# Popular Bio-Technology Lecture Series-2008

Modern biology is a multidisciplinary subject today. The education in this field thus requires a much broader understanding of emerging areas of life sciences as well as their grass root and advance applications. In this context, the Department of Biotechnology, Govt. of India has introduced a programme titled 'Popular Biotechnology Lectures' in order to create awareness on the basic concepts and recent advances of biotechnology and its applications among students and teachers of schools, colleges and general public. Under this programme, a series comprising three popular biotechnology lectures is organized every year by Punjab State Council for Science & Technology. The Council organized 15th Popular Biotechnology Lecture Series this year on 17.2.09 at Govt. Mohindra College, Patiala. About 47 faculty members/teachers and 310 students from 15 institutes including three schools participated in the programme

The lecture series covered popular lectures on "Current trends in cancer research" by Dr. Veena Dhawan, Associate Professor, Deptt. of Experimental Medicine & Biotechnology, PGIMER, Chandigarh, "Aquaculture" by Dr. Asha Dhawan, Zoologist, Deptt. of Fisheries, Guru Angad Dev Veterinary & Animal Sciences University, Ludhiana, and "Biotechnology in Health Care" by Dr. Dapinder Bakshi, Senior Scientific Officer (Biotechnology), Punjab State Council for Science & Technology. The lectures were followed by elaborate discussion session

# CURRENT TRENDS IN CANCER RESEARCH

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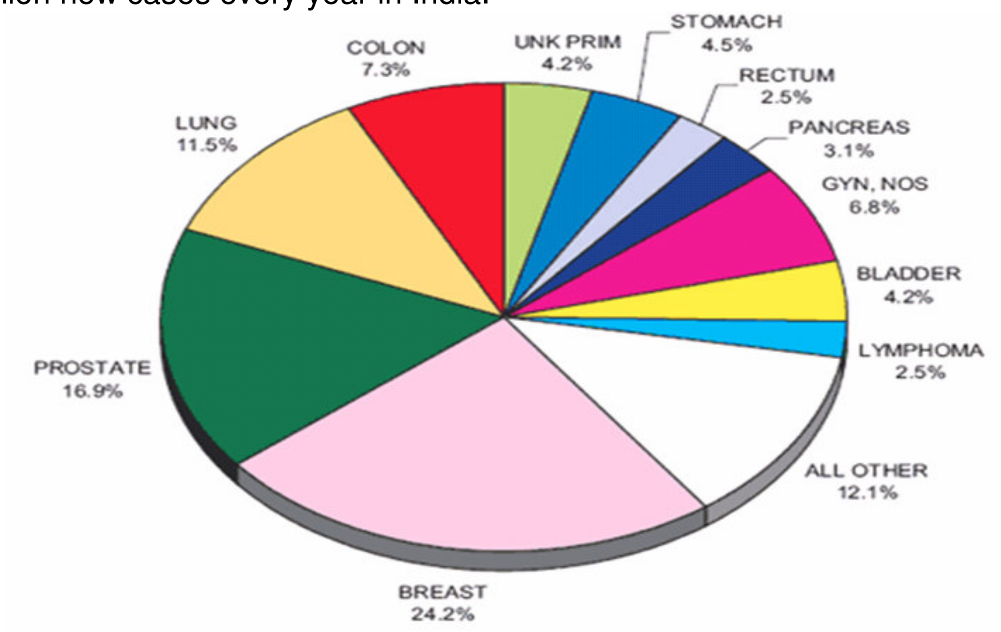
## 1. What is cancer?

**Cancer** (medical term: malignant neoplasm) is a class of diseases in which a group of cells display *uncontrolled growth* (division beyond the normal limits), *invasion* (intrusion on and destruction of adjacent tissues), and sometimes *metastasis* (spread to other locations in the body via lymph or blood).

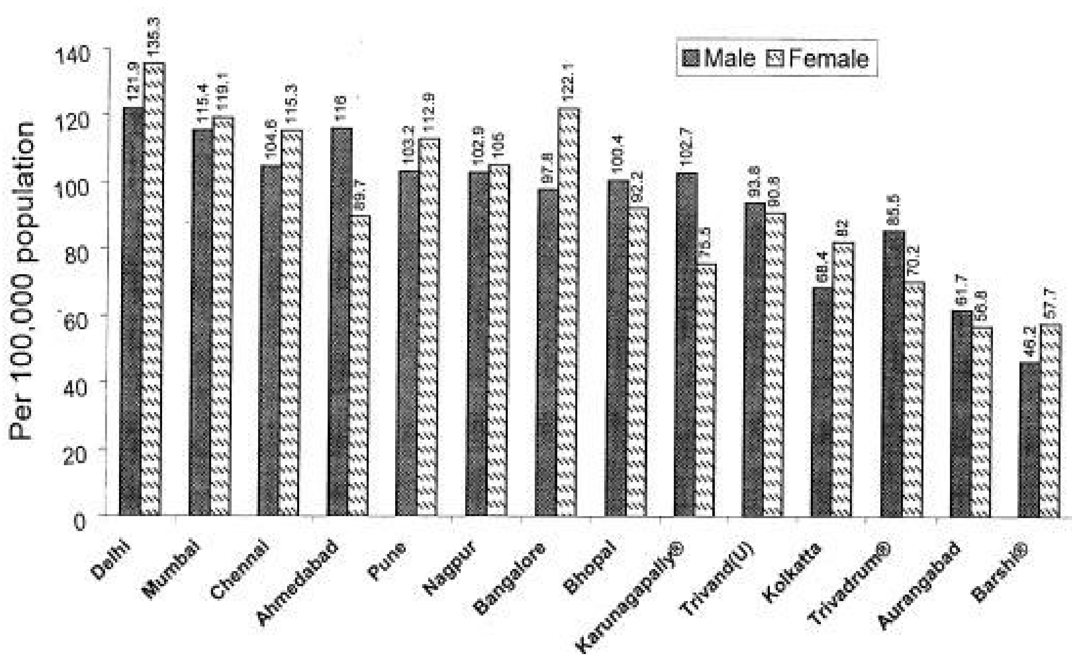
The Greek term carcinoma is the medical term for a malignant tumor derived from epithelial cells. It is Celsus who translated *carcinos* into the Latin *cancer*, also meaning crab. Galen used "*oncos*" to describe *all* tumours, the root for the modern word oncology. Hippocrates described several kinds of cancers. He called benign tumours *oncos*, Greek for swelling, and malignant tumours *carcinos*, Greek for crab or crayfish. He later added the suffix *-oma*, Greek for swelling, giving the name *carcinoma*.

