Advances in Cancer Diagnostics

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Abstract

Malignant transformation of cells into cancer arises due to long term accumulation of genetic and epigenetic events. Early diagnosis of these transformations in cells can improve the prognosis of cancer cases. Cancer screening and surveillance methods include ultrasound, mammography, digital mammography, magnetic resonance imaging, computed tomography, positron emission tomography and magnetic resonance spectroscopy. Other techniques such as immunohistochemistry, in situ hybridization (FISH, CSH), PCR, RT-PCR (real time-PCR), flow cytometry and microarray are used nowadays for diagnosis. Microarray technology is a new and efficient approach to extract data of biomedical relevance for a wide range of applications. In cancer research, it will provide high-throughput and valuable insights into differences in an individual’s tumor as compared with constitutional DNA, mRNA expression, and protein expression and activity. This review highlights the recent developments in cancer diagnostic technologies and describes the eventual use of these technologies for clinical and research applications.

Key words: Cancer, diagnosis, imaging, IHC, PCR, microarray, tumour marker

Introduction

Cancer still remains frequently lethal disease of human as well as animals, especially pet animals, despite the significant progress made in its diagnosis and treatment in recent years. This is a class of diseases in which a group of cells display uncontrolled growth, invasion and sometimes metastasis. Cancer is considered as the second most frequent cause of death in humans and the first one in canines and felines (37). Thirteen percent of the annual deaths worldwide are cancer-related and 70\% of these are in the low and middle income countries (108). Spontaneous cases of tumours in domestic animals especially in canine tumours of which are mostly similar to those of humans, offer an interesting opportunity for comparative studies and to understand cancer biology and drug development (70). The age dependent overall cancer incidence per 100,000 individual per year is approximately 381 in dogs and 264 in cats as against 105 in humans (37, 101). Ministry of health, Brazil (57) found that breast cancer is the predominant cancer in the country, followed by uterine cervix cancer and stomach cancer. The focus of the National Cancer Control Program of India has been on primary prevention, by promoting tobacco control and genital hygiene; secondary prevention by screening for cervical cancer, breast cancer, and oropharyngeal cancer; and palliative care (15). Cancer is generally regarded as a disease of adults. In England only 0.5\% of all cancer cases occur in children less than 15 years of age while in India however, this proportion appears higher at 1.6-4.8\% with variation by place of residence. In dogs the peak age at which tumour cases reported is 8-10 years of age (75, 83).

Tumour markers are the unique attributes of this dynamic process that may reflect the neoplastic process by a high/low level of expression relative to that of normal cells, offering a putative use in diagnosis of cancer. They can be measured quantitatively or qualitatively by chemical, immunological or molecular methods. 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 (IHC) and molecular diagnostics. Non-subjective biological parameters such as tumour ploidy, cell proliferation and hormone receptor status can provide more precise
diagnostic and prognostic information (1). 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 in this review.

**Etiology of Cancer**

Cancer caused by diverse nature of agents such chemical carcinogens, periodic injury (physical, heat, etc.), ionizing radiations, hormones, infectious agents, immunological dysfunction, genetic abnormalities etc. Chemical carcinogens cause DNA mutations are known as mutagens. Tobacco smoking is associated with many forms of cancer (92) and causes 90% of lung cancer (4). Prolonged exposure to asbestos fibers is associated with mesothelioma (62). Decades of research has demonstrated the link between tobacco use and cancer in the lung, larynx, head, neck, stomach, bladder, kidney, oesophagus and pancreas (48). Ionizing radiations also produce mutations such as radon gas and prolonged exposure to ultraviolet radiation from the sun can lead to melanoma and other skin malignancies (19). Non-ionizing radio frequency radiation from mobile phones has also been proposed as a cause of cancer, but there is currently little established evidence of such a link (22).

Many cancers originate from a viral infection; this is especially true in animals such as birds, but also in humans, as viruses are responsible for 15% of human cancers worldwide. The main viruses associated with human cancers are human papillomavirus, hepatitis B and hepatitis C virus, Epstein-Barr virus, and human T-lymphotropic virus. In addition to viruses, researchers have noted a connection between bacteria and certain cancers. The most prominent example is the link between chronic infection of the wall of the stomach with *Helicobacter pylori* and gastric cancer (76, 107).

