ROLE OF TUMOR MARKERS AND RECENT ADVANCES IN CANCER DIAGNOSIS

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INTRODUCTION

The diagnosis of cancer relies primarily on invasive tissue biopsy, as noninvasive diagnostic tests are generally insufficient to define a disease process of cancer. The conventional histopathology based on light microscopy, however, has recently been complemented with ultrastructure, immunohistochemistry and molecular diagnostics. Molecular medicine has led to the discovery and application of molecular tumor markers, which make histology more accurate and additionally help to prognosticate cancer. Simultaneously, oncologic imaging has undergone remarkable advances. The imaging paradigm is shifting from anatomic and spatial 2D and 3D images to a focus on molecular, functional, biologic and genetic imaging. Recent advances in cancer diagnosis are discussed briefly.

CELLULAR AND MOLECULAR PATHOLOGY

The diagnosis of cancer involves the analysis of tissue and cytology specimens obtained through several procedures, including surgical biopsy, endoscopic biopsy, core or aspirational needle biopsy, venipuncture, spinal tap, pleural or ascitic tap, scraping of tissue surfaces and collection of exfoliative cells from urine and sputum.

Light microscopy, assessing morphologic features remained the standard diagnostic method for many years. The use of enzyme histochemistry and electron microscopy expanded the primary microanatomic evaluation to include biochemical and subcellular structural features. More recently, immunohistochemistry, cytogenetics, analysis of DNA content and molecular genetic assays have been added as valuable adjuncts to light microscopy in cancer diagnosis.

Cytology and histopathology in expert hands are highly efficient diagnostic tests. In certain instances, however, these tests by themselves fail to provide a decisive answer as to whether a lesion

is a tumor or not. Special tests like immunohistochemistry (IHC) when complemented with light microscopy facilitate determination of specific tumor types in many such instances. In situations where material is suboptimal, one may have to opt for molecular markers to reliably characterize and diagnose the sample. Strategies have been developed whereby, from single tissue sample, different techniques can be performed in order to fully characterize molecular phenotype and genotype of tumors.

The optimal application of full spectrum of molecular based techniques requires appropriate harvesting of tissues. It is important that tumor material is collected in a way that prevents contamination by other cell types; also contact with RNA- and DNA- degrading enzymes is avoided. Common method to preserve samples for DNA and RNA analysis includes snap freezing in liquid nitrogen. Whenever possible, a part of specimen should be preserved prospectively for advanced diagnostic tests. In routine practice, however, formalin-fixed and paraffin-embedded tissues are still the major sources of material for both diagnostic and research purposes.

Immunohistochemistry

This technique is based on detection of specific protein sequences (antigenic determinants) of tumors by use of antisera and monoclonal antibodies directed against them. The technique has been greatly supported by the increasing number of commercially available antibodies. Everything from surface receptors to intracellular matrix components to hormones can now be determined with relative ease. IHC has greatly reduced the number of unclassified tumors and has been of major assistance in defining metastatic tumors of unknown primary site.

Moreover, it is of great value in the diagnosis of undifferentiated tumors where light microscopy is unable to discern diagnostic features. Examples include:

- Undifferentiated tumors include poorly differentiated carcinoma, anaplastic large cell lymphoma, amelanotic melanoma or, less commonly sarcoma. Expression of cytokeratins strongly suggests an epithelial origin; leukocyte common antigen (LCA) is evidence of lymphoid origin while expression of S 100 protein and HMB 45 is characteristic of malignant melanoma.

- Small round blue cell tumors of childhood pose a diagnostic challenge especially when the tumor presents as a metastasis. Diagnostic possibilities include lymphoma, neuroblastoma, Ewing's sarcoma, peripheral primitive neuro ectodermal tumor, and rhabdomyosarcoma. Positive staining for LCA would suggest lymphoid malignancy. Presence of neural markers like neuron specific enolase and synaptophysin are suggestive of neuro ectodermal tumors, and the markers of skeletal muscle different-iation, desmin and myoglobin, are indicative of rhabdomyosarcoma.

- Benign lymphoid hyperplasia can be distinguished from indolent non-Hodgkin's lymphoma using staining with kappa and lambda antibodies, which detect light chain restriction in the latter.