## 2. Epidemiology

It causes about one-fifth of the deaths in the United States each year. Worldwide, between 100 and 350 of each 100,000 people die of cancer each year. Cancer is due to failures of the mechanisms that usually control the growth and proliferation of cells. Cancer may affect people at all ages, even fetuses, but the risk for most varieties increases with age. Cancer causes about 13% of all deaths. According to the American Cancer Society, 7.6 million people died from cancer in the world during 2007. Cancers can affect all animals. Cancer is gaining increasing importance as a public health issue and affects approximately 0.8 million new cases every year in India.




## Age adjusted incidence rates of all cancers in India




## 3. *Classification*

Cancer is generally classified according to the tissue from which the cancerous cells originate, the primary tumor, as well as the normal cell type they most resemble. These are location and histology, respectively. The following closely related terms may be used to designate abnormal growths:

- **Tumor:** Any abnormal swelling, lump or mass. Currently, the word tumor has become synonymous with neoplasm, specifically solid neoplasm. Some neoplasms, such as leukemia, do not form tumors.
- **Neoplasm:** It can be described as an abnormal proliferation of genetically altered cells. Neoplasms can be benign or malignant:
  - **Malignant neoplasm** or **malignant tumor**: synonymous with **cancer**.
  - **Benign neoplasm** or **benign tumor**: a tumor (solid neoplasm) that stops growing by itself does not invade other tissues and does not form metastases.
- **Invasive** tumor is another synonym of **cancer**. The name refers to invasion of surrounding tissues.
- **Pre-malignancy**, **pre-cancer** or **non-invasive** tumor: A neoplasm that is not invasive but has the potential to progress to cancer (become invasive) if left untreated. These lesions are, in order of increasing potential for cancer, atypia, dysplasia and carcinoma in situ.

Over 200 types of cancer are known; grouped into major categories depending upon the type of tissue from which it has originated:

- Carcinomas – epithelial
- Sarcomas – muscle
- Melanomas - melanocytes
- Teratomas – Germ cells
- Leukemias and Lymphomas – Blood cells

## 4. Signs and symptoms

Symptoms of cancer metastasis depend on location of the tumor. Roughly, cancer symptoms can be divided into three groups:

- ***Local symptoms*****:** unusual lumps or swelling (*tumor*), hemorrhage (bleeding), pain and/or ulceration. Compression of surrounding tissues may cause symptoms such as jaundice (yellowing the eyes and skin).
- ***Symptoms of metastasis (spreading)*****:** enlarged lymph nodes, cough and hemoptysis, hepatomegaly (enlarged liver), bone pain, fracture of affected bones and neurological symptoms. Although advanced cancer may cause pain, it is often not the first symptom.
- ***Systemic symptoms*****:** weight loss, poor appetite, fatigue and cachexia (wasting), excessive sweating (night sweats), anemia and specific

paraneoplastic phenomena, i.e. specific conditions that are due to an active cancer, such as thrombosis or hormonal changes.

## 5. Causes

Cancer is a diverse class of diseases which differ widely in their causes and biology. The common cause is the abnormality in the genetic material of the cancer cell and its progeny. Research into the pathogenesis of cancer can be divided into three broad areas:

1. The agents and events which cause or facilitate genetic changes in cells destined to become cancer.
2. The precise nature of the genetic damage and the genes which are affected by it.
3. The consequences of those genetic changes on the biology of the cell.

### *A. Mutation:*

#### *i Chemical carcinogens*

DNA mutations impact cell growth and metastasis. Substances that cause DNA mutations are known as mutagens and mutagens that cause cancers are known as carcinogens. Particular substances have been linked to specific types of cancer.

**Tobacco smoking** is associated with many forms of cancer i.e. 1 in 3 of all cancers and causes 90% of lung cancer. Decades of research has demonstrated the link between tobacco use and cancer in the lung, larynx, head, neck, stomach, bladder, kidney, oesophagus and pancreas. Tobacco smoke contains over fifty known carcinogens, including nitrosamines and polycyclic aromatic hydrocarbons. Prolonged exposure to asbestos fibers is associated with mesothelioma.

Many mutagens are also carcinogens, but some carcinogens are not mutagens. **Alcohol** is an example of a chemical carcinogen that is not a mutagen. Such chemicals may promote cancers through stimulating the rate of cell division. Faster rates of replication leaves less time for repair enzymes to repair damaged DNA during DNA replication, increasing the likelihood of a mutation.

ii. *Ionizing radiation*

Sources of ionizing radiation, such as radon gas, can cause cancer. Prolonged exposure to ultraviolet radiation from the sun can lead to melanoma and other skin malignancies. Radio-frequency radiation from mobile phones has been proposed as a cause of cancer, but there is little evidence of such a link. Nevertheless, some experts caution against prolonged exposure.

*iii. Viral or bacterial infection*

Some cancers can be caused by infection with pathogens. 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 listed below in **Table-1.** Viruses appear to be the second most important risk factor for cancer development in humans, exceeded only by tobacco usage. Hepatitis viruses, including hepatitis B and hepatitis C, can induce a chronic viral infection that leads to liver cancer in 0.47% of hepatitis B patients per year (especially in Asia, less so in North America), and in 1.4% of hepatitis C carriers per year. Combination of cirrhosis and viral hepatitis presents the highest risk of liver cancer development. Worldwide, liver cancer is one of the most common, and most deadly, cancers due to a huge burden of viral hepatitis transmission and disease.

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. Although only a minority of those infected with *Helicobacter* go on to develop cancer, since this pathogen is quite common it is probably responsible for the majority of these cancers.

**TABLE-1**

| VIRUS | ASSOCIATED TUMORS | AREAS OF HIGH INCIDENCE |
|---|---|---|
| *DNA Viruses* | | |
| Papovavirus family | | |
| Papillomavirus | warts (benign) | worldwide |
| (many distinct strains) | carcinoma of the uterine cervix | worldwide |
| Hepadnavirus family | | |
| Hepatitis-B virus | liver cancer (hepatocellular carcinoma) | Southeast Asia, tropical Africa |
| Herpesvirus family | | |
| Epstein-Barr virus | Burkitt's lymphoma (cancer of B lymphocytes) | West Africa, Papua New Guinea |
| | nasopharyngeal carcinoma | southern China, Greenland |
| *RNA viruses* | | |
| Retrovirus family | | |
| Human T-cell leukemia virus type I (HTLV-1) | adult T-cell leukemia/lymphoma | Japan, West Indies |
| Human immuno-deficiency virus | Kaposi's sarcoma | Central and Southern Africa |

| | | |
|---|---|---|
| (HIV, the AIDS virus) | | |

iv. *Hormonal imbalances*

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.

v. *Immune system dysfunction*

The increased incidence of malignancies in HIV patients points to the breakdown of immune surveillance as a possible etiology of cancer. e.g. HIV is associated with Kaposi's sarcoma, non-Hodgkin's lymphoma, and HPV-associated malignancies such as anal cancer and cervical cancer. Certain other immune deficiency states (e.g. common variable immunodeficiency and IgA deficiency) are also associated with increased risk of malignancy.

