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Molecular Markers in Cancer Diagnosis and Management: A Review

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Abstract

Cancer is a family of diseases that involve uncontrolled cell division and tissue invasiveness. It may affect almost any tissue of the body. Lung, prostate, breast, colorectal and stomach are the five most common cancers in the world. More than 10 million people are diagnosed with cancer every year. Tumour markers are biological substances which can be measured in blood and other body fluids. Increased concentration indicates the presence of a tumour. Many different substances can be used as tumour markers, but the term generally refers to substances produced by the tumour cells and generally found in very low concentration in the body fluids of normal individuals. An ideal tumour marker should be used for screening, diagnosis and monitoring of disease progression. Unfortunately there is no ideal tumour marker. Confusion exists about the value of tumor markers in a patient with cancer of unknown primary. Intuitively, a panel of tumor markers should help to establish the origin of the tumor. Unfortunately, most tumor markers are too nonspecific for this purpose. This review article critically analyses about various molecular markers of cancer and their role in disease diagnosis and treatment evaluation.

Keywords: Molecular markers, tumour, Cancer markers, AFP, CEA,

INTRODUCTION

Cancer is a hyperproliferative disorder that involves transformation, dysregulation of apoptosis, proliferation, invasion, angiogenesis and metastasis. Extensive research during the last 30 years has revealed much about the biology of cancer. Drugs used to treat most cancers are those that can block cell signaling, including growth factor signaling, prostaglandin production, inflammatory cytokines, drug resistance gene products, cell cycle proteins, angiogenesis, invasion, anti-apoptosis and cellular proliferation. Tumour markers are biochemical substances elaborated by tumour cells either due to the cause or effect of malignant process. Tumor markers can be used for one of four purposes: (1) screening a healthy population or a high risk population for the presence of cancer; (2) making a diagnosis of cancer or of a specific type of cancer; (3) determining the prognosis in a patient; (4) monitoring the course in a patient in remission or while receiving surgery, radiation, or chemotherapy.

These markers can be normal endogenous products that are produced at a greater rate in cancer cells or the products of newly switched on genes that remained quiescent in the normal cells. A tumour marker produced by the tumor and when present in significant amounts, indicates the presence of cancer. They may be present intracellular substances in tissues or may be released into the circulation and appear in serum (1-4). Continuing search for suitable tumour markers in serum, tissue and body fluids during neoplastic process is of clinical value in the management of patients with various malignancies. The spectrum of biochemical tumour markers reported to date is very wide.

CLASSIFICATION OF CANCER MARKERS

Tumour markers can be broadly classified as (5)

1. Onco-fetal antigens
2. Tumour associated antigens
3. Hormones
4. Hormone receptors
5. Enzymes and Isoenzymes
6. Serum and tissue proteins
7. Other biomolecules

The qualitative and quantitative evaluation of these markers is possible through modern techniques of sensitive immunoassays using monoclonal or polyclonal antibodies or polymerase chain reaction techniques.

CHARACTERIZATION OF IDEAL TUMOUR MARKERS

An ideal tumour marker theoretical should have the following criteria(6-9)

1. It should be highly sensitive and should have low false negatives
2. It should be highly specific and should have low false positive
3. It should have high positive and negative predictive value.
4. 100% accuracy in differentiating between healthy individuals and tumour patients.
5. It should be able to differentiate between neoplastic and non-neoplastic disease and show positive correlation with tumour volume and extent.
6. It should be either a universal marker for all types of malignancies or specific to one type of malignancy.
7. It should be easily assayable and be able to indicate all changes in cancer patients receiving treatment.

Unfortunately none of the cancer markers reported to date have above ideal characteristics. It is not specific to single malignancy. Every tumour markers is specific to a group of malignancies or a single organ. Malignant process is known to elaborate a group of markers (10). Once the patient is positive for a particular marker before instituting therapy, the effective clinical use becomes evident only after its continued measurement throughout the patients clinical course. The rising or declining value of marker concentration in majority of malignancies predicts progression or remission. The diagnostic efficiency of tumour markers depends on variety of factors such as sensitivity, specificity, positive predictive value and negative predictive value. The review analyses some of the major tumour markers and their significant role in disease diagnosis, prognosis and treatment evaluation.

