Limitations of Conventional Tumor Markers

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1. INTRODUCTION

Tumor markers for malignant diseases can be divided into various stages, namely screening, diagnostic, monitoring and prognosis, and in the foreseeable future, preventive testing. Neither conventional serological tumor markers, spanning from carcinoembryonic antigen (CEA) (1) to prostate-specific antigen (PSA) (2), nor the genomic markers (3) reported in the past 20 yr, have risen to our expectations with regard to early diagnostic or early disease detection.

There are currently about 50 serological tumor markers available (4), some are used for diagnostic purposes in European countries and in Japan. In the United States, however, the application of tumor markers is restricted to monitoring or prognosis rather than diagnosis, except for PSA and nuclear matrix protein (NMP) (5), owing to insufficient sensitivity for early stage detection. Even CA19-9 (6) and CA125 (7) were just approved as diagnostic markers in 2002 for the Medicare reimbursement system.

Relentless efforts have been exerted to find better ways to diagnose cancer. The logistic regression model using multiple makers has been used to improve the sensitivity and accuracy of diagnosis, with only limited success. The proteomics assay (8) especially matrix-assisted laser desorption/ ionization (MALDI) (9) is expected to serve as a new tool for the enhancement of multiple marker assays and for the discovery of new markers.

The simultaneous detection of large numbers of targets such as in proteomics (10) on micro chips (11) as well as in DNA/RNA array will help shed light on the disease process of cancer, but the advantages and efficacies of this technique for diagnostic application will need to be assessed.

Malignant transformations are associated with the mutation or genetic alternation of oncogenes (12) or suppressor genes (13). Suppressor gene alternation is detected in a broader spectrum of cancers than oncogene alternation. More than one gene alternation is required to transform a cell into a cancer cell regardless of whether it is a germ line cell or a somatic cell. In cancer cells, oncogenes mostly involved in growth signal transaction are upregulated from transcriptional stage to phenotypic activity, while suppressor genes involved in DNA repair and genomic integrity are downregulated. Oncogenes are known to be tumor associated rather than tumor specific, in the same way that tumor markers are known to be tumor-associated antigens. From the point of view of clinical application, genomic markers may not be as attractive as anticipated.
On the other hand, through molecular research findings, it has been possible to gain some insight on the molecular pathways that allow malignant growth for some types of cancer. Numerous target cancer drugs are currently under development (13). In pharmacogenomics, a diagnosis is required to identify the cause of cancer for each patient, and new molecular markers are selected for more accurate treatment with fewer side effects when using target cancer drugs instead of conventional cancer drugs. Because each targeting drug requires a specific assay, this field offers interesting prospects of expansion.

Through the advancement of PC-aided graphic imaging technology for cancer diagnostics, it is now possible to detect tumors of less than 10 mm in diameter and determine the exact location of the cancer (14). Medical imaging technology is the most feasible method for the screening or diagnosis of the early stage of cancer and it will likely soon replace serological markers on solid tumors.

It is worth considering imaging technology not only with conventional tracer reagent but also with new tumor markers (15). In the near future, imaging technology is likely to become more cost effective and sensitive, and to detect disease at the earliest stage possible. Cancer diagnosis for prevention or possible risk prediction is the ultimate goal.

2. ADVANTAGES AND LIMITATIONS OF CONVENTIONAL TUMOR MAKERS IN SERUM

The development of tumor specific epitopes or molecules has been elusive in spite of research efforts in science and medicine. Table 1 shows the overall characteristics of popular tumor markers in serum (16–25), selected among over 100 markers reported in the past 50 yr (4). CEA (1) was identified from tumor tissue of colorectal cancer patients through serological adsorption using adjacent normal tissue, a technique similar to the subtraction method using gene cloning.

In terms of sensitivity and specificity at the cutoff point of each marker, CEA is not adequate for diagnostic purposes (16). The α-fetoprotein has a higher sensitivity than CEA with 80%, but its specificity is lower because of the positivity of benign diseases (18).
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CA 19–9, the first tumor marker to be defined by monoclonal antibody, recognizes the sugar chain as specific epitope, not as a whole molecule (6). This specific epitope is present on a wider variety of molecules. It shows about 85% sensitivity and 70% specificity for pancreatic cancer (22). PSA has the highest detection sensitivity with 85% and 85% specificity, and is currently the best serum marker for prostatic cancer (21), CA 15–3 and CA 72–4 both have low sensitivity and high specificity (24,25).