vi. *Heredity*

Most forms of cancer are "sporadic", and have no basis in heredity. There are, however, a number of recognized syndromes of cancer with a hereditary component, often a defective tumor suppressor allele. Famous examples are:

- certain inherited mutations in the genes *BRCA1* and *BRCA2* are associated with an elevated risk of breast cancer and ovarian cancer
- tumors of various endocrine organs in multiple endocrine neoplasia (MEN types 1, 2a, 2b)
- Li-Fraumeni syndrome (various tumors such as osteosarcoma, breast cancer, soft tissue sarcoma, brain tumors) due to mutations of p53
- Turcot syndrome (brain tumors and colonic polyposis)
- Familial adenomatous polyposis an inherited mutation of the *APC* gene that leads to early onset of colon carcinoma.
- Hereditary nonpolyposis colorectal cancer (HNPCC, also known as Lynch syndrome) can include familial cases of colon cancer, uterine cancer, gastric cancer, and ovarian cancer, without a preponderance of colon polyps.
- Retinoblastoma, when occurring in young children, is due to a hereditary mutation in the retinoblastoma gene.
- Down syndrome patients, who have an extra chromosome 21, are known to develop malignancies such as leukemia and testicular cancer, though the reasons for this difference are not well understood.

vii. *Other causes*

Excepting the rare transmissions that occur with pregnancies and only a marginal few organ donors, cancer is generally not a transmissible disease. The main reason for this is tissue graft rejection caused by MHC incompatibility. In humans and other vertebrates, the immune system uses MHC antigens to differentiate between "self" and "non-self" cells because these antigens are different from person to person. When non-self antigens are encountered, the immune system reacts against the appropriate cell. Such reactions may protect against tumour cell engraftment by eliminating implanted cells. In the United States, approximately 3,500 pregnant women have a malignancy annually, and transplacental transmission of acute leukaemia, lymphoma, melanoma and carcinoma from mother to fetus has been observed. The development of donor-derived tumors from organ transplants is exceedingly rare. The main cause of organ transplant associated tumors seems to be malignant melanoma that was undetected at the time of organ harvest.

A few types of cancer in non-humans have been found to be caused by transmission of the tumor cells themselves. This phenomenon is seen in dogs with Sticker's sarcoma, also known as canine transmissible venereal tumor, as well as Devil facial tumour disease in Tasmanian devils.

## 6. Mechanism

Cancers are caused by a series of mutations. Each mutation alters the behavior of a normal cell In order to transform into a cancer cell; genes which regulate cell growth and differentiation must be altered. Genetic changes can occur at many levels, from gain or loss of entire chromosomes to a mutation affecting a single DNA nucleotide.

There are two broad categories of genes which are affected by these changes:

**Oncogenes** may be normal genes which are expressed at inappropriately high levels, or altered genes which have novel properties. In either case, expression of these genes promotes the malignant phenotype of cancer cells.

**Tumor suppressor genes** are genes which inhibit cell division, survival, or other properties of cancer cells. Tumor suppressor genes are often disabled by cancer-promoting genetic changes. Typically, changes in many genes are required to transform a normal cell into a cancer cell.

Various **chromosomal aberrations** which may contribute to the generation of cancer cells:

**Aneuploidy,** the presence of an abnormal number of chromosomes, is one genomic change which is not a mutation, and may involve either gain or loss of one or more chromosomes through errors in mitosis.

Large-scale mutations involve the **deletion or gain of a portion** of a chromosome. **Genomic amplification** occurs when a cell gains many copies (often 20 or more) of a small chromosomal locus, usually containing one or more oncogenes and adjacent genetic material. **Translocation** occurs when two separate chromosomal regions become abnormally fused, often at a characteristic location. A well-known example of this is the Philadelphia chromosome, or translocation of chromosomes 9 and 22, which occurs in chronic myelogenous leukemia, and results in production of the BCR-abl fusion protein, an oncogenic tyrosine kinase.

**Point mutations, deletions, and insertions**: These are small-scale mutations include, which may occur in the promoter of a gene and affect its expression, or may occur in the gene's coding sequence and alter the function or stability of its protein product. Disruption of a single gene may also result from integration of genomic material from a DNA virus or retrovirus, and such an event may also result in the expression of viral oncogenes in the affected cell and its descendants.

A. *Epigenetics*

Epigenetics is the study of the regulation of gene expression through chemical, non-mutational changes in DNA structure. The theory of epigenetics in cancer pathogenesis is that non-mutational changes to DNA can lead to alterations in gene expression. Known mechanisms of epigenetic change include:

i) DNA methylation
ii) Methylation or acetylation of histone proteins bound to chromosomal DNA at specific locations.

Regulators of epigenetic signalling in cancer cell:

i) Histone Deacetylase inhibitors and DNA methyltransferase inhibitors

**B.** ***Oncogenes***

Mutations in **proto-oncogenes,** which are the normally quiescent counterparts of oncogenes, can modify their expression and function, increasing the amount or activity of the product protein. When this happens, the proto-oncogenes become oncogenes, and this transition upsets the normal balance of cell cycle regulation in the cell, making uncontrolled growth possible.

Oncogenes promote cell growth through a variety of ways. Many can produce hormones, a "chemical messenger" between cells which encourage mitosis, the effect of which depends on the signal transduction of the receiving tissue or cells. Some oncogenes are part of the signal transduction system itself, or the signal receptors in cells and tissues themselves, thus controlling the sensitivity to such hormones. Oncogenes often produce mitogens, or are involved in transcription of DNA in protein synthesis, which creates the proteins and enzymes responsible for producing the products and biochemicals cells use and interact with.

One of the first oncogenes to be defined in cancer research is the ras oncogene. Mutations in the Ras family of proto-oncogenes (comprising H-Ras, N-Ras and K-Ras) are very common, being found in 20% to 30% of all human tumours.

C. *Tumor suppressor genes*

Tumor suppressor genes code for anti-proliferation signals and proteins that suppress mitosis and cell growth. Generally, tumor suppressors are transcription factors that are activated by cellular stress or DNA damage. Often DNA damage will cause the presence of free-floating genetic material as well as other signs, and will trigger enzymes and pathways which lead to the activation of tumor suppressor genes. The functions of such genes is to arrest the progression of the cell cycle in order to carry out DNA repair, preventing mutations from being passed on to daughter cells. The p53 protein, one of the most important studied tumor suppressor genes, is a transcription factor activated by many cellular stressors including hypoxia and ultraviolet radiation damage.

However, a mutation can damage the tumor suppressor gene itself, or the signal pathway which activates it, "switching it off". The invariable consequence of this is that DNA repair is hindered or inhibited: DNA damage accumulates without repair, inevitably leading to cancer. Mutations of tumor suppressor genes that occur in germline cells are passed along to offspring, and increase the likelihood for cancer diagnoses in subsequent generations. Members of these families have increased incidence and decreased latency of multiple tumors.