Some hormones can act in a similar manner to non-mutagenic carcinogens in that they may stimulate excessive cell growth. A well-established example is the role of hyperestrogenic states in promoting endometrial cancer. There are a number of recognized syndromes where an inherited predisposition to cancer, often due to a defect in a gene that protects against tumor formation. For examples, certain inherited mutations in the genes *BRCA1* and *BRCA2* are associated with an elevated risk of breast cancer and ovarian cancer. Other causes include few types of cancer caused by transmission of the tumor cells themselves. This phenomenon is seen in dogs with canine transmissible venereal tumor (58) and in the Tasmanian devil facial tumour disease (61).

**Diagnosis**

The diagnosis of cancer involves the analysis of tissue and cytology specimens obtained through several procedures, including surgical biopsy, core or aspirational needle biopsy, venipuncture, pleural or ascitic tap, scraping of tissue surfaces and collection of exfoliative cells from urine and sputum. Conventional histopathology based on assessing morphology has remained the standard diagnostic method for many years but development of advanced sophisticated technologies like mass spectrometry, microarray and automated DNA sequencing have opened new avenues in cancer diagnosis and therapeutics. The use of enzyme histochemistry and electron microscopy expanded the primary micro-anatomic evaluation to include biochemical and sub-cellular ultra-structural features.

1. **Clinical Symptoms**

Clinical symptoms of cancer vary according to the type and nature of the cancer and its location in different organs. These include gastrointestinal obstruction which may be accompanied by bleeding which is presented as diarrhea and vomiting (commonly associated with tumors invading the stomach, small intestine, large intestine, or colon), hematuria (in tumors of the kidney or bladder), Cushing's disease, hypoglycemia, etc. (in hormone-producing tumors such as some pancreatic, thymic and hepatic tumors), hematological disturbances (95) as anemia, polycythemia, granulocytosis etc. and neurologic symptoms (11, 84) such as loss of coordination or seizures (in tumors of the brain or spinal cord). Cancers producing non-specific symptoms are extremely difficult to be diagnosed for their location, referred to as paraneoplastic disorders. These include weight loss, low-grade fever, seizures, lethargy, loss of appetite, diarrhea, skin rash, hair loss, and general arthritic-like symptoms. These types of cancers require specialized diagnostic techniques such as laboratory screening tests, X-rays, CT scan, MRI etc. which can provide a means for earlier diagnosis and perhaps better long-term prognosis.

2. **Imaging**

A diagnosis of 'malignancy' is frequently suspected based on imaging information, later confirmed on histology. Until now, exploratory surgery or limited radiologic evaluations are most commonly used techniques 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. Molecular imaging with magnetic resonance spectroscopy (MRS) and positron emission tomography (PET) is currently possible in clinical practice. These modalities permit functional, biochemical and physiologic assessment of important aspects of malignancy. 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. Conventional radiography provides the easiest way to diagnose the tumours of gastro intestinal tract, lungs, brain, liver, urinary bladder, breast, bone, joints etc in the pet and domestic animals (9, 17, 89, 96, 110).
2.1 Ultrasound

Ultrasoundography uses high frequency broadband sound waves in the megahertz range that are reflected by tissue to varying degrees to produce images (up to 3D). It used in the diagnosis of cancers of abdominal organs, heart, breast, muscles, tendons, arteries and veins. It is useful in aiding the characterization of lesions (shape, size, density) found on screening mammograms in women with dense breasts (43) and in bitches having mammary tumours (26). While it may provide less anatomical detail than techniques such as CT or MRI, it has several advantages which make it ideal in numerous situations, in particular that it studies the function of moving structures in real-time, emits no ionizing radiation, and contains speckle that can be used in elastography. Ultrasound has also been used for the diagnosis of the tumourous conditions of the abdominal organs of domestic and wild animals (21). Tumours of the hollow organs such as urinary bladder can be diagnosed easily by the ultrasonographic technique (33). Singh et al. (94) used ultrasound guided biopsies (USGB) and ultrasound guided fine needle aspiration biopsies (USGFNAB) to diagnose the hepatic affections in dogs and benefits of these techniques can be utilized for the diagnosis of tumours of other internal organs. High resolution ultrasound can be used to evaluate the tumour volume accurately in the murine orthotopic tumour models without sacrifice (47, 77).

