

DIAGNOSIS OF CANCER

Valivarthi Harika*, Kondi Vanitha, Yasam Harika,
Jampani Yamini and Thotakonda Sravani

Department of Pharmaceutics, Vishnu Institute of Pharmaceutical
Education and Research, Narsapur – 502 313, Hyderabad, Telangana, India.

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 immuno histochemistry, *in situ* hybridization (FISH), 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.

Keywords: Cancer, diagnosis, imaging, IHC, PCR, microarray, tumour marker.

INTRODUCTION

Cancer Definition

Cancer is not just one disease, but a large group of almost 100 diseases. Its two main characteristics are uncontrolled growth of the cells in the human body and the ability of these cells to migrate from the original site and spread to distant sites. If the spread is not controlled, cancer can result in death.

The major risk factors for cancer are: tobacco, alcohol, diet, sexual and reproductive behavior, infectious agents, family history, occupation, environment and pollution.

Cancer types can be grouped into broader categories. The main categories of cancer include:

- Carcinoma

Cancer that begins in the skin or in tissues that line or cover internal organs. There are a number of subtypes of carcinoma, including adenocarcinoma, basal cell carcinoma, squamous cell carcinoma, and transitional cell carcinoma.

- Sarcoma

Cancer that begins in bone, cartilage, fat, muscle, blood vessels, or other connective or supportive tissue.

- Leukemia

Cancer that starts in blood-forming tissue such as the bone marrow and causes large numbers of abnormal blood cells to be produced and enter the blood.

- lymphoma and myeloma

Cancers that begin in the cells of the immune system.

- central nervous system cancers

Cancers that begin in the tissues of the brain and spinal cord.

METHODS

Diagnosis

Diagnosis begins with a thorough Physical examination and a complete medical history. The doctor will observe, feel and palpate (apply pressure by touch) different parts of the body in or

der to identify any variations from the normal size, feel, and texture of the organ or tissue. Different methods are used for the diagnosis of cancer those are

Histological Methods

These methods are based on microscopic examination of properly fixed tissues, supported with complete clinical and investigative data. These methods are most valuable in arriving at the accurate diagnosis. These diagnosis by either of these methods is made on the basis of that cytological features of benign tumours resemble those of normal tissues and that they are unable to invade and metastasise, while malignant tumours are identified by lack of differentiation in cancer cell termed an aplasia (a condition whereby cells lose the morphological characteristics of mature cells and their orientation with respect to each other and to endothelial cells.) or cellular atypia (it is a pathologic term for a structural abnormality in a cell) and may invade as well as metastasis.

Cytological Methods

These methods for diagnosis consist of study of cells shed off into body cavities and study of cell by putting a fine needle introduced under vacuum into the lesion (fine needle aspiration cytology FNAC).

Fine Needle Aspiration Cytology

Fine Needle Aspiration Cytology (FNAC) is a diagnostic procedure where a needle is inserted into your body, and a small amount of tissue is sucked out for examination under a microscope.

It is a quicker and less painful procedure than a Biopsy, but doctors still prefer a biopsy for lymphomas as it gives surer results.

Application

This type of sampling is performed for one of two reasons

1. A biopsy is performed on a lump or a tissue mass when its nature is in question.
2. For known tumors, this biopsy is performed to assess the effect of treatment or to obtain tissue for special studies.

When the lump can be felt, the biopsy is usually performed by a Cytopathologist or a Surgeon. In this case, the procedure is usually short and simple. Otherwise, it may be performed by an interventional radiologist, a doctor with training in performing such biopsies under X-ray or ultrasound guidance. In this case, the procedure may require more extensive preparation and take more time to perform.

Also, fine-needle aspiration is the main method used for chorionic villus sampling as well as for many types of body fluid sampling, as shown in Fig 1 and Fig 2.

Histochemistry and Cytochemistry

These are additional diagnosis tools which helps the pathologist in identifying the chemical composition of cell. Their constituents and their products by special staining methods.