Carcino-Embryonic Antigen (CEA)

Carcinoembryonic antigen, first described in 1965 by Gold and Freedman, was characterized as a glycoprotein of 200KD. Subsequent development of a radioimmunoassay made it possible to detect very low concentrations of CEA in blood, other body fluids, and also in normal and diseased tissues. Although CEA was first identified in colon cancer, an abnormal CEA blood level is specific neither for colon cancer nor for malignancy in general. Elevated CEA levels are found in a variety of cancers other than colonic, including pancreatic, gastric, lung, and breast. It is also detected in benign conditions including cirrhosis, inflammatory bowel disease, chronic lung disease, and pancreatitis. The CEA was found to be elevated in up to 19 percent of smokers and in 3 percent of a healthy control population. Thus, the test for CEA cannot substitute for a pathological diagnosis (11,12).

As a screening test, the CEA is also inadequate. Since cancer prevalence in a healthy population is low, an elevated CEA has an unacceptably low positive predictive value, with excess false positives. Also, since elevated CEA occurs in the advanced stage of incurable cancer but is low in the early, curable disease, the likelihood of a positive result affecting a patient's survival is diminished. The CEA has been suggested as having prognostic value for patients with colon cancer. Preoperative CEA values have been positively correlated with stage and negatively correlated with disease free survival.

Although not satisfactory for screening a healthy population, CEA has been used to monitor recurrence. Early data suggested that CEA preceded clinical relapse by several months. Subsequently, several investigators have examined intensive, serial CEA monitoring as an indicator for second look surgery in the hope that relapse could be detected at a time when surgical resection for cure was still possible. Criteria for reoperation included a significant rise of CEA above a base line level on serial determinations and absence of obvious unresectable disease on staging workup. Determinations of CEA should be done frequently: at a minimum of every 3 months and if possible every 1 month to 2 months. The CEA is of some use as a monitor in treatment. Usually the CEA returns to normal within 1 to 2 months of surgery, but if it returns elevated persistent disease may be indicated. The test is not infallible in patients treated with radiotherapy and chemotherapy but can be useful in those whose tumor is not measurable. The CEA is often positive in malignancies other than colonic. In cancer of the breast, lung, pancreas, stomach, and ovary the CEA may be elevated and can be used to monitor the progress of disease or response to treatment (13,14).

Alpha Fetoprotein (AFP)

Alpha-Fetoprotein is a normal fetal serum protein synthesized by the liver, yolk sac, and gastrointestinal tract that shares sequence homology with albumin. It is a major component of fetal plasma, reaching a peak concentration of 3 mg/ml at 12 weeks of gestation. Following birth, it clears rapidly from the circulation, having a half life of 3.5 days, and its concentration in adult serum is less than 20 ng/ml.

AFP is of importance in diagnosing hepatocellular carcinoma and may be useful in screening procedures. AFP elevation is more common in areas where hepatocellular carcinoma is endemic, such as Africa and in patients who are HBsAg positive. AFP is elevated in normal pregnancy, benign liver disease (hepatitis, cirrhosis), as well as in cancer (15,16). An elevated AFP has been termed by Sell "the single most discriminating laboratory test indicative of malignant disease now available." As such, it could be valuable in screening for hepatocellular carcinoma in high risk populations. AFP is elevated in testicular germ cell tumors containing embryonal or endodermal sinus elements. A defenitive positive marker value is highly sensitive in indicating relapse or response to treatment.

The AFP is less frequently elevated in other malignancies such as pancreatic cancers, gastric cancers, colonic cancers, and bronchogenic cancers. This elevation was not necessarily associated with liver metastases. The AFP is rarely elevated in healthy persons, and a rise is seen in only a few disease states. Elevation occurs in certain liver diseases, especially acute viral or drug induced hepatitis and conditions associated with hepatic regeneration. In general, the elevations are under 500 ng/ml and do not denote hepatocellular carcinoma. It is also elevated in ataxia-telangiectasia and in hereditary tyrosinosis. Thus, AFP is a useful marker in hepatocellular carcinoma and germ cell tumors, the only conditions associated with extreme elevations greater than 500 ng/ml. In both tumors it has value in diagnosis and monitoring of therapy (17,18). In the former, which is one of the most common tumors worldwide, AFP may be of use in screening.