2.2 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 cancerous lesions in pulmonary organs and liver prior to metastasectomy and for surgical planning of pancreatic and renal cancer treatment. Cases of nasal tumours in dogs (44) and horses (103) can be diagnosed and evaluated on the basis of the CT scan. New roles for spiral CT include the detection of pulmonary emboli, CT angiography and endoscopic viewing of hollow organs. Dogs and cats are prone to brain and spine tumours which are life threatening and need to be diagnosed as early as possible before they attain incurable stage. Brain tumours includes the tumours of pituitary, cerebrum, cerebellum, hypothalamus etc. and tumours of spine divide as extradural, intradural-extramedullary, or intramedullary, among them 50% are extradural, whereas 35% and 15% are intradural-extramedullary and intramedullary, respectively (49). So it is certain that CT scan can play an important role in the diagnosis of tumours of brain and spine in dogs and cats (17, 36, 55, 79).

2.3 Magnetic Resonance Imaging

In this technique powerful magnets are used to polarize and excite hydrogen nuclei in water molecules in tissue, producing a detectable signal which is spatially encoded, resulting in images of the body. 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. Recent advances with the use of supercoils have resulted in an increase in sensitivity and specificity (40). Contrast agents used in it are chelates of gadolinium, a lanthamide with three unpaired electrons, which has a very strong magnetic field. MRI is the imaging technique of choice for evaluating tumours 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. Breast MRI has been shown to be capable of detecting early breast cancer, with sensitivities in the range of 95–100%, i.e. a low false-negative rate (30, 64). In veterinary field MRI has been used frequently in detecting macrotumours of brain and spine in dogs (18, 42). Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) is a newer technique by which one can evaluate the kinetic parameters such as blood flow, perfusion, vascular permeability, and the fraction of interstitial space within a tumour. These parameters derived from DCE-MRI present information which are appropriate to noninvasively differentiate canine brain tumors (111).

2.4 Metabolic and Functional Imaging

Functional imaging is a recent tool used in oncology and its uses includes characterization of indeterminate lesions on conventional imaging, cancer staging and monitoring response to treatment.

2.4.1 Positron emission tomography (PET)

PET can detect biologic changes in vivo using radiolabeled tracers. It represents the metabolic activity of underlying tissue processes such as glucose, oxygen and amino acid metabolism or measures receptor density status. Fluorine-18 fluorodeoxyglucose (18F-FDG) is used most commonly, and closely mimics endogenous molecules (27, 41). 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 <1 cm in size 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. Limitation with PET to tumor detection is that increased FDG uptake can also be demonstrated in inflammatory tissue.
2.4.2 Magnetic resonance spectroscopy

MRS is a non-invasive method for studying tumor biochemistry and physiology. It measures signals from chemical compounds within tissues; $P_{31}$ MRS provides information on tissue energetics and pH while $H_1$- 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. In a review by Katz-Brull et al. (41) in a study of five breast H-MRS performed in four independent centers around the world to date, the combined analysis of the data from a total of 153 tumors demonstrated sensitivity and specificity as high as 92% in distinguishing malignant from benign tumors using the choline signal. Of special interest, in a subgroup of 20 younger women the sensitivity and specificity of the method approached 100% (41).

3. Cytologic and Histopathological Technique

Histopathology is still a gold standard for diagnosis of tumors but it alone does not provide sufficient details of the cellular changes which could predict the clinical behaviour of the tumour. Even then histopathological examination of the tumour cells by any expert oncologist can give an accurate diagnosis about the type of tumor and possible malignancy status. Serous effusions from pleural, peritoneal or pelvic cavity can act as biopsy material for diagnosis of status. Serous effusions from pleural, peritoneal or other abnormalities (38). 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.

Apart from the diagnosis, tumor marker levels reflect the stage of the disease and possible prognosis. 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.

New tumour markers are identified by the oncologists time to time to improve the prognosis and to evaluate the behaviour of the tumours. SC6- Ag is a new tumour marker in GIT tumours which is considered as a valuable marker for the diagnosis of pancreatic cancer before and after surgery (52). Similarly, a range of new potential tumour markers is evolving such as Y-Box-binding Protein-1 for neuroblastoma (104), adhesion molecule L1 in oesophageal adenocarcinoma (81), M2 pyruvate kinase (102) etc.