Though immunohistochemical techniques are more useful for tumour diagnosis, histochemical and cytochemical methods are still employed for this purpose.

Immunohistochemistry

This is an immunological method of recognizing a cell by one or more of its specific components in the cytoplasm, cell membrane or nucleus. These cell components are combine with specific antibodies on the formaline fixed paraffin section. The complex of antigen and antibody on the slide is made visible for light microscopic identification by either fluorescent dyes or by enzymes, as shown in Fig 3.

The list of immunochemical stains is ever increasing; one important group of such antibody stains is directed against various classes of intermediate filaments which is useful in classification of poorly-differentiated tumours of epithelial or mesenchymal origin. List of tumours and stains are shown in Table 1.

Electron Microscopy

Ultra structural examination of tumour call offers selective role in diagnostic pathology

1. Cell junctions-their presence and type.
2. Cell surface eg: presence of microvilli.
3. Cell shape and cytoplasmic extinction.
4. Shape of the nucleus and features of nuclear membrane.
5. nucleoli-size and density.
6. cytoplasmic organelles-their number is generally reduced.
7. Dense bodies in the cytoplasm.
- 8 any other secretory products in the cytoplasm eg: melanosomes in melanoma and membrane bound granules in endocrine tumours.

Tumour Markers

Tumor markers are substances that are produced by cancer or by other cells of the body in response to cancer or certain benign (noncancerous) conditions. Most tumor markers are made by normal cells as well as by cancer cells; however, they are produced at

much higher levels in cancerous conditions. These substances can be found in the blood, urine, stool, tumor tissue, or other tissues or bodily fluids of some patients with cancer. Most tumor markers are proteins. However, more recently, patterns of gene expression and changes to DNA have also begun to be used as tumor markers. Markers of the latter type are assessed in tumor tissue specifically.

Thus far, more than 20 different tumor markers have been characterized and are in clinical use. Some are associated with only one type of cancer, whereas others are associated with two or more cancer types. There is no "universal" tumor marker that can detect any type of cancer.

Tumor markers are used to help detect, diagnose, and manage some types of cancer. Although an elevated level of a tumor marker may suggest the presence of cancer, this alone is not enough to diagnose cancer. Therefore, measurements of tumor markers are usually combined with other tests, such as biopsies, to diagnose cancer.

A doctor takes a sample of tumor tissue or bodily fluid and sends it to a laboratory, where various methods are used to measure the level of the tumor marker. List of tumours markers for different cancers are listed in Table 2.

Flow Cytometry

In biotechnology, flow cytometry is a laser-based, biophysical technology employed in cell counting, cell sorting, biomarkers detection and protein engineering, by suspending cells in a stream of fluid and passing them by an electronic detection apparatus. It allows simultaneous multiparametric analysis of the physical and chemical characteristics of up to thousands of particles per second.

Flow cytometry is routinely used in the diagnosis of health disorders, especially blood cancers, but has many other applications in basic research, clinical practice and clinical trials. A common variation is to physically sort particles based on their properties, so as to purify populations of interest.

Flow cytometry is a technology that is used to analyse the physical and chemical characteristics of particles in a fluid as it passes through at least one laser. Cell components are fluorescently labeled and then excited by the laser to emit light at varying wavelengths.

The fluorescence can then be measured to determine the amount and type of cells present in a sample. Up to thousands of particles per second can be analysed as they

pass through the liquid stream. Which is shown in Fig 4 and Fig 5.

Applications

This laser-based technology is used to perform several procedures including:

- Cell counting
- Cell sorting
- Detection of biomarkers
- Protein engineering

Flow cytometry has numerous applications in science, including those relevant to healthcare. The technology has been widely used in the diagnosis of health conditions, particularly diseases of the blood such as leukemia, although it is also commonly used in the various different fields of clinical practice as well as in basic research and clinical trials.