Prostate Specific Antigens (PSA)

Prostate specific antigen termed earlier as gamma-seminoprotein due to its presence of seminal plasma. PSA is 34 KD single chain glycoprotein consisting of 93% amino acids and 7% carbohydrate. It is a monomer made up of 240 amino acid resides. The PSA screening test is a blood test that looks for a specific tumor marker. In general, tumor markers are produced by the tumor itself or by our body in response to the presence of cancer or non-cancerous conditions. The most commonly tested tumor marker for the prostate gland is prostate specific antigen (19). It is normally present in low levels in the blood of all adult men. The normal range is 0 to 4 ng/ml. PSA is prostate-specific, not cancer-specific. A variety of conditions can raise PSA levels: prostatitis (prostate inflammation), benign prostatic hypertrophy (prostate enlargement), and prostate cancer. PSA levels can also be influenced by a number of other things. Some prostate glands normally produce more PSA than others. PSA levels tend to increase with age. And, PSA levels can vary with race: African Americans often have higher PSA levels; Asian men often have lower PSA levels(20).

PSA seems to have the capability of achieving at least one of the characteristics of ideal tumor marker- tissue specificity; it is found in normal prostatic epithelium and secretions but not in other tissues. It is a glycoprotein, whose function may be to lyse the seminal clot. PSA is highly sensitive for the presence of

prostatic cancer. The elevation correlated with stage and tumor volume. It is predictive of recurrence and response to treatment. Finally, the antigen has prognostic value in patients with very high values prior to surgery are likely to relapse. Unfortunately, PSA is detectable in normal men and often is elevated in benign prostatic hypertrophy, which may limit its value as a screening tool for prostate cancer. A recent study has shown that PSA combined with rectal exam is a better method of detecting prostate cancer than rectal exam alone (21,22).

Cancer Antigen (CA-125)

Cancer antigen-125, is a protein that is found at levels in most ovarian cancer cells that are elevated compared to normal cells. CA-125 is produced on the surface of cells and is released in the blood stream. CA125 is an antigen present on 80 percent of nonmucinous ovarian carcinomas. It is defined by a monoclonal antibody (OC125) that was generated by immunizing laboratory mice with a cell line established from human ovarian carcinoma. It circulates in the serum of patients with ovarian carcinoma and was therefore investigated for possible use as a marker (23,24).

A study of about 22,000 post menopausal women 45 years or older screened about 11,000 with the CA-125 test. 468 patients with elevated CA-125 levels were given an ultrasonography test. Of those patients, 29 underwent surgical procedures. 6 had ovarian cancers, 2 had adenocarcinoma of unknown origin, 14 had benign tumors, 4 had fibroids, and 3 had no abnormalities. CA-125 test has a lower specificity in premenopausal women than postmenopausal women (25).

The use of CA 125 in screening for ovarian cancer is perhaps the most controversial aspect of CA 125. Screening of premenopausal women for ovarian cancer was not recommended by the NIH Consensus Panel, except for women who have a family history of a first degree relative with ovarian cancer or an individual who has one of the hereditary cancer syndromes. These women should have annual physical and pelvic exams, a CA 125 test, and a transvaginal ultrasonography. Screening of postmenopausal women with current diagnostic modalities has been criticized on the basis of the low positive predictive value resulting from the low sensitivity and the low specificity (high false-positive rate) of the screening program, and the observation that only about one-half of the early-stage ovarian cancer patients will have an increased CA 125 value (26-29). There is little benefit to the early detection of late-stage cancers. However, efforts at improving the performance of cancer screening is being attempted with the use of nonlinear algorithms and multiple tumor marker tests. With surgical resection or chemotherapy, the level correlates with patient response. Thus, it is superior to other markers such as CEA. The CA125 is elevated in other cancers including endometrial, pancreatic, lung, breast, and colon cancer, and in menstruation, pregnancy, endometriosis, and other gynecologic and non gynecologic conditions (30).

Tissue Polypeptide Antigen

Serological tumour marker composed of a molecular complex of cytokeratins 8, 18, and 19. It is used in the diagnosis and staging of bronchogenic carcinoma. TPA, which is regarded as a marker of cell proliferation, is a mixture of proteolytic fragments containing the relatively stable α-helical rod domains of simple epithelium-type cytokeratins. These fragments are probably released during necrosis and lysis of the carcinoma cells (31,32). Thus TPA should be regarded as a broad-spectrum epithelial tumour marker and not as a specific molecular marker for epithelial neoplasms. The moderate elevation in TPA occurs in many diseases and in pregnancy. TPA is known to be sensitive but non-specific tumour marker. However, TPA along with CEA is of some help for monitoring lung, bladder and breast, colorectal and ovarian carcinomas (33).