- Flow cytometric analysis of CD antigens in hematological malignancies can serve to characterize disease type more specifically. For example, mantle cell lymphoma and chronic lymphocytic leukemia can usually be distinguished by CD23 expression, which is negative in mantle cell lymphoma.

- The presence of micro metastatic disease in bone marrow and lymphnode is suggested by the detection of epithelial proteins in these samples.

  Besides diagnosis, IHC is helpful in assessing prognosis in many cancers. For example:

- Antibodies directed against the proteins involved in the regulation of cell cycle like cyclin D1 and E have been reported to be of prognostic significance in breast cancer and squamous cell carcinoma of head and neck.

- Determination of estrogen, progesterone and Her-2 Neu receptor status in breast cancer helps in predicting response to therapy.

All the advantages of IHC can be nullified if it is used without an expertise. Strict adherence to laboratory practices is essential. A panel of antibodies is generally recommended to characterize a diagnostic problem. Results need to be interpreted in appropriate context; possibility of false positive and false negative remains.

Molecular Oncology

At a molecular level, a cancer cell may be distinguished from its normal counterpart by abnormalities in structure or expression of

certain genes. These abnormalities, directly or indirectly, result in disturbance of cell cycle regulation and induce dysregulated growth in cancer cells. Molecular changes developing during multistep carcinogenesis follow a general pattern, with some alterations being early (essential for tumor development), while others are late events (required for progression or invasion). While leukemias and lymphomas are associated with relatively few but highly specific cytogenetic and molecular genetic abnormalities, solid tumors frequently contain multiple specific and nonspecific changes.

Molecular oncology studies the alterations in genetic and biochemical processes at the molecular level. It helps in establishing a definitive diagnosis and classification of tumors based on the recognition of complex profiles ('finger-prints') or unique molecular alteration that occur in specific tumor types. The changes can be studied on chromosomes, DNA or RNA.

Chromosome analysis

Chromosome abnormalities are frequently found in malignant cells. Many abnormalities may be specific to tumor types. Chromosome rearrangements can be duplications (addition of chromosome), deletions (loss of whole or parts of chromosomes), segmental amplifications (random reiteration of segments or extra fragments), translocations (exchange between chromosomes) and inversions (reversal of orientation).

Analysis of chromosome abnormalities in solid tumors has historically been difficult using tissue sections. In hematological malignancies, individual abnormalities can be easily analyzed on bone marrow aspirate samples.

Detection of chromosome abnormalities has traditionally been performed through banding analysis of metaphase chromosome. The time required for culture and analysis varies; average turn around time is 2 to 3 days for bone marrow, 4 to 7 days for blood and upto 3 weeks for solid tissue biopsies. The method has several disadvantages and subtle abnormalities may be missed. Fluorescence in situ hybridization (FISH) technique is applicable to interphase cells and is more sensitive compared to conventional cytogenetics. It involves hybridization of conjugated probes to chromosomes, and visualization of the probe by fluorescent microscopy. Comparative genomic hybridization (CGH) is a newly described method that globally assays for chromosomal gains

and losses in genomic complement. The molecular cytogenetic techniques like FISH and CGH are increasingly been used in addition to conventional cytogenetics to properly discern various chromosomal abnormalities in tumor samples.

DNA and RNA analysis

Not all mutations in cancer genes are apparent at cytogenetic level, so it has become increasingly important to identify genes themselves, and relevant changes within their structure. The gene alterations can be broadly classified into following:

- Oncogenes: normal cell proteins that become abnormally activated.
- Tumor suppressor genes: normal antiproliferative genes or their products that become inactivated.
- DNA repair genes: become inactivated, causing accumulation of potentially damaging mutations.
- Regulators of apoptosis: inactivation of pro-apoptotic genes or activation of anti-apoptotic genes promotes cell survival.

Molecular detection methods involve the analysis of nucleotide sequences within nucleic acid to detect the presence of malignant cells in fluids and tissues. DNA is, in general, stable in tissues and cells after removal. RNA, on the other hand, is unstable and highly degradable by endogenous RNAses released by lysosomes or dead cells.