Some examples of the fields this technology is used in include molecular biology, immunology, pathology, marine science and plant biology. In medicine, flow cytometry is a vital laboratory process used in transplantation, oncology, hematology, genetics and prenatal diagnosis. In marine biology, the abundance and distribution of photosynthetic plankton can be analysed. Size of particles is 1-50 micron size. Flow cytometry can also be used in the field of protein engineering, to help identify cell surface protein variants.

This is a computerized technique by which the detailed characteristics of individual tumour cell are recognized and quantified and the data can be stored for subsequent comparison too.

In Situ Hybridization

This is a molecular technique by which nucleic acid sequences can be localized by specifically labeled nucleic acid probe directly in the intact cell rather than by DNA extraction. It is shown in Fig 6.

Molecular Diagnostic Technique:

The group of molecular biologic methods in the tumour diagnostic laboratory are a variety of DNA/RNA-based molecular technique in which the DNA/RNA are extracted from the cell and then analysed. The molecular methods in tumour diagnosis can be applied in hematologic as well as non-hematologic malignancies by

- Analysis of molecular cytogenetic abnormalities.
- Mutational analysis.
- Antigen receptor gene rearrangement and
- By study of the oncogenic viruses at molecular level.

DNA Microarray Analysis of Tumours

Currently, it is possible to perform molecular profiling of a tumour by use of gene chip technology which allows measurement of level of expression of several thousand genes simultaneously.

The core principle behind microarrays is hybridization between two DNA strands, the property of complementary nucleic acid sequences to specifically pair with each other by forming hydrogen bonds between complementary nucleotide. A high number of complementary base pairs in a nucleotide sequence mean tighter non-covalent bonding between the two strands. After washing off non-specific bonding sequences, only strongly paired strands will remain hybridized. Fluorescently labeled target sequences that

bind to a probe sequence generate a signal that depends on the hybridization conditions (such as temperature), and washing after hybridization. Total strength of the signal, from a spot (feature), depends upon the amount of target sample binding to the probes present on that spot. Microarrays use relative quantitation in which the intensity of a feature is compared to the intensity of the same feature under a different condition, and the identity of the feature is known by its position. It is observed in Fig 7,8.

DNA microarray technology is a promising approach that allows both qualitative and quantitative screening for sequence variations in the genomic DNA of cancer cell. DNA microarray based samples.