CA 19 and CA 15-3

Cancer antigen 19-9 (CA 19-9) is a protein that exists on the surface of certain cancer cells. CA19-9 is tumour marker of first choice for cancer pancreas and cancer gall bladder. The marker is 210 KD tumour associated glycoprotein antigen present as carbohydrate determinant on glycolipid and glycoprotein. CA 19-9 is elevated in about 70% of people with advanced pancreatic cancer, but it may also be elevated in other cancers, conditions, and diseases such as colorectal cancer, lung cancer, gall bladder cancer, bile duct obstruction (e.g., gallstones), pancreatitis, cystic fibrosis, and liver disease. Small amounts of CA 19-9 are present in the blood of healthy people (34, 35).

CA15-3 is a heterogenous 300KD glycoprotein antigen was defined by using two monoclonal antibodies 115D8 and DF3 raised against breast carcinoma cells. The diagnostic sensitivity of the CA15-3 for breast carcinoma is low as its elevated levels are also observed in benign breast diseases and liver cirrhosis. In general, the higher the CA 15-3 level, the more advanced the breast cancer and the larger the tumor burden. CA 15-3 concentrations tend to increase as the cancer grows. In metastatic breast cancer, the highest levels of CA 15-3 often are seen when the cancer has spread to the bones and/or the liver. Mild to moderate elevations of CA 15-3 are seen in a variety of conditions, including liver and pancreatic cancer, cirrhosis, and benign breast disorders as well as in a certain percentage of apparently healthy individuals. The CA 15-3 elevations seen in non-cancerous conditions tend to be stable over time. Normal CA 15-3 levels do not ensure that a person does not have localized or metastatic breast cancer. It may be too soon in the disease for elevated levels of CA 15-3 to be detected or the person may be one of the 25% to 30% of individuals with advanced breast cancer whose tumors do not shed CA 15-3. Increasing concentrations of CA 15-3 over time may indicate that a patient is not responding to treatment or that the cancer is recurring (36, 37).

BRCA1 And BRCA2

BRCA1 (breast cancer 1) is a human tumor suppressor gene, which produces a protein, called breast cancer type 1 susceptibility protein. It originally stood for Berkeley, California, as the first evidence for the existence of the gene was provided by the King lab at UC Berkeley in 1990. BRCA1 is expressed in the cells of breast and other tissue, where it helps repair damaged DNA, or destroy cells if DNA cannot be repaired. If BRCA1 itself is damaged, damaged DNA is not repaired properly and this increases risks for cancers. The protein encoded by the BRCA1 gene combines with other tumor suppressors, DNA damage sensors, and signal transducers to form a large multi-subunit protein complex known as the BRCA1-associated genome surveillance complex (BASC) (38). The BRCA1 protein associates with RNA polymerase II, and through the C-terminal domain, also interacts with histone deacetylase complexes. This protein thus plays a role in transcription, DNA repair of double-stranded breaks ubiquitination, transcriptional regulation as well as other functions (39).

BRCA2 belongs to the tumor suppressor gene family and the protein encoded by this gene is involved in the repair of chromosomal damage with an important role in the error-free repair of DNA double strand breaks. Although the structures of the BRCA1 and BRCA2 genes are very different, at least some functions are interrelated. The proteins made by both genes are essential for repairing damaged DNA. The BRCA2 protein binds to and regulates the protein produced by the RAD51 gene to fix breaks in DNA (40). These breaks can be caused by natural and medical radiation or other environmental exposures, but also occur when chromosomes exchange genetic material during a special type of cell division that creates sperm and eggs (meiosis). The BRCA1 protein also interacts with the RAD51 protein. By repairing DNA, these three proteins play a role in maintaining the stability of the human genome and prevent dangerous gene rearrangements that can lead to hematologic cancer. Like BRCA1, BRCA2 probably regulates the activity of other genes and plays a critical role in embryo development (41).