The analysis is usually done on total cellular DNA using southern blot (SB) procedure or polymerase chain reaction (PCR) in which regions of DNA are amplified and more easily identified. Messenger RNA (mRNA) detection of genes and their products is done by the techniques like northern blot, reverse transcription-PCR (RT-PCR) and in situ hybridization. Relatively newer techniques like micro-array methods, that allow measurement of differential expression of all the genes including those of low abundance, are still experimental. All these methods are highly sensitive but false positives remain a major problem.

Diagnostic molecular markers for some malignancies are depicted in table 1. One common abnormality is translocation involving an IG locus or T-cell receptor. The result of juxtaposing of distant genes to one of these loci, results in continuous transcriptional drive to B and

T lymphoid cells. Genes translocated to these loci are:

- c-myc in Burkitt's lymphoma
- Cyclin D1 (PRAD1) in mantle cell lymphoma
- BCL2 in follicular lymphoma
- BCL6 in large cell lymphoma
- BCL10 in mucosa-associated lymphoid tissue (MALT) lymphoma

Table 1: Selected molecular genetic markers in cancer diagnosis

Disease	Marker	Method
CML	t(9;22) (q34;q11) [BCR/ABL]	SB, RT-PCR, FISH
CLL	Trisomy 12	FISH
ALL	t(9;22) [BCR/ABL] t(1;19) [E2A/PBX] t(8;14), t(2;8), t(8;22) t(4;11)	RT-PCR, FISH SB, FISH RT-PCR, FISH

AML
    M2
    M3
    M4 Eo
	t(8;21) [AML1/ETO]
	t(15;17) [PML/RARA]
	inv 16 [MYH11/CBFb]
		SB, RT-PCR, FISH

NHL all cases	Antigen receptor gene rearrangement	SB, PCR
Follicular NHL	t(14;18) [BCL2/IGH]	SB, PCR
Burkitt's lymphoma	t(8;14), t(2;8), t(8;22) [MYC;IGH/IGK/IGL]	SB, FISH
Ewings family of tumors	t(11;22) [FL11/EWS]	SB, FISH
Neuroblastoma	MYCN amplification	SB, FISH
Breast cancer	HER2/NEU/ERBB2 amplification	SB, FISH
Bladder cancer	TP53 mutation	PCR
Head & neck cancers	TP53 mutation	PCR
Colon	KRAS mutation	PCR

- CML-chronic myelogenous leukemia, CLL-chronic lymphocytic leukemia, ALL-acute lymphoblastic leukemia, AML-acute myeloid leukemia, NHL- non-Hodgkin's lymphoma, SB-southern blot hybridization, FISH- fluorescent in-situ hybridization, RT-PCR (reverse transcriptase- polymerase chain reaction), PCR-polymerase chain reaction.
- Genes are represented in capital letters within parantheses.Table2: Selected important tumor markers.

Another important example is detection of Philadelphia (Ph) chromosome t (9; 22) (q34; q11) in 95% of chronic myeloid leukemia cases. This translocation involves joining of 5' portion of BCR gene on chromosome 22 to 3' portion of ABL gene on chromosome 9. Conventional cytogenetics, FISH, SB and PCR methods can detect this marker. Even when Ph chromosome is cytogenetically absent, PCR can reveal BCR-ABL abnormality.

TUMOR MARKERS

Tumor markers are biologic or biochemical substances produced by tumors and secreted into blood, urine, other body fluids or body tissues of some patients with certain types of cancer in higher than normal amounts. A tumor marker may be produced by tumor itself, or by the body in response to the presence of cancer or certain non-cancerous conditions. Tumor markers can be detected by various methods including antigen-antibody based techniques (enzyme linked immunosorbent assay, radio-immunoassay, precipitin tests, flow-cytometry, immunohistochemistry, immunoscintigraphy), spectrophotometry, chromatographic techniques and molecular genetic methods.

Measurement of tumor markers levels, when used along with other diagnostic tests, can be useful in the detection and diagnosis of some type of cancers. Some examples of the most commonly measured tumor markers are presented in table 2.