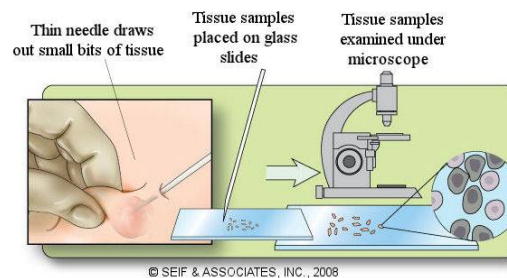


Fig. 1: FNAC method for taking of samples from tissues

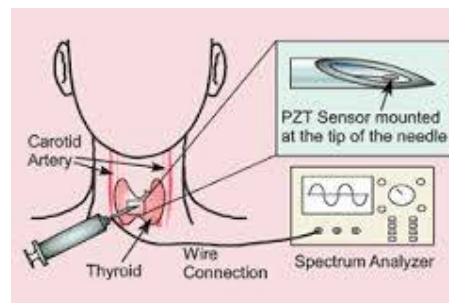


Fig. 2: FNAC method for taking of samples from thyroid tissues

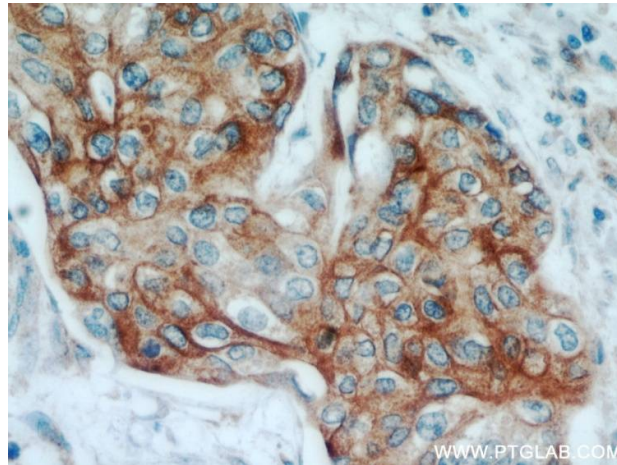


Fig. 3: The formaline fixed paraffine section

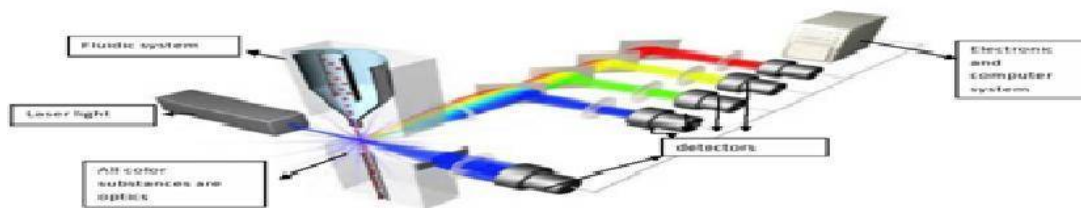


Fig. 4: Flow cytometry

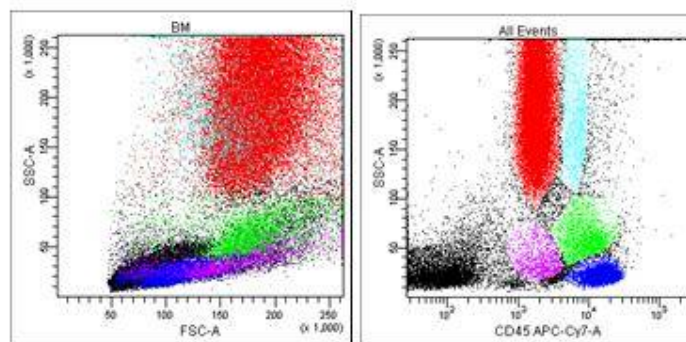
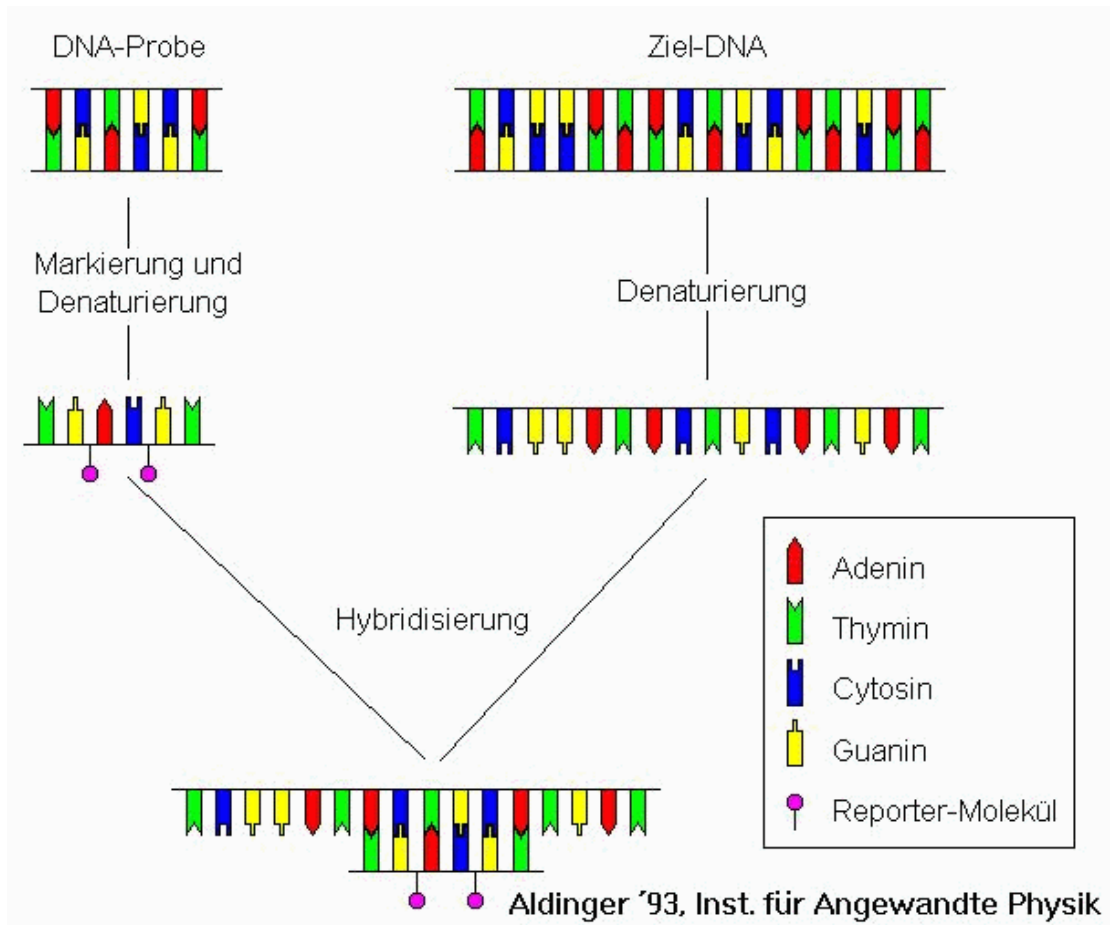