Even typical markers shown here are not sensitive or specific enough for diagnostic and screening purposes. PSA, the most sensitive marker, is highly organ specific, squamous cell carcinoma (SCC) is rather tissue specific (20), and all other markers are less organ specific.

Figure 1 shows the brief relation between detection sensitivity and the stage of diseases at standard classifications for various markers. Most markers increase in serum concentration with the size of the tumor, and the detection sensitivity percent is proportional to the advancement of the tumor. If specific tumor markers without the cutoff value were developed in the near future, more sensitive detection methodology would be required to detect the earliest stage of disease.

Only PSA shows a relatively better sensitivity at the early stage (26,27). PSA is elevated in serum even in tumors of less than 1 cm in diameter (21). NMP bladder cancer marker using urine as specimen has the same sensitivity as PSA at the early stage (28,29).

To improve diagnostic sensitivity and accuracy, an algorithm based on a combination of multiple markers has been applied to various tumors, some achieved improvement on detection sensitivity; however, decisive results have not been obtained (30).

The search for a more sensitive and reliable method is now turning to proteomics analysis (10,31), which allows for the simultaneous measurement of hundreds of test parameters in serum on a chip. However, only two reports have shown proteomic assays as a promising tool for cancer diagnostics.

An assay based on mass spectrometry, surface-enhanced laser desorption/ionization (SELDI), was able to detect ovarian cancer at 100% sensitivity with 95% specificity, including all 18 cases of stage I patients as shown by cluster pattern analysis (32). SELDI assay showed 93% sensitivity for all breast cancer cases at 91% specificity using 3 makers se-
lected from among 147 markers (10). SELDI is a promising method for the discovery of new biological markers. However, further data using serum as specimen must be collected to assess the value of this technology for practical applications in the future. The sensitivity of existing markers at the early stage of diseases is inadequate for diagnostic or screening, but acceptable for monitoring of cancer or prognosis. Following the discovery of PSA 15 yr ago, no other adequate marker has been reported, and therefore we need to look into new genomic related markers as suitable tumor marker candidates. Only in vitro diagnostics using biomarkers can predict the risk of cancer prior to its onset, not imaging diagnostics.

3. NUCLEIC ACID MOLECULE DETERMINATION AS TUMOR MARKERS

Molecular science and gene analysis have contributed to the understanding of cancer biology (33). A single gene defect, developing several hereditary tumors has been reported, and more than hundreds of oncogenes and suppressor genes have been identified (3).

Thus, too much has been expected of gene markers for cancer diagnostics; the development of most cancers involves a complex series of steps (34), and the genes themselves do not reflect any physiological state in the body. A recent study on identical twins has revealed that cancer is weakly influenced by genetic makeup. That means nurture is more important than nature’s encoding (35). The possible targets of nucleic acid determination with regards to cancer so far are as follows:

- Oncogenes/suppressor genes mutation.
- Inactivation of suppressor genes through methylation on CpG island or instability of promoter region.
- mRNA profile of somatic cell.
- Microsatellite mutation (instability).
- Telomere stability and telomerase activity.

Most oncogenes related with intracellular signal-transduction pathways cause cancer by overexpression as a result of mutation, especially in the case of ras-related genes. However, oncogenes or oncogene profiles for cancer diagnostics have proven inadequate for clinical application in spite of intensive investigation.

The mutation of MIC, KRAS/RAF, Her2/EGFR, BCL-2 and other genes is found in a certain percentage of various malignancies (36,37). The combination of oncogenes with conventional tumor markers, such as K-ras mutation with CA19-9 for colorectal and pancreatic cancer improves detection sensitivity and prognostic value (38). The mutation of suppressor genes such as p53 and BR is more common in overall cancers than that of oncogenes.