5. Serological Methods

Serological methods used in estimation of serum tumour markers are ELISA and RIA (93, 109). The ELISA is typically used to detect and quantify antigen within biological fluids, in which the Dual-Antibody Sandwich ELISA is being used for measuring the concentration of 80% of tumor markers in blood or serum. RIA is one of the most sensitive technique for detecting antigen or antibody. The principle involves competitive binding of radiolabelled antigen and unlabelled antigen to a high-affinity antibody. Gamma emitting isotope such as Iodine and beta emitting isotope such as tritium are also routinely used as labels. The important step in the RIA is the determination of the amount of antibody needed to bind 60% - 70% of a fixed quantity of radioactive antigen. Determination of amount of bound labeled antigen can be done by precipitating the Ag- Ab complex to separate it from free antigen and the radio activity in the precipitate can be measured (7).

The presence of CEA, AFP, PSA and other markers in the serum of the cancer patients can be
detected with the help of ELFA (Enzyme linked fluorescent assay). The test measures the amount of CEA that may appear in the blood of some people who have certain kinds of cancers, especially large intestine (colon and rectal) and breast cancer. It may also be present in people with cancer of the pancreas, ovary, or lung (2, 100).

6. Immunohistochemistry (IHC)

IHC is based on detection of specific antigenic determinants present in the cells of the tissues by use of polyclonal or monoclonal antibodies (80). IHC has a 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 such as poorly differentiated carcinoma, anaplastic large cell lymphoma, amelanotic melanoma or, less commonly sarcoma. For examples, expression of leukocyte common antigen (LCA) is evidence of lymphoid origin, cytokeratins suggest an epithelial origin (50) while expression of S100 protein and HMB 45 is characteristic of malignant melanoma. This technique has been utilized extensively to determine estrogen (39), progesterone and Her-2 (c-erbB2) receptor status in breast cancer in predicting response to therapy (14). Detection of overexpression of c-erbB2 oncoprotein by IHC also reported in canine mammary tumours (74, 88) and in chemically induced rat mammary tumours (56). Yet other antibodies directed against 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. Expression of other oncoproteins p53, c-myc, c-met, LKB1 etc. readily found in human lung cancer (13, 32), bladder cancer and head and neck cancers. Animal cancers such as ovine pulmonary adenocarcinoma, canine mammary tumours, canine skin tumours, buffalo cutaneous histocytoma and chemically induced rat tumours found positive for different oncoprotein expression and tumour proliferative markers (PCNA, Ki67) by various researchers using IHC technique (68, 69, 71, 72, 74, 75, 82). Presence of neural markers like neuron specific enolase and synaptophysin are suggestive of neuroectodermal tumors, and the markers of skeletal muscle differentiation, desmin and myoglobin, are indicative of rhabdomyosarcoma.