Human Chorionic Gonodotrophin (β-HCG)

HCG is a marker of germ cell tumours and trophoblastic disease, is a 45KD glycoprotein, comprised of two dissimilar subunits the alpha chain (14KD) and beta chain (24KD). It contains 30 % carbohydrate. The beta subunit determines the immunological and hormone specificity. Beta-hCG is expressed in human fetal tissue and cancer cells of various histologic types. It is degraded to the beta-core fragment, which is concentrated in urine and is also known as urinary gonadotropin peptide. Urinary gonadotropin fragment and lipid-associated sialic acid levels are elevated in up to 60% of patients with endometrial cancer (42).

Increased levels of beta-hCG occur in patients with choriocarcinoma of the uterus, embryonal carcinomas, polyembryomas, mixed cell tumors, and, less commonly, dysgerminomas. Both beta-hCG and human placental lactogen (hPL) are the most useful markers for trophoblastic disease and can be localized in syncytiotrophoblasts of partial and complete hydatidiform moles. The intensity and pattern of reactivity for these antigens differ in partial and complete moles. Gestational choriocarcinomas demonstrate variable but positive staining results for beta-hCG and hPL. The hPL immunostaining differentiates placental-site trophoblastic tumors from choriocarcinomas. The use of beta-hCG is not limited to trophoblastic diseases because it has been localized in a wide array of nontrophoblastic gynecologic neoplasms (43).

Human Telomerase Reverse Transcriptase (hTERT)

Human telomerase reverse transcriptase (hTERT) is a novel and newly available biomarker for patients with ovarian and uterine cancers. The hTERT mRNA level has a significant correlation with CA-125 and with histological findings in ovarian cancer. Serum hTERT mRNA is useful for diagnosing gynecologic cancer and is superior to conventional tumor markers. Upregulation of hTERT may play an important role in the development of cervical intraepithelial neoplasia (CIN) and cervical cancer; hTERT could be used as an early diagnostic biomarker for cervical cancer in the future (44).

Inhibin

Inhibin is a peptide hormone normally produced by ovarian granulosa cells. It inhibits the secretion of follicle-stimulating hormone (FSH) by the anterior pituitary gland. It reaches a peak of 772 +/- 38 U/L in the follicular phase of the menstrual cycle and is normally undetectable in the serum of menopausal women. Granulosa-cell tumors produce inhibin and its serum levels reflect the the tumor burden. Measurement of inhibin can be used as a marker for primary as well as recurrent granulosa cell tumor. The recent availability of markers of ovarian stroma, including melan-A and inhibin-alpha, has provided a means for the positive identification of ovarian stromal tumors, which can manifest in a myriad of histological appearances (45).

The hormonal activity of granulosa cell tumors permits the use of a variety of serum tumor markers in the diagnostic evaluation. Clinically, the most useful serum marker for granulosa cell tumors is inhibin. Inhibin exists in 2 different isoforms, inhibin A and inhibin B. Both isoforms consist of a dimer of 2 subunits, the alpha and beta subunits. The alpha subunit is the same for both isoforms, while the beta subunits differ (beta A and beta B) and show about 64% homology. The 3 subunits (alpha, beta A, beta B) are produced on separate genes located on chromosomes 2 (alpha and beta B) and 7 (beta A). Inhibin usually becomes nondetectable after menopause. However, certain ovarian tumors, mostly mucinous epithelial ovarian carcinomas and granulosa cell tumors, produce inhibin. An elevated inhibin level in a postmenopausal woman or a premenopausal woman presenting with amenorrhea and infertility is suggestive of the presence of a granulosa cell tumor, but not specific. Inhibin levels can also be used for tumor surveillance after treatment to assess for residual or recurrent disease. Although most commercial laboratories only provide assays for inhibin A, serum levels of inhibin B seem to be more frequently elevated. Whenever available, the use of assays is suggested that detect both isoforms. The free alpha subunit can also be measured (46).

CONCLUSION

Scientists continue to study tumor markers and their possible role in the early detection and diagnosis of cancer. The NCI is currently conducting the Prostate, Lung, Colorectal, and Ovarian Cancer screening trial, or PLCO trial, to determine if certain screening tests reduce the number of deaths from these cancers. Along with other screening tools, PLCO researchers are studying the use of PSA to screen for prostate cancer and CA 125 to screen for ovarian cancer. Final results from this study are expected in several years. Cancer researchers are turning to proteomics in hopes of developing better cancer screening and treatment options. Proteomics technology is being used to search for proteins that may serve as markers of disease in its early stages or to predict the effectiveness of treatment or the chance of the disease returning after treatment has ended.

CONFLICT OF INTEREST: Nil

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