Fig. 5: Graphical representation of different components detected by using flow cytometry



Aldinger '93, Inst. für Angewandte Physik
Fig. 6: In situ hybridizations

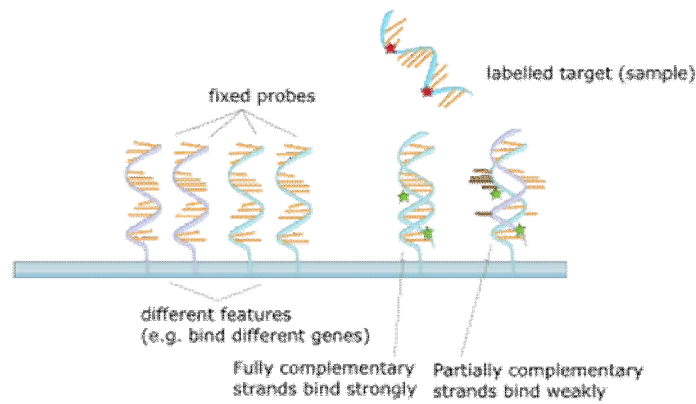


Fig. 7: Hybridization of the target to the probe

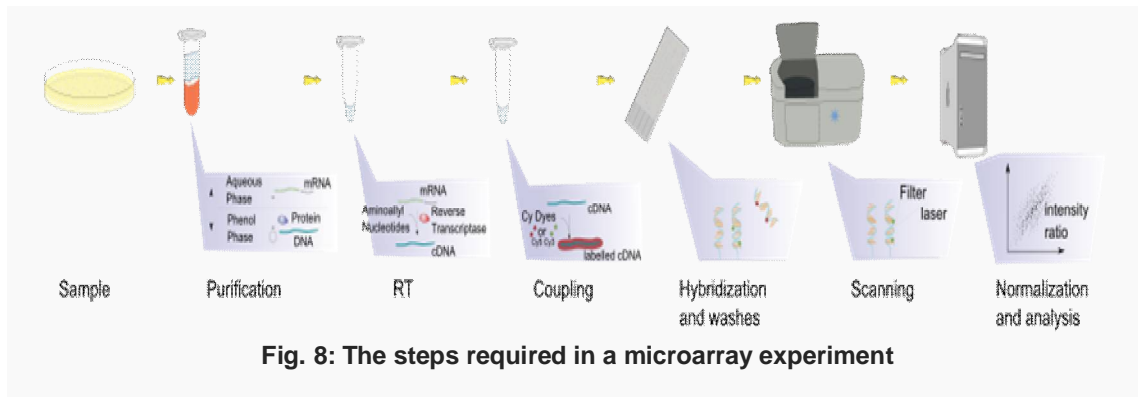


Fig. 8: The steps required in a microarray experiment

Table 1: List of stains

Intermediate filaments	Tumour
Keratin	Carcinomas,mesenchyomas,some germ cell tumours
Vimentin	Sarcomas,melanomas,lymphoma
Desmin	Myogenic tumours
Neurofilaments	Neuraltumours
Glial fibrillary acid protein(GFAP)	Glia tumours

Table 2: List of tumour markers

Markers	Cancer
Oncfoetal antigen Alpha-foetalprotien(afa) Carcinoembryonicantigen(cea) CA19-9	Hepatocellular carcinoma,non –seminomatousgerm cell tumour of testis. Cancer of bowel,pancreas,breast. Gastrointestinal adenocarcinoma,pancreatic tumor
Cytoplasmic proteins Imm unoglobulin Prostatespecific antigen Granules of melanin Actin Cytokeratin Factor III Glial fibrillaryprotien	Multiple myeloma,othergam mopathies Prostate carcinoma Melanoma Sarcoma Epithelial tumor Endothelial cell in vascular tumor Astrocytoma
Enzymes Prostate acid phaosphatase Neuron specific enolase Galactosyltransferasel	Prostatic carcinoma Neuroblastoma,oat cell carcinoma lung Colon cancer,pancreatic cancer
Hormones Human chorionic gonadotropin Calcitonin Catecholamines and vanillylmandelic acid Insulin probe uction Ectopic hormone production	Trophoblastic tumours,nonseminomatous germ cell tumours of testis Medullary carcinoma thyroid Neuroblastoma,pheochromocytoma Islet cell tumor Paraneoplastic syndromes
Secreted cancer antigen CA-125 CA-15-3	Ovary Breast
Serum and tissue protiens	β -2microglobulin,S-100
Other biomolecules	Polyamines

REFERENCES

1. Malati T. Tumour markers: an overview. Indian Journal of Clinical Biochemistry. 2007;22(2): 17-31.
2. Tumor Markers. <http://www.patient.co.uk/doctor/Tumour-Markers.htm>.
3. Perkins GL, Slater ED, Sanders GK and Prichard JG. Serum tumor markers. American family physician. 2003;68(6):1075-1088.
4. Novaković S. Tumor markers in clinical oncology. Radiology and Oncology. 2004;38(2).
5. Harsh Mohan's. Text book of pathology fifth edition fore word Ivan damjanoy. Basics of pathology.
6. Mischel PS, Cloughesy TF and Nelson SF. DNA-microarray analysis of brain cancer: molecular classification for therapy. Nature Reviews Neuroscience. 2004;5(10):782-792
7. Green ED, Guyer MS. Institute N. H. G. R. Charting a course for genomic medicine from base pairs to bedside. Nature. 2011;470(7333):204-213.
8. Tang YW, Procop GW and Persing DH. Molecular diagnostics of infectious diseases. Clinical Chemistry. 1997;43(11):2021-2038.
9. Netto GJ, Saad RD and Dysert PA. Diagnostic molecular pathology: current techniques and clinical applications, part I. Proceedings (Baylor University. Medical Center). 2003;16(4):379.
10. Cobo F. Application of Molecular Diagnostic Techniques for Viral Testing. The open virology journal. 2012;supp1(6):104.