<table>
<thead>
<tr>
<th>TYPE OF TUMOUR MARKER</th>
<th>TUMOURS SHOWING MARKERS</th>
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<tbody>
<tr>
<td><strong>Tumour antigen</strong></td>
<td></td>
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<tr>
<td>Carcinoembryonic Antigen</td>
<td>Cancer of colon, breast, lung, pancreas, stomach, and ovary</td>
</tr>
<tr>
<td>Alpha-Fetoprotein</td>
<td>Hepatocellular carcinoma, testicular germ cell tumors</td>
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<tr>
<td>Prostate Specific Antigen</td>
<td>Prostate cancer</td>
</tr>
<tr>
<td>CA 125</td>
<td>Non mucinous ovarian carcinomas</td>
</tr>
<tr>
<td>CA19-9</td>
<td>Gastrointestinal adenocarcinoma, pancreatic tumours</td>
</tr>
<tr>
<td><strong>Cytoplasmic proteins</strong></td>
<td></td>
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<tr>
<td>Granules of melanin</td>
<td>Melanoma</td>
</tr>
<tr>
<td>Actin</td>
<td>Sarcoma</td>
</tr>
<tr>
<td>Cytokeratin</td>
<td>Epithelial tumours</td>
</tr>
<tr>
<td>Factor III</td>
<td>Endothelial cell in vascular tumour</td>
</tr>
<tr>
<td>Glial fibrillary protein</td>
<td>Astrocytoma</td>
</tr>
<tr>
<td><strong>Hormones</strong></td>
<td></td>
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<tr>
<td>Human chorionic gonadotropin</td>
<td>Trophoblastic tumour, choriocarcinoma, Nonseminomatous testicular tumour</td>
</tr>
<tr>
<td>Calcitonin</td>
<td>Medullary carcinoma of thyroid</td>
</tr>
<tr>
<td>Catecholamines and metabolites</td>
<td>Pheochromocytoma</td>
</tr>
<tr>
<td>Insulin production</td>
<td>Islet cell tumor</td>
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<tr>
<td><strong>Enzymes</strong></td>
<td></td>
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<tr>
<td>Prostatic acid phosphatases</td>
<td>Prostate cancer</td>
</tr>
<tr>
<td>Neuron specific enolase</td>
<td>Small cell cancer of lung, neuroblastoma</td>
</tr>
<tr>
<td>Galactosyl Transferase II</td>
<td>Colon cancer, Pancreatic cancer</td>
</tr>
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Rodrigues et al. (86) found that COX-2 and TGF-beta proteins may cooperate in the process of prostate tumorigenesis in dog as their expression in neoplastic and preneoplastic lesions were much higher. Martins et al. (54) used confocal microscopy to evaluate the immunohistochemical expression of Connexins 43, 26 and 32 in normal, hyperplastic and neoplastic perianal dog glands and found that decrease of connexin expression and the eventual decrease of gap junction intercellular communication capacity coincide with the progress of the carcinogenic process. On the
other hand, Cx32 was not observed in normal, hyperplastic or neoplastic glands.

7. Flow Cytometry

Over the decade, flow cytometry has evolved as an indispensable tool in the diagnosis of hematologic malignancies. Many new antibodies, improved gating strategies, and routine use of multiparameter techniques have dramatically improved the diagnostic utility of flow cytometry. Typically, light scatter is combined with staining for tumor-specific antigen combinations (97). With the addition of technologies to fix cells for permeabilization, intracellular antigens can also be detected by flow cytometry (16). For example TdT only expressed in T cells that reside in the thymus and a limited number of bone marrow cells. The majority of cases of ALL and lymphoblastic lymphoma express TdT. Therefore, if TdT cells are found in the peripheral blood or cerebrospinal fluid, one can identify them as malignant cells. The majority of B-lineage ALL cells expresses TdT, CD19, and CD10, with a smaller number expressing CD34. Any combination of these markers (all of which are found on normal cells in the bone marrow) with the addition of certain aberrant markers such as CD13, CD33, or CD15 may uniquely identify the ALL cells from normal bone marrow or peripheral blood cells. CLL can also be identified in the peripheral blood and bone marrow by flow cytometry. Coexpression of CD5 with either CD20 or CD19 or CD5 with kappa and lambda light chain may detect minimal residual disease to less than 5% sensitivity (85). Three-color flow analysis using CD5, CD20, or CD19 with kappa and lambda may increase this sensitivity to less than 1%. The use of flow cytometry in the veterinary clinical laboratory for the diagnosis of blood malignancies had increased considerably during the past decade (3). The most common applications of flow cytometry in small animal oncology are measurement of DNA content in tumours and immunophenotyping of haematopoietic malignancies (10).

8. Fluorescence In Situ Hybridization (Fish) Technique

This technique involved the specific hybridization of a labeled nucleic acid probe to complementary gene sequence and subsequent visualization by autoradiographic or immunocytochemical method in tissue section, smears or cytocentrifuged cell suspensions. Chromosome abnormalities are frequently found in malignant cells. 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). It is applicable to interphase cells and is more sensitive compared to conventional cytogenetic.

Comparative genomic hybridization (CGH) is a newly described method developed in 1992 and used globally for studies of chromosomal gains and losses in genomic complement. In CGH, test and reference genomic DNA are first differentially labeled with different fluorescent dyes and co hybridized to normal metaphase chromosomes. Then, fluorescent signals along each chromosome are examined and analyzed to provide a cytogenetic pattern of gains and losses. Several investigators have found this method to be useful in cancer studies, suggesting that different tumor types or different stages of tumor progression have distinct CGH patterns (23). These quantitative changes are related to modification in expression level of genes located in the target region. They found a substantial degree of correlation between the two levels of information. To increase resolution, several groups have adapted array technology to CGH, leading to so-called array-CGH (78). Array-CGH has now been established as a new method for molecular characterization of cancers. Moreover, it can serve as a starting point for further screening investigations, such as genome-wide gene expression profiling.