Table 2 shows the suppressor genes that might be important for possible application on cancer testing (3,39–45). These can be divided into categories according to function of transcription related activity (40), cell-cycle controller, and DNA repair (45,46). These functions require normal cells to prevent disturbing factors such as mismatch accumulation, abnormal cell growth, and cell transformation.

The loss-of-function of single suppressor genes in germline mutations such as p53, RB, BLM causes hereditary cancer. Mutations of BLM (44) and BRCA1/BRCA2 cause colon (42), breast, and ovarian cancer respectively resulting from a defect in DNA repair. PETEN mutation (46), a lack of activity reducing the amount of kinase Akt product, is found in various cancers. KLF6 gene (41) of transcription factor is mutated in 77% of primary prostate cancers.
Various factors trigger the inactivation of suppressor genes. Figure 2 shows various factors for the inactivation of suppressor genes that are sleeping or switched off with methylation on the CpG island within the promoter region (47), other than the existing concepts of deletion or mutation of suppressor genes. Current research reveals the importance of the silencing of suppressor gene by promoter methylation on tumors.

DNMT1/DNMT3b encoded methyltransferase is a key molecule responsible for the methylation of suppressor genes (43). RERb2, retinoic acid receptor gene silenced by methylation (48), which causes acute promyelocytic leukemia (APL) or acute myeloid leukemia (AML), has been confirmed in experimental models. In addition, cases of colon, lung, lymphoma, and breast cancer with a high percentage of methylation have been reported. Tumor suppressor genes play a crucial role in anticancer defense. Suppressor gene somatic cells have, in principle, the same potential to inform on risk of cancer onset and early stages of cancer than oncogenes.
However, whether methylation for genes silenced by the methyltransferase expression product of DNMT1/DNMT2 is specific to the suppressor gene locus or to global genes remains to be solved, and how many genes are involved in the methylation of suppressor genes in cancer in addition to DNMT1/ DNMT3b also remains unclear (43).

Microarray applied on gene expression profile uses tissue or cell as specimen. To improve prognosis and classification of prostate cancer, Saravana et al., analyzed thousands of genes using transcription profile, from normal adjacent prostate to metastatic cancer for over 700 clinical specimens. The expression of hepsin and pim-1 transcript screen showed the correlation with clinical outcome reflecting the progression of the disease (49). In a subsequent study, they identified enhancer of zeste homolog 2 (EZH2) as a candidate of most specific markers for prostatic cancer with regard to the metastatic and lethal progression of the disease (50,51). In addition, gene-expression analysis of solid tumors between primary tumors and metasteses showed a set of genes associated with metastasis and metastatic cells being present in primary tumors.

Prostasin, a potential serum marker for ovarian cancer was also identified through microarray technology (51). The transcription profile-method using microarray is a useful tool for the discovery of new markers. This technology, although more complicated than routine diagnostic application, provides more precise data and should be used in conjunction with pathological diagnosis.

The somatic mutation of microsatellite instability is comparable to a molecular clock showing tumor cell divisions through changes in diversity (53). Current reports show that microsatellite diversity is detected even in the serum of cancer patients using PCR amplification of DNA.

Microsatellite mutation in serum is detected in 71% of patients with small cell lung cancer (53) as well as colon, head, and neck cancers (54), among others. DNA analysis of microsatellite instability in serum, detected 71% of hepatocellular carcinoma in cancer patients with an α-fetoprotein serum concentration of less than cutoff level, and overall hepatocellular carcinoma at 100% sensitivity and 81% specificity when using the profile of 19 marker DNAs (55). Thus, microsatellite instability is a unique potential marker in serum for nonorgan specific cancer, offering the possibility of detecting the early stage of events in cancer. Telomerase activity has been detected in over 90% of human cancers from tissue specimen (56,57) and normal germ cells but not in normal adjacent tumor tissues and is associated with more aggressive tumor behavior.

Telomerase activity measured by telomeric repeat amplification protocol was positive in 81% of colorectal cancers and in 70% of gastric cancers (58,59); another study detected positive telomerase activity in 93% of overall breast cancers (60), including 68% of primary tumors and 95% of advanced stage tumors, among surgically resected samples.

