction of a new and strong analytical tool (Koizumi et al 1992). The discipline of large-scale and high-throughput biology has seen extraordinary progress in the last decade, contributing to a new era of technology development. The completion of numerous genome-sequencing projects, the discovery of oncogenes and tumor-suppressor genes, and current breakthroughs in genomic and proteomic technology, as well as strong bioinformatics tools, will have a direct and significant impact on how cancer biomarkers are discovered. The overexpression of CEA was one of the first cancer biomarkers to be discovered, and it was based on actual data (von Kleist 1986). Modern technologies allow for concurrent rather than serial investigations, and they can play a vitol role in the recognition of distinct patterns and many markers rather than just one; such methods are a key component and a paradigm change in the hunt for novel biomarkers (figure 1).

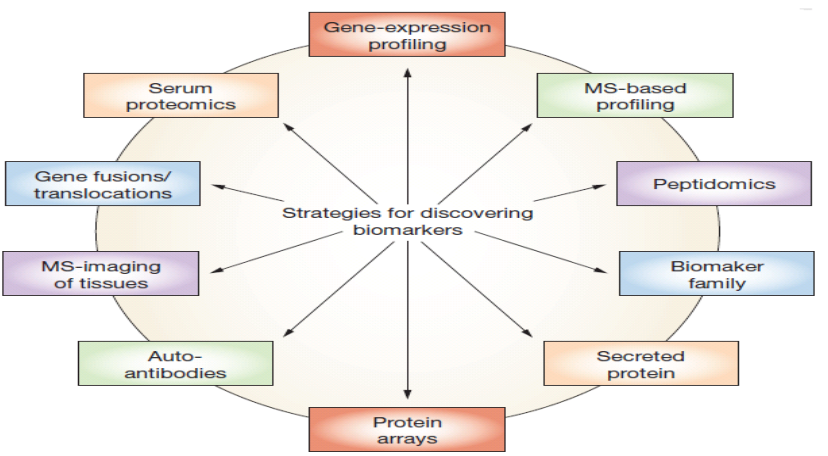

Figure 1: *Strategies for discovering biomarkers.(Adopted from* http://www.sinobiological.com/Cancer-Biomarker-Discovery-a-5782.html#Strategy)

Tumor markers are chemicals produced by cancer cells or other cells in the body in response to cancer or certain non-cancerous situations. Bence-Jones described the first tumour marker when he reported the presence of aberrant proteins in a patient's urine, which is today known as multiple myeloma (Heo et al., 2012). The majority of tumour markers are produced by both normal and malignant cells, although they are produced at a higher level in cancerous situations. Some cancer patients' blood, urine, stool, tumour tissue, and other tissues or body fluids may include these compounds. Proteins are the most common tumour markers, although gene expression patterns and DNA alterations have lately gained popularity as tumour markers. The latter sort of marker is evaluated in tumour tissue specifically (Watanapa et al., 2002).

A perfect and ideal tumour marker would allow for a simple blood test to screen for cancer, with the stage of tumour progression being linked to the marker's levels (Imai et al., 1993). A tumour marker could assist in diagnosis, early detection, staging, prognosis, and treatment follow-up. However, no single marker now available works well in all circumstances due to a lack of sensitivity and specificity; performance characteristics are dependent on unique factors of each malignancy, such as prevalence, tumour heterogeneity, and treatment response. Technological advancements in molecular biology are paving the way for new approaches to detect tumour markers, ushering in a discipline that is rapidly evolving. Recognizing that the ideal tumour marker has yet to be discovered (Imai et al., 1993), it is critical to look at the qualities of what would be an ideal tumour marker for malignancies.

More than 20 distinct tumour markers have been found, described, and are now being used in clinical trials. Only a few of them are related with a single type of cancer, while others are linked to two or more. Unfortunately, there is no such thing as a "universal" tumour marker that can detect any or all cancers (Watanapa et al., 2002).

The use of tumour markers is not without its drawbacks. In noncancerous or benign diseases, certain tumour markers may have an elevated level. Furthermore, having a higher level of the tumour marker linked with a particular form of cancer is not a need. Watanapa et al. (2002) found that tumour markers have not been identified for all types of malignancies.

Tumour markers can play an important role in the early detection of cancer, making them useful in the detection, diagnosis, and management

of certain cancers. Although a rising level of a tumour marker in the blood suggests that the tumour is progressing, this alone is not enough to diagnose malignancy. As a result, tumour marker readings are frequently used in conjunction with other procedures to diagnose cancer, such as biopsies, ultrasonic imaging, and so on (Duffi 2001).

The level of tumour markers can be measured in a variety of ways. It can be measured prior to treatment to assist clinicians in determining the best course of action. It can be used as a prognostic marker in some types of cancer; in this situation, the level of a tumour marker reflects the stage of the disease.