9. Polymerase Chain Reaction (PCR)

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. Microsatellite markers, also known as simple sequence repeats or SSRs (51) are scattered widely within the biological genomes and closely linked with many important genes. In carcinogenesis, microsatellites often display loss of heterozygosity (LOH) as tumour suppressor genes. These are highly polymorphic repetitive DNA sequences that are randomly distributed throughout eukaryotic genomes displaying high levels of variation and are having high mutation rates (1-4 per generation) (107). The PCR process was originally developed to amplify short segments of a longer DNA molecule (85). PCR allows early diagnosis of malignant diseases such as leukemia and lymphomas, which is currently the highest developed in cancer research and is already being used routinely. PCR assays can be performed directly on genomic DNA samples to detect translocation-specific malignant cells at a sensitivity which is at least 10,000 fold higher than other methods. Quantitative PCR methods allow the estimation of the amount of a given sequence present in a sample a technique often applied to quantitatively determine levels of gene expression. Real-time PCR is an established tool for DNA quantification that measures the accumulation of DNA product after each round of PCR amplification.

Mackay et al. (53) opined that Real-time quantitative PCR is a very powerful and accurate technique to examine expression patterns of different oncogene, suppressor genes in different cancerous conditions. Real-time PCR has engendered wider acceptance of the PCR due to its improved rapidity.
sensitivity, reproducibility and the reduced risk of carry-over contamination. There are currently five main chemistries used for the detection of PCR product during real-time PCR.

PCR act as an important tool for the diagnosis of the virus induced tumours of the animals such as cutaneous papillomatosis in cattle, urinary bladder tumours of cattle (12, 67) and buffaloes (99), equine sarcoids (60), papillomatosis in dogs (25, 59) etc.

10. Microarray

Microarray has emerged as a powerful tool to increase the potential of standard methods through genome wide biology studies. DNA microarray technology is a promising approach that allows both qualitative and quantitative screening for sequence variations in the genomic DNA of cancer cells. DNA microarray-based sequence analysis uses comparative hybridization to obtain information ranging from mutational detection to polymorphism genotyping. Sequencing by hybridization is conceptually based on the construction of unknown sequences from hybridization data (105). Labeled DNA for analysis binds strongly only to those targets that are fully complementary to one of its subsequences. Specific binding profile is further checked by a computational algorithm to deduce the whole original sequence.

Mutational Analysis

Detection of mutations in cancer is of major importance for both basic understanding of the disease process and clinical practice. High-density oligonucleotide arrays are commonly used to achieve this purpose. Many early applications of this method concerned breast cancer–associated genes BRCA1 and BRCA2 (20, 28, 29). From this initial success, one can easily predict the impact of specific “mutation arrays,” which test for a variety of known mutations in numerous oncogenes, tumor suppressors, and other genes shown to be of interest in cancer.

Polymorphism Genotyping

Microarray is an appropriate tool to understand how sequence polymorphism may impact biologic functions and be associated with heritable phenotypes. Single nucleotide polymorphisms (SNPs) are the most abundant form of DNA polymorphisms. SNP microarray is an oligoarray in which SNPs are screened by a set of oligonucleotide probes. In a first approach, different oligonucleotides can be used to identify several thousand SNPs and then specific oligonucleotides can be used to genotype these SNPs in various samples (91). SNP microarrays have potential applications in loss-of heterozygosity (LOH) analysis, in disease susceptibility and pharmaco- and toxicogenetic studies.

Screening of Genomic Imbalance

Among genetic changes occurring during carcinogenesis, chromosomal rearrangements with gene copy number fluctuations (including gains and losses of nucleic material) occur frequently. Amplification of oncogene and/or deletion of tumor suppressor gene is a key event in several kinds of cancer. In array-CGH, targets are cloned DNA (bacterial artificial chromosome, yeast artificial chromosome, cosmids, or cDNA) arrayed onto microscopic glass slides. They allow locus-by-locus screening of copy number changes. Bruder et al. (5) applied array-CGH to neurofibromatosis type 2 locus involved in schwannoma.