Telomerase detection in body fluids, such as ascites, bronchial washings, and urine has also been demonstrated in various cancers (61). Telomerase enzyme activity was detected in 94.7% of bladder cancers using bladder washes as specimen. Telomerase activity in cancer has no organ specificity, but detection sensitivity and specificity is high compared with other tumor markers in serum.

4. PHARMACOGENOMIC TESTING OF CANCER FOR TARGETING DRUGS

Current advancements in pharmacogenomics are anticipated to lead toward a tailored cancer therapy targeting specific molecules that cause particular tumors (12). Target drugs pro-
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Conventional tumor markers are often used for accurate treatment, offering a judicious, and cost-effective alternative to conventional drugs; they ensure that the optimum dose of the right drug is given to the right patient with fewer adverse effects. Each new target drug needs a specific test to identify the pharmacogenomic background of the patient prior to dose administration. For instance, Herceptin, a breast cancer drug already on the market, is restricted to patients with an overexpression of HER-2/neu. Patients who carry an overexpressed HER-2/neu receptor protein, about 20% of all cancer patients, must be differentiated from others for therapy (62).

Table 3 shows a number of target drugs and their brief characteristics. Gleevec is an Food and Drug Administration (FDA)-approved drug (63,64). Bcr-Abl tyrosine kinase inhibitor is about 90% effective in the early stage of chronic myelogenous leukemia (CML) caused by Bcr-Abl tyrosine kinase abnormality. Iressa (65,66); EGFR tyrosine kinase inhibitor, has only been approved for nonsmall lung cell cancer in Japan.

Both avastin (67), a monoclonal antibody to VEGF protein, and CCI-779 (68,69), an inhibitor to mTOR/S6 kinase signal transaction in PTEN-deficient tumors, are in clinical trial stage. The next target drug should focus on the genes associated with the invasion or metastasis of cancer. Pharmacogenomics might thus provide the chance to develop new molecular marker testing methods that will provide detailed information on the functioning mechanism of targeting drugs. All targeting drugs listed in the table require a new cancer testing approach that characterizes the tumor of each patient with molecular markers, thereby reducing side effects and avoiding the unnecessary use of expensive drugs (70).

5. IMPACT OF IMAGING DIAGNOSTICS ON CANCER IN RELATION WITH TUMOR MARKERS

Tumor biomarkers have improved with time for prognosis and monitoring purposes mainly, but for earlier detection and risk assessment their development have not yet reached a level adequate for clinical applications. In the past two decades, noninvasive imaging technology based on various platforms for cancer diagnostics has made significant advances with the surrounding computer-aided technologies, not only for clinical applications but also for basic research (13).

Table 3
Target Drugs to Specific Genes for Cancer Therapy

<table>
<thead>
<tr>
<th>Drug</th>
<th>Target Gene</th>
<th>Function</th>
<th>Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herceptin</td>
<td>HER-2</td>
<td>Monoclonal antibody to HER-2 protein TK inactivation</td>
<td>Breast</td>
</tr>
<tr>
<td>Gleevec</td>
<td>bcr/abl</td>
<td>Tyrosinekinase Inhibitor</td>
<td>Leukemia</td>
</tr>
<tr>
<td>Iressa</td>
<td>EGFR</td>
<td>Tyrosinekinase Inhibitor</td>
<td>Lung</td>
</tr>
<tr>
<td>bevacizumab</td>
<td>VEGF</td>
<td>Monoclonal antibody to VEGF for anti-angiogenesis</td>
<td>Breast, Colorectal</td>
</tr>
<tr>
<td>CC1-779</td>
<td>PETN/mTOR-S6</td>
<td>mTOR/S6 kinase Inhibitor in PTEN-deficient tumor</td>
<td>Prostate, Brain</td>
</tr>
</tbody>
</table>
In the case of lung cancer, early detection is needed to improve prognosis because most cancers are metastasized when first detected by biomarkers or by cytological assessment of sputum. Mass screening for lung cancer with low-dose X-ray spiral computerized tomography (CT) in mobile units was performed on 5483 individuals from the general population in Japan (13). The detection rate with CT was 0.48%, including cancers of less than 10 mm in diameter, whereas that of standard mass screenings done previously in the same area were 0.03–0.05%, 10 times less sensitive. This high-resolution CT also constitutes an excellent tool for confirmatory discrimination of subtypes of small peripheral lung peripheral adenocarcinomas (71). Magnetic resonance imaging (MRI) on preoperative local staging of patients with pancreatic cancer was applied to discriminate resectability. MRI results showed 98% sensitivity, 92% specificity, and 96% accuracy in patients with suspected pancreatic tumor (72). MRI-guided intervention is now expanding to diagnosis and treatment of prostate cancer, as in the case of fiber-guided endoscopy for colorectal or gastric cancer.