Development of cancer was proposed in 1971 to depend on at least two mutational events. In what became known as the **Knudson two-hit hypothesis**, an inherited, germ-line mutation in a tumor suppressor gene would only cause cancer if another mutation event occurred later in the organism's life, inactivating the other allele of that tumor suppressor gene.[29]

Usually, oncogenes are dominant, as they contain gain-of-function mutations, while mutated tumor suppressors are recessive, as they contain loss-of-function mutations. Each cell has two copies of the same gene, one from each parent, and under most cases gain of function mutations in just one copy of a particular proto-oncogene is enough to make that gene a true oncogene. On the other hand, loss of function mutations needs to happen in both copies of a tumor suppressor gene to render that gene completely non-functional. However, cases exist in which one mutated copy of a tumor suppressor gene can render the other, wild-type copy non-functional. This phenomenon is called the *dominant negative effect* and is observed in many p53 mutations.

D. *Cancer cell biology*

Often, the multiple genetic changes which result in cancer may take many years to accumulate. During this time, the biological behavior of the pre-malignant cells slowly changes from the properties of normal cells to cancer-like properties. Pre-malignant tissue can have a distinctive appearance under the microscope. Among the distinguishing traits are an increased number of dividing cells, variation in nuclear size and shape, variation in cell size and shape, loss of specialized cell features, and loss of normal tissue organization. Dysplasia is an abnormal type of excessive cell proliferation characterized by loss of normal tissue arrangement and cell structure in pre-malignant cells. These early neoplastic changes must be distinguished from hyperplasia, a reversible increase in cell division caused by an external stimulus, such as a hormonal imbalance or chronic irritation.

**i. *Clonal evolution***

The process by which normal tissue becomes malignant is a process of somatic evolution within the body. Millions of years of biological evolution insure that the cellular metabolic changes that enable cancer to grow occur only very rarely. Most changes in cellular metabolism that allow cells to grow in a disorderly fashion lead to cell death. Cancer cells undergo a process of natural selection, in that the few cells with new genetic changes that enhance their survival or reproduction continue to multiply, and soon come to dominate the growing tumor, as cells with less favorable genetic change are out-competed. This process is called clonal evolution. Tumors often continue to evolve in response to chemotherapy treatments, and on occasion aberrant cells may acquire resistance to particular anti-cancer pharmaceuticals.

### ii. *Biological properties of cancer cells*

In a 2000 article by Hanahan and Weinberg, the biological properties of malignant tumor cells were summarized as follows:

- Acquisition of self-sufficiency in growth signals, leading to unchecked growth.
- Loss of sensitivity to anti-growth signals, also leading to unchecked growth.
- Loss of capacity for apoptosis, in order to allow growth despite genetic errors and external anti-growth signals.
- Loss of capacity for senescence, leading to limitless replicative potential (immortality)
- Acquisition of sustained angiogenesis, allowing the tumor to grow beyond the limitations of passive nutrient diffusion.
- Acquisition of ability to invade neighbouring tissues, the defining property of invasive carcinoma.
- Acquisition of ability to build metastases at distant sites, the classical property of malignant tumors (carcinomas or others).

The completion of these multiple steps would be a very rare event without :

- Loss of capacity to repair genetic errors, leading to an increased mutation rate (genomic instability), thus accelerating all the other changes.

## E. Telomeres, telomerase and cancer

Cancer cells require **limitless replicative potential** to expand to numbers that vastly exceed the number of cells in the human body. Normal human cell types have the capacity for 60–70 doublings (Hayflick limit). Thus the generational limit of normal somatic cells may be a barrier to cancer development.

**Telomeres:** Telomeres are simple-sequence DNA repeats found at the end of chromosomes. Human telomeres contain 250-1500 copies (6-12 kb) of the sequence, **TTAGGG.** At each cell division, 50–100 bp of telomeric DNA are lost from the ends of every chromosome. DNA polymerases are unable to completely replicate the 3′ ends. Progressive shortening of the telomeres occurs with each division; eventually the telomeres lose the ability to protect the ends of the chromosomes. This results in end to end chromosomal fusion and the death of the affected cell. Telomere maintenance occurs in just about all malignant cells.

The majority (85%–90%) possess upregulated expression of an enzyme called **telomerase** which adds hexanucleotide repeats onto the ends of telomeric DNA. The telomeres are thus kept at a length above a critical threshold which allows for unlimited multiplication of descendant cells.

## *7.* Prevention

Cancer prevention is defined as active measures to decrease the incidence of cancer. The epidemiological concept of "prevention" is usually defined as either primary prevention, for people who have not been diagnosed with a particular disease, or secondary prevention, aimed at reducing recurrence or complications of a previously diagnosed illness. This can be accomplished by avoiding carcinogens or altering their metabolism, pursuing a lifestyle or diet that modifies cancer-causing factors and/or medical intervention (chemoprevention, treatment of pre-malignant lesions).

*A. Modifiable ("lifestyle") risk factors*

The vast majority of cancer risk factors are environmental or lifestyle-related in nature, leading to the claim that cancer is a largely preventable disease. Examples of modifiable cancer risk factors include:

- Alcohol consumption (associated with increased risk of oral, esophageal, breast, and other cancers),
- Smoking (although 20% of women with lung cancer have never smoked, versus 10% of men),
- Physical inactivity (associated with increased risk of colon, breast, and possibly other cancers),
- Overweight (associated with colon, breast, endometrial, and possibly other cancers).

Other lifestyle and environmental factors known to affect cancer risk (either beneficially or detrimentally) include certain sexually transmitted diseases (such as those conveyed by HPV), the use of exogenous hormones, exposure to ionizing radiation and ultraviolet radiation, and certain occupational and chemical exposures.

B. *Diet*

The consensus on diet and cancer is that obesity increases the risk of developing cancer. Particular dietary practices often explain differences in cancer incidence in different countries (e.g. gastric cancer is more common in Japan, while colon cancer is more common in the United States). Proposed dietary interventions for primary cancer risk reduction generally gain support from epidemiological association studies. Examples of such studies include:

- Reduced meat consumption is associated with decreased risk of colon cancer,
- Consumption of coffee is associated with a reduced risk of liver cancer.

- Consumption of grilled meat to an increased risk of stomach cancer, colon cancer, breast cancer, and pancreatic cancer,
- Presence of carcinogens such as benzopyrene in foods cooked at high temperatures.
- Consumption of a plant-based diet and lifestyle changes resulted in a reduction in cancer markers in a group of men with prostate cancer who were using no conventional treatments at the time.
- Women on the low fat diet were found to have a markedly lower risk of breast cancer recurrence, in the interim report of December, 2006.
- Reducing intake of refined sugars and starches as cancer prevention regimens.