Tumor markers may be measured on a regular basis during cancer treatment. A drop in the level of a tumour marker or a return to the normal level of the marker may suggest that the cancer is responding to treatment; however, no change or an increase may indicate that the disease is not responding. After therapy has concluded, tumour markers can be examined to assess for recurrence (Watanapa et al., 2002).

Methods for determining tumour markers

The level of the tumour marker can be measured using a variety of methods, including tumour tissue or body fluid. The level of a tumour marker will be assessed in several samples obtained over time if the goal of the marker is to establish treatment effectiveness or whether there is a recurrence. These "serial measurements," which reveal whether a marker's level is rising, falling, or keeping the same, are usually more useful than a single measurement.

Tumor markers currently being used for primary liver cancer

For a variety of cancer forms, many tumour markers are presently used in clinical practice. Some of these markers can be examined in laboratories that satisfy the Clinical Laboratory Improvement Amendments' criteria, but others can't, thus they're believed experimental. Alpha-fetoprotein (AFP), a tumour marker with 60-70 percent specificity and sensitivity, is currently in use for hepatocellular carcinoma; however, its sensitivity increases dramatically with tumour size (Shaib et al., 2005). It's been used to help diagnose HCC and track treatment response; to assess the stage, prognosis, and response to therapy of germ cell tumours; and to assess the stage, prognosis, and response to treatment of germ cell tumours (Watanapa et al., 2002). To date, there is no specific tumour marker for early detection of cholangiocarcinoma, but CA19-9 and Carcinoembryonic antigen (CEA) are used alone or in combination with

other markers, with average sensitivity and specificity of 71 percent and 51 percent and 78 percent and 88 percent, respectively, for CA19-9 and CEA (Green et al., 1991).

Use of tumor markers in cancer screening

For early cancer screening and detection, a tumour marker should have an excellent accuracy (capability to clearly identify persons with the disease) and precision (capability to recognize people who do not have the disease). Since tumour markers might be used to determine a tumor's response to therapy and outcome, felt very confident that they could also be beneficial in cancer screening tests at initial stages, earlier, there were many symptoms However, no tumour marker discovered so far is accurate or specialised enough to be utilized alone for cancer screening. When an indicator is very reactive, it will identify the majority of persons who have the condition, with relatively little inaccurate data. but when the marker is extremely specialized, only a tiny proportion of people who might not have the illness will show positive result for it—moreover, there will be very few completely bogus findings. (De Groen et al., 1999).

The prostate-specific antigen, for example, is a blood test which is used to examine men for prostate cancer. It evaluates the quantity of PSA in the bloodstream. Raised PSA level, on the other hand, can be produced by harmless prostate problems, and the majority of men with an elevated PSA level do not possess prostate tumours. PSA testing, according to preliminary findings from two large randomized controlled studies, only contributes to a minor decline in the amount of prostate tumour fatalities. Additionally, it is unclear if the advantages of PSA screening outweigh the risks of subsequent diagnostic testing and therapies for malignancies that would not have endangered lives in many circumstances (De Groen et al., 1999, Sripa et al., 2007), moreover,the specificity of PSA as diagnostic biomarker for prostate carcinoma is very low (Salman et al., 2015).

CA-125, a tumour indicator for ovarian malignancy that is sometimes raised in the women's blood with ovarian malignancy but can even be enhanced in women with inflammatory conditions, is not responsive or precise enough to be used in combination with transvaginal ultrasound to detect ovarian cancer in women at high risk of the disease.

The susceptibility of CA-125 was discovered through an examination of 28 putative ovarian cancer indicators in blood from women who developed later the disease, which revealed that none of these indicators scored even close to CA-125 in diagnosing the disease in women at moderate risk. (Ganeshan et al., 2012, Khan et al., 2002).

Several enzymes are involved in degradation and breakdown of basement membrane and extracellular matrix components (Tan et al., 2001), these enzymes are termed as metalloproteinase (MMPs) and cancer cells usually breach the basal layer by suppressing external matrix-digesting proteins, MMP-9 was indicated in the sera of patients with gastric malignancy where as the up regulation of MMP-7 was observed in patients suffering from ovarian, renal and colorectal cancer (Sahin et al., 1995, Lee et al., 2003, Finn, O.J. 2005, Koziol et al., 2003), expression of MMPs has been observed in surgically resected cholangiocarcinoma specimens by immunohistochemical technique (Kao et al., 2001). But the detection of blood circulatory MMPs might be helpful in CCA diagnosis and prognosis as proposed by Leelawat K et al. by observing the sensitivities and specificities of MMP-9 and MMP-7 at different cut-off values (Suzuki et al., 2005). A 4204 Da peptide (fragment of prothrombin) has detected with 75.8% sensitivity in a comparative study of biomarkers in biliary tract cancer patients (Kikkawa et al., 2012), the sensitivities of CEA and CA19-9 were 50% and 61.3% respectively (Houghton & A.N., 1994), but in this study CCA and gall bladder carcinoma were analyzed under the head of BTCs. So a separate study is a prime need to discover more specific biomarkers for CCA and antibody based study would be a necessary step for further verification and identification.