A recently developed method, optical coherence tomography (OCT) combined with optical fiber may differentiate flat malignant from inflammatory lesions, without requiring biopsy. Ultrasonography with improved resolution is now a common screening tool for the physical check-up of healthy individuals. Ultrasonography used as a second-line test to serum CA125 shows an increased detection sensitivity in ovarian cancer (73), similar to the combination of occult blood testing with fiber endoscopy in colorectal cancer.

Positron emission tomography (PET), which produces images by detecting the radiation given off by tracer molecules, has improved in terms of sensitivity; its applications have also been expanded through the development of new tracers responding to specific targets. PET using tracers responding to target genes are being adapted to gene expression study in experimental animals (14). PET imaging of HSV1-tk expression using thymidine analog called FIAU labeled with radioisotope was used in a clinical gene-therapy trial.

Imaging technologies in diagnostics of cancer have acquired a strong position in relation to molecular and biomarkers in serum or tissue. Advancements in imaging technology will soon lead to the development of a downsized and more cost-effective system with higher resolution that will be easier to use. Without additional improvement, most tumor markers could serve as supplemental tools to imaging diagnosis in the near future. The development of tumor markers as possible tracers with the intervention of imaging analysis, a form of functional molecular imaging analysis, and the development of new primary test markers prior to the application of imaging diagnostics, are promising paths to explore for cancer testing. However, imaging technologies are limited in the sense that they cannot predict cancer risk or provide information on the aggravation of the disease (74).

6. SUMMARY

New biomarkers in serum that make up for the limitations of conventional tumor markers need to be improved both in terms of organ specificity and detection sensitivity at the early stage of disease. The proteomics assay, especially that based on SELDI, and the gene expression assay are considered promising tools for the discovery of new biomarkers. The development of new biomarkers that reflect both aggravation and metastasis of tumors is highly desirable.

Compared with phenotypic biomarkers, oncogenes/suppressor genes are not very specific in cancer somatic cells, in spite of initial expectations, and they are not likely to become
mainstream in routine cancer diagnostics. Further studies on suppressor gene silencing as a result of methylation of the promoter region and microsatellite instability need to be evaluated as possible markers to a broad spectrum of cancers. Telomerase activity expressed in somatic cancer cells and associated with aggressive tumor behavior, is the most specific marker. Telomerase activity measured by telomeric repeat has been detected in over 90% of tissue specimens in solid tumors and body fluids. If greater detection sensitivity for telomerase activity is achieved, serum rather than tissue will be used as specimen. The early stage detection of solid tumors in routine screening is thus foreseeable with telomerase activity combined with imaging analysis.

Pharmacogenomic testing for targeting cancer drugs is the most promising emerging routine diagnostics tool. In contrast to conventional cancer drugs, new targeting drugs require corresponding molecular testing to select the right dose for the right patient with fewer adverse effects and effective cost containment.

Imaging technologies based on various platforms have progressed significantly with the aid of computer-aided technologies. Imaging diagnostics can detect as little as 10 mm in diameter of solid tumor in addition to showing localization. Most conventional tumor markers for diagnostics are likely to disappear in the future. The development of new biomarkers either for primary testing prior to imaging diagnostics or for specific tracers of imaging analysis is one option worth investigating for early cancer diagnostics. Only in vitro diagnostics using biomarkers can predict the risk of cancer prior to its onset, not imaging diagnostics.

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