The WCRF/AICR Expert Report lists some recommendations that people can follow to help reduce their risk of developing cancer, including the following dietary guidelines:

- Reducing intake of foods and drinks that promote weight gain, namely energy-dense foods and sugary drinks,
- Eating mostly foods of plant origin,
- Limiting intake of red meat and avoiding processed meat,
- Limiting consumption of alcoholic beverages,
- Reducing intake of salt and avoiding mouldy cereals (grains) or pulses (legumes).

C. *Vitamins*

The idea that cancer can be prevented through vitamin supplementation stems from early observations correlating human disease with vitamin deficiency, such as pernicious anemia with vitamin B12 deficiency, and scurvy with Vitamin C deficiency. This has largely not been proven to be the case with cancer, and vitamin supplementation is largely not proving effective in preventing cancer.

Epidemiological studies have shown that low vitamin D status is correlated to increased cancer risk. The possibility that Vitamin D might protect against cancer has been contrasted with the risk of malignancy from sun exposure.

Epidemiologists observed that high levels of beta-carotene were associated with a protective effect, reducing the risk of cancer particularly lung cancer. This hypothesis led to a series of large randomized clinical trials conducted in both Finland and the United States (CARET study) during the 1980s and 1990s. Contrary to expectation, these tests found no benefit of beta-carotene supplementation in reducing lung cancer incidence and mortality. In fact, the risk of lung cancer was slightly, but not significantly, *increased* by beta-carotene, leading to an early termination of the study.

Recent reports indicate that folic acid supplementation is not effective in preventing colon cancer, and folate consumers may be more likely to form colon polyps.

D. *Chemoprevention*

The concept that medications could be used to prevent cancer is an attractive one, and many high-quality clinical trials support the use of such chemoprevention in defined circumstances.

Daily use of tamoxifen/raloxifene, typically for 5 years, has been demonstrated to reduce the risk of developing breast cancer in high-risk women by about 50%.

Finasteride, a 5-alpha-reductase inhibitor, has been shown to lower the risk of prostate cancer, though it seems to mostly prevent low-grade tumors.

The effect of COX-2 inhibitors such as rofecoxib and celecoxib upon the risk of colon polyps have been studied in familial adenomatous polyposis patients and in the general population but this came at the price of increased cardiovascular toxicity.

E. *Genetic testing*

Genetic testing for high-risk individuals is already available for certain cancer-related genetic mutations. Carriers of genetic mutations that increase risk for cancer incidence can undergo enhanced surveillance, chemoprevention, or risk-reducing surgery. Early identification of inherited genetic risk for cancer, along with cancer-preventing interventions such as surgery or enhanced surveillance, can be lifesaving for high-risk individuals.

| **Gene** | **Cancer types** | **Availability** |
|---|---|---|
| BRCA1, BRCA2 | Breast, ovarian, pancreatic | Commercially available for clinical specimens |
| MLH1, MSH2, MSH6, PMS1, PMS2 | Colon, uterine, small bowel, stomach, urinary tract | Commercially available for clinical specimens |

F. *Vaccination*

Prophylactic vaccines have been developed to prevent infection by oncogenic infectious agents such as viruses, and therapeutic vaccines are in development to stimulate an immune response against cancer-specific epitopes. e.g.

**Dendritic-cell Vaccines:**

- Harvest dendritic cells from the patient.
- Expose these *in vitro* to antigens associated with the type of tumor in the patient.
- Inject the "pulsed" dendritic cells back into the patient.
- Have shown promise against melanomas, prostate cancer and lymphoma.

**Tumor-specific Antigen Vaccines:**

- Tumor cells are removed from the patient.
- Treated with heat or chemicals so they are not viable
- Mixed with an adjuvant (such as BCG) and injected back into the patient.

Such vaccines are currently in clinical trials for use against chronic myelogenous leukemia CML).

G. *Screening*

Cancer screening is an attempt to detect unsuspected cancers in an asymptomatic population. Screening tests suitable for large numbers of healthy people must be relatively affordable, safe, noninvasive procedures with acceptably low rates of false positive results. If signs of cancer are detected, more definitive and invasive follow up tests are performed to confirm the diagnosis.

Screening for cancer can lead to earlier diagnosis in specific cases. Early diagnosis may lead to extended life, but may also falsely prolong the lead time to death through lead time bias or length time bias. A number of different screening tests have been developed for different malignancies.

- Breast cancer screening can be done by breast self-examination, and with mammograms. It has been shown to reduce the average stage of diagnosis of breast cancer in a population.
- Colorectal cancer can be detected through fecal occult blood testing and colonoscopy, which reduces both colon cancer incidence and mortality, presumably through the detection and removal of pre-malignant polyps.
- Cervical cytology testing (using the Pap smear) leads to the identification and excision of precancerous lesions. Over time, such testing has been followed by a dramatic reduction of cervical cancer incidence and mortality.
- Testicular self-examination is recommended for men beginning at the age of 15 years to detect testicular cancer.
- Prostate cancer can be screened using a digital rectal exam along with prostate specific antigen (PSA) blood testing, though some authorities (such as the US Preventive Services Task Force) recommend against routinely screening all men.

## 8. Diagnosis

Most cancers are initially recognized either because signs or symptoms appear or through screening. Neither of these led to a definitive diagnosis, which usually requires the opinion of a pathologist, a type of physician (medical doctor) who specializes in the diagnosis of cancer and other diseases.

A. *Investigation*

People with suspected cancer are investigated with medical tests. These commonly include blood tests, X-rays, CT scans and endoscopy.

B. *Biopsy*

A cancer may be suspected for a variety of reasons, but the definitive diagnosis of most malignancies must be confirmed by histological examination of the cancerous cells by a pathologist. Tissue can be obtained from a biopsy or surgery.

The tissue diagnosis given by the pathologist indicates the type of cell that is proliferating, its histological grade and other features of the tumor. Together, this information is useful to evaluate the prognosis of this patient and to choose the best treatment.

Cytogenetics and immunohistochemistry are other types of testing that the pathologist may perform on the tissue specimen. These tests may provide information about future behavior of the cancer (prognosis) and best treatment. Diagnostic medicine is being transformed by our new-found ability to monitor large number of cell characteristics. The traditional methods (mainly microscopy of stained cells) will be augmented or replaced by current technologies like DNA microarray analysis which permits measurements of the expression of tens of thousands of genes. In the future, techniques for systematically measuring protein production, modification and localization (important measures of cell states), will give us even more refined portraits of cells which will allow for more successful tailored treatments.

## 9. *Treatment*

The choice of therapy depends upon the location and grade of the tumor and the stage of the disease, as well as the general state of the patient (performance status). Whatever the treatment, the main goal is the complete removal of the cancer without damage to the rest of the body. Because "cancer" refers to a class of diseases, it is unlikely that there will ever be a single "cure for cancer" any more than there will be a single treatment for all infectious diseases. A number of experimental cancer treatments are also under development.