However, in most instances tumor marker levels alone are not sufficient to diagnose cancer for the following reasons:

● False elevation may occur in non-neoplastic conditions as many tumor markers are proteins, over expressed not only by cancer cells, but also by normal tissues e.g. CA-125 is also elevated in conditions like endometriosis and non-malignant ascites besides epithelial ovarian cancer.

● Some markers may be elevated in more than one type of cancer, thereby decreasing the diagnostic accuracy e.g. elevated CEA levels are found in multiple malignancies of gastrointestinal origin. Also many markers share cross-reacting epitopes with products of normal tissues, which leads to errors in their quantitative estimation.

Table 2: Selected important tumor markers

Tumor Marker	Half-life	Malignancies	Nonmalignant conditions	conditions
AFP (Alpha feto protein)	5-7 days	Hepatoblastoma, nonseminomatous germ cell tumor (NSGCT) testis, non-dysgerminomatous germ cell tumor of ovary, hepatocellular carcinoma (HCC), others like gastric, pancreatic and lung.	Cirrhosis, hepatitis	Levels >1000ng/ml in large HCC while 40% with small resectable tumors have normal levels. ~40% of patients with NSGCT have elevated AFP. Levels of AFP along with â-hCG and LDH help in risk stratification of germ cell tumors of testis.
B-hCG (Human chorionic gonadotropin)	18-48 hrs	Choriocarcinoma, hydatidiform mole, testicular germ cell tumors, others like bladder, prostate and kidney.	Hypogonadism	High levels (>103mIU/ml) in choriocarcinoma and H-mole. Used for risk stratificaton of gestational trophoblastic neoplasms.
CEA (Carcino embryonic antigen)	2 weeks	Colorectal cancers, others like breast, cholangiocarcinoma, and stomach. Also in liver metastases, malignant ascites and pleural effusion.	Smokers, fatty liver, hepatitis	Used to follow up the colorectal cancers rather than diagnose.
CA-125	6 days	Epithelial ovarian cancer	Pregnancy, menstruation, endometriosis, ascites and pleural effusion	Levels> 500 U/ml are mostly found in ovarian cancers. Sensitivity of detection in advanced disease is almost 90%while only 50% in stage 1 disease. More useful in the follow-up of patients.
PSA (Prostate specific antigen)	3 days	Prostate cancer	Prostatitis, benign prostatic hyperplasia, prostatic manipulation	Mainly used for screening, though role is controversial

- Tumor marker levels are not elevated in every person with cancer- especially in the early stage of disease.

- No simple tests are yet available with sufficient sensitivity and specificity to detect the presence of a cancer. The field of tumor markers is ever expanding with many new candidate markers either in clinical use or under active evaluation.

More importantly (apart from diagnosis) tumor marker levels reflect the extent (stage) of the disease at diagnosis and can be useful in predicting how well the disease will respond to treatment. If measured serially during the treatment, a decrease or return to normal in the level of tumor marker may indicate a favorable response to treatment while a rising level may indicate that the cancer is growing. Finally, tumor marker assays may be used after treatment has ended as a part of follow up care to check for recurrence.

American Society of Clinical Oncology (ASCO) guidelines state that standard use of a marker in routine clinical practice should only be recommended if the marker reliably adds to the clinician's judgment during clinical decision-making, resulting in a more favorable clinical outcome such as increased survival, improved quality of life and/or reduced cost.

IMAGING

Until recently, there were limited options, besides exploratory surgery or limited radiologic evaluation, available for cancer diagnosis and staging. With the advent of computed tomography (CT) and magnetic resonance imaging (MRI), it became possible to obtain important structural and anatomic information. These techniques often allow differentiation of benign from malignant lesions and assist in accurate staging of disease. During the past decade, in concurrence with the developments in basic molecular biology, remarkable advances have been made in imaging technology and methodology. Molecular imaging with magnetic resonance spectroscopy (MRS) and positrom emission tomography (PET) is currently possible in clinical practice. These modalities permit functional, biochemical and physiologic assessment of important aspects of malignancy.

A diagnosis of 'malignancy' is frequently suspected based on imaging information, later confirmed on histology. Imaging is essential for the diagnosis of all tumors that are not accessible to clinical

evaluation or endoscopy. Sites in which imaging plays a key role for the diagnosis include brain, breast, lung and mediastinum, the tumors arising from the abdominal organs, retro peritoneum and bones.