In another comparative study of measurement of serum tumor biomarkers, a novel tumour marker RCAS1 has been proposed by Enjoji M et al. as more significant marker than CA19-9 (Old et al., 1998, Pan et al., 2012). This group studied three different panels of cholangiocarcinoma patients according to the type of treatment, prior to any anti-cancer therapy the sensitivity of serum RCAS1 and CA19-9 values were 74.4% and 59.0% respectively. After therapy, the significantly declined level of both of these two markers observed. This indicates that after effective chemotherapy and surgical resection, the serum RCAS1 level decreases or reached to its normal value.

A significant up-regulation of platelet-derived growth factor (PDGF) has been observed in sera of CCA patients and this elevated level has also been observed in 84.6% adjacent tumours tissues which is an indication of the fact that expression of this gene is involved in tumorigenesis (Zhang et al., 2009). Furthermore, overexpression of this gene is also reported in 80% of opisthorchiasis-associated CCA patients (Liu et al., 2011). On the basis of above mentioned findings PDGF might be use as promising candidate biomarker for diagnosis, prognosis prediction and treatment strategies of CCA. In sera of HCC patients, Bugti et al., 2017 observed the different protein bands of 70 kDa, 32 kDa, 50 kDa and 90 kDa protein bands on SDS-PAGE which might correspond to the AFP, Calreticulin (CRT), Tu translation elongation factor (TUFM) and protein phosphate 2A (p90/CIP2A) respectively (Bugti et al., 2017).

Specificity in tumour markers

A protein or tumor indicator expressing just in tumor cells is known as a precise tumor indicator. Union proteins related to vicious processes where a malignancy is transmitted and attached to a gene's active promoter, is the best example of this type of marker. The continuously vigorous construction of the blended protein leads towards the growth of an evil clone (Zhu et al., 2013). Creation and destruction of fusion genes may take place by different DNA recombination methods, DNA sequences can be merged through structure, insertions and convergences, the genes' behavioural effect may also be involved with. Haematological diseases and some dense tumours of mesoblastic origin are formed as a result of these types of mechanisms (Anderson et al., 2005).

Additional types of markers are oncofetal antigens which are less compromising but still very meaningful. These antigenic markers are produced during the developing phases of embryo and then in cancer cells. Alpha fetoprotein, which is most commonly used marker for HCC diagnosis is expressed during early embryonic development and then disappears, its over expression has been observed in malignancy development (Zhang et al., 2003) but its specificity lies between 60%-70% (Liu et al., 2011) and it also expressed in ovarian and testicular cancer (Liu et al., 2011). CAE is another widely used oncofetal antigen, expressed in many cancers including all gastrointestinal tumors (Bradford et al., 2006).

Serum concentration of few proteins is of a great value in terms of their expression because several proteins express normally by differentiated cells but in the corresponding tumour cells, their expression rate increases which causes a relative increase in serum concentrations. This higher concentration of proteins can be used as a tumour marker in sera of the patients. A well known example of cell specific protein is tyrosinase protein used for the diagnosis of melanocytes (Draghici et al., 2005) similarly (Prostate Specific Antigen) is used as an airing indicator for prostate cancer (Gorg et al., 1987).

Sera from cancer patients comprise antibodies that respond with a completely distinctive group of TAAs, however the short rate of advantageous reactions against any person antigen does not consent the usage of autoantibodies as beneficial analytical markers. In a group of cancer patients, autoantibody reactivity to particular TAAs was shown to be rarely greater than 20% to 30% (Zhang et al., 2009, Zhang et al., 2003, Feldman et al., 2002, Bragazzi et al., 2011). However, when TAAs are added to a panel of antigens over time, the number of positive antibody reactions rises in cancer patients, but not in healthy people. In a panel of known TAAs, Koziol and coworkers discovered the presence of serum autoantibodies in a variety of patient malignancies (Koziol et al., 2003).
In a study, antibody frequencies for seven TAAs, (p62, cyclin B1, IMP1, c-myc, Koc, surviving and p53) remained investigated in 527 cancer patients (45 colorectal cancers , 56 lung cancers, 64 breast cancer patients, 65 hepatocellular carcinomas, 91 gastric cancers, and 206 prostate cancers) and 346 healthy controls. The 7-panel TAA was subdivided into subsets that distinguished between tumours and controls via recursive separation, then these subsets stayed distinctive to each cancer group. Antibody frequency to any one TAA was found to be changeable, but seldom exceeded 15% to 20%. There was a gradual increase in positive antibody reactions as TAAs were added to a final total of 7 antigens, up to a range of 44 percent to 68 percent. Various antigen tests can give precise and important apparatuses for tumor recognition and early identification, according to this study (Chen et al., 2019).