Evaluation of Gene Expression

Microarray-based expression comparison indicates a panel of up or downregulated genes that are considered as molecular markers for cancer. Expression of genes differ in different types of tumours and may explain some traits i.e. iodothyronine deiodinase mRNA was overexpressed in a human hemangiomia (35) conferring severe hypothyroidism to the young patient bearing this tumor. From complex changes accompanied by human B-cell chronic lymphocytic leukemia progression, Stratowa et al. (98) proposed a list of cancer markers significantly associated with disease staging and patient survival as decreases in expression of interleukin (IL)-1-beta, IL-8, and early growth response protein-1 (EGR1) indicate late stage and combination of decreased expression of L-selectin, integrin beta-2, IL-1-beta, IL-8, and EGR1 and high expression of the TCF1 gene is indicative of low survival. Another example, cDNA microarray screening has identified 176 genes that share a distinct expression pattern between mutated BRCA1 and BRCA2 hereditary breast cancers (31).

Molecular classification of cancer

Ross et al. (62) analyzed gene expression profiles in 60 cancer cell lines and found that cell lines grouped together in agreement with organ type. These relationships are governed by specific expression profiles of peculiar clusters of genes. Other markers are related to the properties of the tissue from which the tumor derived. Specific basal epithelial genes included troponin I, matrix metalloproteinase 14, laminins gamma-2 and alpha-3, annexin VIII, keratins 5 and 17, and integrin beta-4; luminal epithelial related genes were X-Box binding protein 1, GATA binding protein 3, estrogen receptor 1, and annexin XXXI. Furthermore, alterations in the expression of a specific gene provide leads for further investigation into the basis of the tumorigenic phenotype of the cell.

Tissue Array

Relevance of cancer markers identified by genomic or proteomic analysis in the diagnostic, prognostic, and therapeutic of cancer can be evaluated with tissue microarrays or tissue chips (33). This consist of a set of small cylindrical sections (600 μm in diameter, 5 μm thick) acquired from formalin-fixed tissues and arrayed on a glass slide. Typical tissue microarrays contain 500 to 1,000 sections. They are used in large-scale screening of tissue specimens for in situ detection of DNA, RNA, and protein targets or to
survey gene amplification. IHC of arrayed tissue allows measurement of protein levels and has become a mainstay in a two-phase strategy with microarray based gene expression profiling. Indeed, tissue arrays may become a validation tool used in a second analysis to focus on individual targets differentially expressed in cancer by global methods. Camp et al. (5) in his study on 38 breast carcinomas screened for three antigens (estrogen receptor, progesterone receptor, and Her2/new) found similar results in the whole section when they tested at least two samples. Furthermore, they validated the use of archival fixed and embedded tissue to construct tissue arrays in tissue-based molecular research.

Conclusion

There is increasing interest in the development newer and sensitive techniques to screen and diagnose the patients with cancer as early as possible, especially in high-risk groups where conventional technology falls short. Although histopathology remains the standard conventional method for cancer diagnosis but recent techniques such as imaging (MRI, CT, MRS), IHC, PCR, flow cytometry, FISH, CSH and microarray contribute a major breakthrough in diagnosis, prognosis and therapeutics of cancer. Ultrasonography is also limited in resolution, and its diagnostic application is as an adjunct to mammography in mammary tumours. MRI, currently the next most widely used adjunct imaging modality, has been demonstrated to have efficacy in local staging, in evaluating extent of disease and, more importantly, in using architectural enhancement to differentiate benign from malignant lesions. IHC enables to detect the expression of tumour markers and oncoproteins. Similarly molecular techniques used to detect cellular DNA mutations, genetic alterations, abnormal expression of certain genes are Polymerase Chain Reaction (PCR), reverse transcription-PCR (RT-PCR) and in situ hybridization. The most exciting application of this modality is, perhaps, for screening and early detection in high-risk populations. It is likely that, as our understanding of functional and molecular characteristics of tumors improves, a multimodal imaging approach will evolve, enhancing diagnostic accuracy and lowering the current threshold for detection, thus minimizing the loss of lives due to cancer.

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