The choice for a given patient, as to which mode of imaging would be most suitable, depends upon its physical capabilities. Conventional radiography provides the highest spatial resolution and is best suited to study gastro intestinal tract (barium studies), breast and bone. CT scan conversely has higher density discrimination than conventional radiology and is best suited for distinguishing small differences in density between soft tissue, fluid, fat and other structures that cannot be demonstrated on conventional film. Ultrasound has the major advantage that ionization radiation is not used, and is often the initial diagnostic modality for evaluating thyroid, parotid, testicle, liver, kidney and pelvic organs.

Advances in computed tomography

Recent innovations include spiral (helical) CT, multiphase imaging and multi detector scanning. Potential patient benefits include rapid data acquisition and improved detection and characterization of lesions. Spiral CT currently is the preferred technique for detecting pulmonary and liver lesions prior to metastasectomy and for surgical planning of pancreatic and renal cancer treatment. New roles for spiral CT include the detection of pulmonary emboli, CT angiography and endoscopic viewing of hollow organs.

Advances in magnetic resonance imaging

MRI has a number of imaging benefits including superb soft tissue contrast, multiplanar and 3D image acquisition, freedom from ionizing radiation and bony artifacts, and ability to acquire biological and physiological information. MRI is the imaging technique of choice for evaluating tumors in brain, head and neck, spine, breast (when mammography is technically difficult owing to dense breast, silicone implants and scarring due to surgery / trauma), liver and adrenal glands. Recent advances include increased speed of data acquisition and the ability to visualize function superimposed on anatomical changes.

Functional and metabolic imaging

Clinical application of functional imaging in oncology includes characterization of indeterminate lesions on conventional imaging,

disease staging and monitoring response to treatment.

a. Positron emission tomography

PET creates tomographic images that represent the metabolic activity of underlying tissue processes such as glucose, oxygen and amino acid metabolism or measures receptor density status. The most commonly used PET radiotracer for tumor imaging is the glucose analogue 2 [F-18] fluoro 2deoxyDglucose (18-FDG). FDG enters cells and is phosphorylated to FDG-6-phosphate, which becomes trapped within malignant tumor cells with high glucose metabolism.

PET is the most accurate non-invasive technique for detecting and staging lung cancer. It is superior to CT arterial portography in detecting intrahepatic metastases in colorectal cancers and can identify metastatic deposits in lymph nodes that are still small (< 1 cm) and considered benign by CT. In contrast, PET may recognize large masses, such as post therapy fibrotic tissue, as benign if minimal FDG uptake is demonstrated. The most serious limitation to tumor detection with PET is that increased FDG uptake can also be demonstrated in inflammatory tissue.

b. Magnetic resonance spectroscopy

MRS is a powerful, non-invasive method for studying tumor biochemistry and physiology. It measures signals from chemical compounds within tissues; 31 P MRS provides information on tissue energetics and pH while I H- MRS conveys information on cell membrane synthesis and degradation, reflecting cellular proliferation and necrosis.

MRS resonances can provide diagnostic information on tumor grade and are used to monitor tumor response to therapy. MRS has great potential to follow the pharmacokinetics of some chemotherapeutic agents. The "fusion" of functional data with improved imaging capabilities will prove to be more beneficial.

Suggested reading

1. Crook T, Perry AR, Osen P et al. Molecular and cellular pathology of cancer. In Souhami RL, Tannock I, Hohenberger P, Eds. Oxford textbook of oncology, 2nd edition, 2002. Oxford

university press Inc. New York; 227-240.

2.   Rustin GJS. Circulating tumor markers. In Souhami RL, Tannock I, Hohenberger P, Eds. Oxford textbook of oncology 2nd edition 2002. Oxford university press Inc. New York: 303-314

3.   Fishman EK, Urban BA. Cancer diagnosis: imaging. In De Vita VT, Hellman S, Rosenberg SA, Eds. Cancer principles and practice of oncology, 6th edition, 2001. Lippincot Williams and Wilkins, Philadelphia: 659-689.