**A. Surgery**

- Some cancer cells remain at the original site.
- Others may have already started to metastasise to distant organs.

**B. Radiation therapy**

- Eradicates cells by inducing apoptosis.
- Can be directed very specifically to where the primary tumor was located in order to destroy any remaining cancer cells. However, undetected metastases elsewhere go untreated.

**C. Chemotherapy**

- Designed to curtail division and proliferation.

- Many normal cells with high turnover rates, such as skin, hair and blood cells, are affected along with the cancer cells.
- Sometimes, cancer cells develop resistance to chemotherapy.

Current therapeutic approaches are thus fairly “blunder bus-like”.

**D.** *Targeted therapies*

Targeted therapy constitutes the use of agents specific for the deregulated proteins of cancer cells and has a significant impact in the treatment of some types of cancer, and is currently a very active research area. Small molecule targeted therapy drugs are generally inhibitors of enzymatic domains on mutated, overexpressed, or otherwise critical proteins within the cancer cell. Prominent examples are the tyrosine kinase inhibitors imatinib (Gleevec/Glivec) and gefitinib (Iressa).

Monoclonal antibody therapy is another strategy in which the therapeutic agent is an antibody which specifically binds to a protein on the surface of the cancer cells. Examples include the anti-HER2/neu antibody trastuzumab (Herceptin) used in breast cancer, and the anti-CD20 antibody rituximab, used in a variety of B-cell malignancies.

Targeted therapy can also involve small peptides as "homing devices" which can bind to cell surface receptors or affected extracellular matrix surrounding the tumor. Radionuclides which are attached to these peptides (e.g. RGDs) eventually kill the cancer cell if the nuclide decays in the vicinity of the cell. Especially oligo- or multimers of these binding motifs are of great interest, since this can lead to enhanced tumor specificity and avidity.

Photodynamic therapy (PDT) is a ternary treatment for cancer involving a photosensitizer, tissue oxygen, and light (often using lasers). PDT can be used as treatment for basal cell carcinoma (BCC) or lung cancer; PDT can also be useful in removing traces of malignant tissue after surgical removal of large tumors.

**E.** *Immunotherapy*

**Boost the patient's immune system and direct it against:**

- **Molecules expressed on the cancer cells but not on healthy cells.**
- **Tumor-specific antigens have been hard to identify.**
- **Many of the immune agents now in use target healthy cells as well.**
- **In the test tube, immune cells are effective in killing the cancer cells.**
- **Unfortunately, *in vivo* once the immune cells meet the cancer cells, nothing further seems to happen.**
- **The immune cells fail to mediate any attack.**

**Doxorubicin (Adriamycin):**

Induces the expression of both Fas and FasL on cancer cells and causes cancer cells to kill themselves by inducing apoptosis.

**Immunostimulants:**

- Injecting the bacterial preparation BCG seems to be effective in early stage bladder tumors.
- With IL-2 or IFN-α has been beneficial in some instances.
- Generally attempts to stimulate the host immune system sufficiently to beat off the cancer have been disappointing.

**F.** *Hormonal therapy*

The growth of some cancers can be inhibited by providing or blocking certain hormones. Common examples of hormone-sensitive tumors include certain types of breast and prostate cancers. Removing or blocking estrogen or testosterone is often an important additional treatment. In certain cancers, administration of hormone agonists, such as progestogens may be therapeutically beneficial.

***G.*** *Angiogenesis inhibitors*

- **Two angiogenesis inhibitors with potential clinical applications are** Angiostatin **(an amino-terminal fragment of plasminogen) and** Endostatin **(20kDa protein derived from carboxyl-terminal domain of collagen XVIII). Both have inhibited angiogenesis in laboratory animals.**
- Antisense constructs against VEGF inhibit experimental angiogenesis.
- Mab's against VEGF-R have also been successful in stopping angiogenesis.
- Genetically engineered cells secreting a soluble form of VEGF-R have been shown capable of inhibiting angiogenesis at distant sites.
- **Small molecule inhibitor of receptor tyrosine phosphorylation** inhibits the tyrosine phosphorylation of VEGF-R and PDGF-R and thus inhibits signalling. They have potent anti-angiogenic effects in pre-clinical models and are undergoing clinical trial.
- Various interferons (e.g. IFNα-2a) are also reported to induce dramatic decreases in urinary excretion of FGF, and complete tumor regression over a three year treatment-free interval.

**Correct Mutated Signalling Pathways:**

Recent experimental strategies attempt to compensate for or correct defective genes. e.g. *ras*

- The Ras protein has to be post-translationally modified; as a lipid molecule attached to it.

- This is catalyzed by an enzyme called farnesyl transferase.
- Inhibitors of farnesyl transferase are being tested in clinical trials as anti–cancer agents.

**Restore Growth Inhibitory Pathways:**
Another (theoretical) approach is to restore the function of growth-inhibiting genes inactivated through mutation. Gene therapy can replace the mutated gene with a functioning one. To date the clinical results have been disappointing.

## *10.* Prognosis

Cancer has a reputation for being a deadly disease. Some types of cancer have a prognosis that is substantially better than nonmalignant diseases such as heart failure and stroke.

Progressive and disseminated malignant disease has a substantial impact on a cancer patient's quality of life, and many cancer treatments (such as chemotherapy) may have severe side-effects. In the advanced stages of cancer, many patients need extensive care, affecting family members and friends. Palliative care solutions may include permanent or "respite" hospice nursing.

- *Emotional impact*

The World Health Organization has promoted National Cancer Control Programme (NCCP) and India is one of the few countries that have actively taken up this initiative. District hospitals in India have the services of specialists and provide reasonable services. These hospitals can have a 'Cancer Detection and Prevention Clinic', which will provide diagnostic services and minimal treatment. Many local organizations offer a variety of practical and support services to people with cancer etc. Support can take the form of support groups, counseling, advice, and financial assistance, transportation to and from treatment, films or information about cancer. Neighborhood organizations, local health care providers, or area hospitals may have resources or services available. Counseling can provide emotional support to cancer patients and help them better understand their illness.

## 11. Research directions

Cancer research is the intense scientific effort to understand disease processes and discover possible therapies. The improved understanding of molecular biology and cellular biology due to cancer research has led to a number of new, effective treatments for cancer since President Nixon declared "War on Cancer" in 1971. Since 1971 the United States has invested over $200 billion on cancer research, that total includes money invested by public and private sectors and foundations.[102] Leading cancer research organizations and projects include the American Association for Cancer Research, the American Cancer Society (ACS), the American Society of Clinical Oncology, the European Organisation for Research and Treatment of Cancer, the National Cancer

Institute, the National Comprehensive Cancer Network, and The Cancer Genome Atlas project at the NCI.

# AQUACULTURE - A STEP TOWARDS DIVERSIFICATION

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Fish is the fastest growing source of food in developing countries. Out of total 95 mt., 60 mt. are used for human consumption. World over, fish constitutes around 20% of total animal protein. Global per capita fish consumption is around 19 kg, whereas in India it is only 9 kg. In India Kerala has four times more consumption than national average.