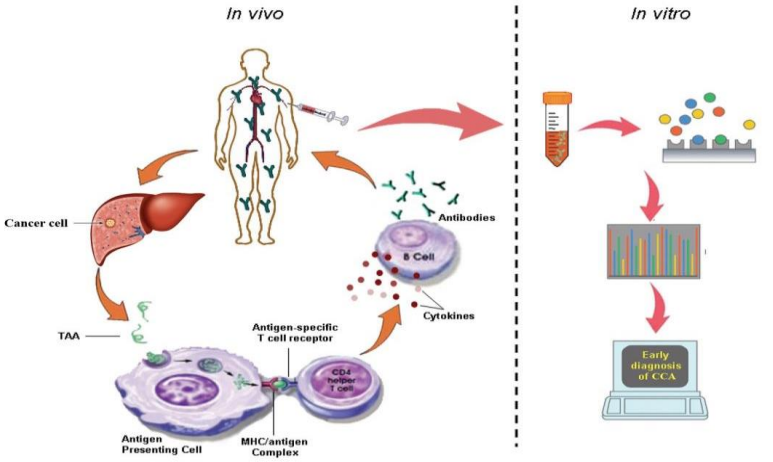

Figure 2 Autoantibody as a tumor biomarker is depicted. Small quantities of growth-associated antigens are found in vivo (TAA), the component proteins (antigens) generated by cholangiocarcinoma (CCA) are absorbed by an antigen-presenting cell (APC) and broken into fragments and displayed on the cell's surface. Antigen fragments bind to the major histocompatibility complex (MHC) proteins on the surface of APCs, also known as human leukocyte antigen (HLA) molecules. This complex subsequently attaches to a T-cell receptor on the surface of the CD4 helper T cell, a different type of immune cell. This combination allows T cells to concentrate their immunological responses on a single protein. CD4 helper T cells that recognise antigens divide and multiply though secreting cytokines, which promote inflammation and aid in the activation of other immune cells. The antigen-specific B cell is one of the activated cells, capable of producing and releasing antibodies into the circulation system to deactivate and help eradicate antigens from the body. As a result, the body's own immune system can act as a natural "amplification technique" to respond to malignant tumor antigens in small amounts (*in vitro*) [Figure is adopted from; Wang 2006].

Autoantibody immunity to tumor-related proteins has gotten a lot of attention recently. Scientists have begun to discover clinical applications of malignant tumor associated autoantibody as a marker for malignancy recognition, as a tool to screen therapy, or as a sign of disease prediction prognosis as antibody immunity to tumor antigens has become more routine. The innovation of malignancy linked autoantibody signatures may thus become a useful tool for malignance analysis and prognosis, given the

wide spread list of autoantibodies to TAAs (Zhu et al., 2013, Feldman et al., 2002, Malhi et al., 2006).

P53 is a cancer suppressor protein that is a phosphoprotein and hardly visible in the nucleus of normal cells and is one of the most thoroughly investigated TAA. Development of Cell-cycle can be halted by p53 in response to cellular stress, such as DNA destruction (Makarov, A. 2000), to allow the DNA to be repaired or to initiate a process that can lead to cell death (Wilkinson & J. M. 1986). Autoantibodies to p53 in melanoma patients were originally identified in 1982, and many more investigations have since confirmed and expanded on this discovery (Rodriguez et al., 2008, Viner et al., 2009).

Anti-p53 antibodies have been detected in a variety of cancers, including oral (Wilson et al., 2008), lung (Jedrychowski et al., 2011), esophageal (Swaney et al., 2008), hepatic (Nagaraj et al., 2012), colon (Ytes et al., 2009), breast (Swaney et al., 2008) and gastric gastric (Phanstiel et al., 2008) cancers. A downregulation or even deletion of p53 antibody planes has been observed for several tumor locations a few weeks following surgical removal of the tumor, which is consistent with the idea that ongoing activation of the immune system by the antigen is required to maintain high antibody levels (Sciascia et al., 2017, Reuschenbach et al., 2009. Lancaster et al., 2011, Tan et al., 2009). These findings lead to the hypothesis that antibody serology may be used to detect illness reappearance (Dumstrei et al., 2016).

Conclusions

Cancer markers can be utilized for one of the four purposes listed below:

1. To check for the existence of cancer in healthy or high-risk individuals.
2. To create a tumor or a specific sort of tumor analysis.
3. To predict the patient's prognosis.
4. To track the progress of a patient who is in remission or is undergoing surgery, radiation, or chemotherapy.

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