## ECONOMIC IMPORTANCE OF FISH

**Nutritive value**

- Raw fish has 15-25% protein having all essential amino acids particularly lysine.
- Fish tissue and fish oil in particular are rich in PUFA (Omega n3 & n6- Eicosapentaenole, EPA & Decosahexaenoic acid, DHA) which forms 15-25% of total fatty acids.
- 65% portion of fish is edible.
- Fish is easily digestible (digestibility >90%) due to less connective tissue (3-5%).
- In addition fish flesh is rich in Vitamin B12, A, D, E, K and minerals (Ca, P, I).

**Medicinal Value**:

- ***"Sea food is heart food"***. Amino acids like proline & analin in a particular ratio & Omega 3 fatty acid has the property to arrest cholesterol level, prevent blood clotting, check blood pressure & heart attack.
- ***"Fish as brain food"*** Fish is good for pregnant women & lactating mothers as it contain DHA which makes baby (in womb) more intelligent,

good for sight and development. Mother's milk contains DHA which accounts for 25% of fat contents of brain.

- Fish oil (Tuna, Cod) lower blood sugar & blood pressure in diabetic patients.
- Oily fish (mackerel, salmon, sardines) ward off cancer of prostate, colon, rectum & ovary.
- Fish also reduce risk of arthrosclerosis & tumor growth.
- Prevent neurological disorder such as Alzheimer's disease, asthma and reduce depression.

**Fish products & byproducts**

- Fish body oil is used for the manufacture of insecticidal soap, candles, paints, varnishes, plastic, waterproof material.
- Fish (Tuna, Cod) liver oil has medicinal value
- Fishmeal is used as poultry, fish & animal feed.
- Fish floor is used to prepare bread, biscuits & cakes.
- Fish roe is used in the preparation of food items like sauces, hams & macaroni.
- Fish glue is used as adhesive for paper, wood & leather.

In addition fish act as bait, biological control agent, scavenger & for recreation.

## FISHERIES

Fisheries is an industry which involves exploitation of water bodies for plants and animals of economic importance. It is classified into Capture Fisheries & Culture Fisheries.

***Capture fisheries*** involves the capturing of fishes and other aquatic organisms from water bodies. It is further classified into Inland capture fisheries and Marine capture fisheries. *Inland capture fisheries* involve capturing of fishes and other aquatic animals and plants from inland water bodies. It is further divided into freshwater capture fisheries (rivers, canals, lakes, reservoirs etc) and brackish water capture fisheries (estuaries).

*Marine capture fisheries* involves capturing fishes and other aquatic animals & plants from marine resources like ocean (off shore, deep sea), backwaters, lagoons, bays etc

***Culture Fisheries or Aquaculture*** involves culturing of aquatic plants and animals of economic importance in water bodies under controlled conditions. It is further divided into *Inland aquaculture* (freshwater aquaculture & brackish water aquaculture) and *mariculture*. It is an emerging industry and has tremendous potential.

**FISHERIES RESOURCES OF INDIA AND PRODUCTION**

India has vast fisheries resources in the form of coastline (8118 km), Exclusive Economic Zone, EEZ (2.03 million sq km), continental shelf (0.506 million sq km), rivers & canals (29000 km), reservoirs & lakes(3.15 million ha), ponds & tanks (2.36 million ha), bheels & derelict waters (1.3 million ha) and brackishwater (1.2 million ha). Present fish production of the country is 6.4 mmt (3.4mmt from inland resources and 3.0 mmt from marine resources). Kerala is contributing maximum (21%) from marine resources and west Bengal (29%) from inland resources. India is exporting aquatic animals and plants worth Rs 8000 crores. India contributing 4.7% in global production having 3$^{rd}$ position in capture fisheries and 2$^{nd}$ in aquaculture.

**AQUACULTURE**

The average aquaculture production of the country is around 2.2 million tones, growing @ 6%.

**IMPORTANCE OF AQUACULTURE**

- Source of nutritive food
- Utilization of available water resources & waste land
- Utilization of animal , agricultural & agro-industrial products, by-products and wastes

- Integration of aquaculture with livestock farming and agriculture to utilize maximum on farm resources
- Productive venture
- Reduce pressure on capture fisheries
- Diversification of agriculture
- Easy culture practices
- No marketing problem
- No credit system
- Employment generation
- Foregin exchange
- Source of recreation

## TYPES OF AQUACULTURE

**Depending upon water salinity**, aquaculture is categorized into fresh water, brackish water and marine water culture/mari-culture. Freshwater aquaculture contributes 95% (mostly carp culture) whereas brackish water and mari-culture contributes only 5% (mostly shrimps in brackish-water).

**Depending upon management practices** aquaculture is categorized into extensive, semi-intensive and intensive aquaculture

**Depending upon number of species stocked** aquaculture is categorized into mono culture (single species) & polyculture (more than one species)

**Depending upon water movement** aquaculture is categorized into standing water (ponds, paddy field, sewage ponds) and running water (cage culture, pen culture, re-circulatory system, flow through system)

**Depending upon water temperature** aquaculture is categorized into cold water and warm water aquaculture

## CRITERIA FOR SELECTION OF CULTURABLE SPECIES

- Economically important (high food value)
- Fast growth rate
- Compatible for polyculture

- Marketable size before attaining maturity
- Feed upon locally available ingredients
- High Feed Conversion Rate (FCR)
- Hardy to environment & handling
- Disease resistant
- Early maturity, easy to breed in captivity & high fecundity

**SOME ECOMONICALLY IMPORTANT CULTURABLE SPECIES**

Carps, catfishes, prawns and mussels form important component of culture.

**Freshwater species** include both warm water & cold water species.

**Warm water species**

- India is basically a carp country and contributes 84% of India's aquaculture production. Carps include Indian major carps like *Catla catla* (catla), *Labeo rohita* (rohu) & *Cirrhinus mrigala* (mrigal) and Exotic carps like *Cyprinus carpio* (common carp), *Ctenopharyngodon idella* (grass carp) & *Hypophthalmichthis molitrix* (silver carp). These are cultured in ponds in polyculture system.
- Catfishes, owing to their unique taste are considered a delicacy for fish consumers. These includes air breathing fishes like *Clarias batrachus* (Indian magur), *Heteropneustes fossilis* (singhi) and non airbreathing fishes like *Wallago attu* (mulley), *Mystus seenghala* (seenghara), *Pangasius pangasius* (pangas), *Ompok pabda* (pabda), *O. bimaculatus.* Air breathing catfishes have a greater potential to utilize shallow, swampy, marshy water bodies, whereas, non air breathing catfishes can be well suited to normal pond environment.
- Murrels include *Channa* spp (*C. punctatus, C. striatus, C. marulius*)
- *Notopterus* spp (*N. notopterus, N. chitala*)
- Ornamental fishes
- Freshwater prawn is a priced commodity. It includes *Macrobrachium rosenbergii* (gaint freshwater prawn) & *M. malcolmsonii* (Indian river prawn)

- Mussels include *Lamellidens* spp & *Hyriopsis* spp
- Aquatic plants like *Trapa bispinosa* (water chestnut), *Eurale ferox* (makhana), highly priced *Spirulina* (blue green algae), biofertilizers like *Azolla* & duckweed like *Lemna, Spirodella* & *Wolffia*.

**Coldwater species**

Indigenous species viz. *Schizothorax richardsonii* (snow trout), *Tor putitora* (golden mahseer), *Tor tor* (red fin mahseer) and Exotic species viz *Salmo trutta fario* (brown trout), *Salmon gairdnerii* (rainbow trout), *Cyprinus carpio* var *communis* (scale carp), *C. carpio* var *nudes* (leather carp).

**Brackish water species**

*Mugil cephalis* (grey mullet), *Chanos chanos* (milk fish), *Etroplus suratensis* (pearl spot), *Lates calcarifer* (sea bass), *Penaeus indicus* (Indian white prawn) & *P. monodon* (black tiger prawn) and *Scylla serrata* (mud crab)

**Marine species**

*Pinctada fucuta* (pearl oyster), *Perna virids* (green mussel) & *P. indica* (brown mussel), *Crassostrea madrasensis* (edible oyster), *P. monodon* (black tiger prawn), Panulirus homarus (lobster), *Scylla serrata* (mud crab), Holothuria scabra (sea cucumber), Gracilaria edulis (agar yielding sea weed), and finfishes like *Mugil cephalis* (grey mullet), *Etroplus suratensis* (pearl spot), *Lates calcarifer* (sea bass) and ornamental fishes

## AQUACULTURE TECHNIQUES

### For freshwater aquaculture

Freshwater aquaculture accounts over about 30% of the total fish production of the country and 70% of the total inland fish production. Mainly carps and freshwater prawn are cultured in earthen ponds in composite/ polyculture system. In addition fish culture (carps) is also integrated with agriculture & livestock (dairy, poultry, piggery, duckery) farming which offers great efficiency in resource utilization. Fish (high value species like catfishes & prawn) culture in cages & pens (in large water bodies like lakes & reservoirs), in re-circulatory and running water (flow through) system are in its infancy (not commercialized yet). Waste water aquaculture or sewage fed aquaculture is commercialized in Kolkata in about 10000 ha area. Paddy cum fish farming is age long practice in coastal states. In mountain areas of the country, fish culture is carried out in tanks, ponds and running water raceways as a small scale activity.

**For brackish water aquaculture**

Brackish water aquaculture is confined mainly to bheries (manmade impoundments in coastal wetlands) of west Bengal and pokkali (salt resistant deepwater paddy) fields along the Kerala coast. Natural seed of fish & shrimp is trapped in these systems. Now along the coastline, 0.152 million ha has bought under shrimp culture (*P. monodon*) (5% of the aquaculture production) having excellent export value. Commercial cultivation of brackish water finfish (sea bass, milk fish, pearl spot, mullets, mud crab) is almost non existent.

**For mari-culture**

Mari-culture is the rearing of aquatic organisms in coastal & off shore waters in cages & pens, rafts, long lines & racks. It is in its infancy & expected to be a major activity in the years to come.

## AQUACULTURE ALLIED INDUSTRY

In addition to carry out culture and breeding of aquatic organisms, other activities involve

- Transportation & Marketing (including export & import)
- Preservation, processing & value addition
- Fishing gears & crafts
- Fish feed, fertilizers, chemicals, medicines etc
- Human Resource Development

India utilizes only about 40% of the available 2.36 million ha of ponds & tanks for freshwater aquaculture, 15% of total potential brackish water resources of 1.2 million ha. Productivity from capture fisheries is only 20 kg/ha/yr against potential of more than 100 kg/ha/yr and productivity from aquaculture is 2.4 tonnes/ha/yr against potential of 15 tonnes/ha/yr. So there is room for both horizontal & vertical expansion of these resources.

# BIOTECHNOLOGY IN HEALTH CARE

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Biotechnology may be defined as any technological application that make use of biological systems, living organisms, their derivatives in order to make or modify products or processes for specific use. Conventional biotechnology has been practiced worldwide for centuries in the form of traditional fermentation techniques where microorganisms were used for preparation of bread, cheese, beer etc. and traditional animal and plant breeding techniques where plants and animals were selectively bred for useful traits. Past decades have witnessed the ushering of modern biotechnology, which with the advent of Genetic Engineering Tools and Recombinant DNA Biotechnology has made it possible to alter and modify the genetic makeup of organisms and production of unique individuals / traits.

**Branches of Biotechnolgy :** There are several branches of biotechnolgy

1. ***Agricultural Biotechnology (Green Biotechnology)*** is biotechnology applied to agricultural processes e.g. selection and domestication of plants via micropropagation and designing of transgenic plants.
2. ***Industrial Biotechnology (White Biotechnology)*** is the application of biotechnology for industrial purposes, including manufacturing, alternative energy and biomaterials. It includes the practice of using cells or components of cells like enzymes to generate industrially useful products.
3. ***Medical Biotechnology (Red Biotechnology)*** is the application of biotechnology for medical processes e.g. development of drugs, vaccines and use of gene therapy for treatment of genetic disorders.
4. ***Marine Biotechnology (Blue Biotechnology)*** is biotechnology applied for marine and aquatic applications.

## Status of Biotechnology in India

The enormous economic potential of biotechnology has been well recognized by India. The first endeavour in this direction was creation of National Biotechnology Board (NBB) under the Ministry of Science and Technology in 1982 for the planning, promotion and coordination of biotechnology in the country. NBB was further upgraded in 1986 to the Department of Biotechnology (DBT) with mandate for development of infrastructure, manpower, research & development along with boost to the Biotech industry in the country. The funding and initiatives of DBT have been successful in generating a rich pool of academicians and scientists in the country.

Further, initiative in this regard has been development of **National Biotechnology Development Strategy** in 2007 by Government of India after two-year-long nationwide consultation process with multiple stakeholders. The salient features of strategy are:

- **Regulation :** Setting up of National Biotechnology Regulatory Authority for single window mechanism for biosafety clearance of GM products and processes.
- **Promoting Biotech Industry :** 30% of DBT's Budget to be spent on Public-private partnership progammes.
- **Building world class Human Capital**
  - Star Colleges in Life Sciences : Lending luster to learning
  - Autonomous institutes to promote excellence in R & D
  - UNESCO Centre globally oriented training
  - Centre of Excellence in Biotechnology
  - New Initiatives for Tech transfer and IP capacity building

## Biotech industry in India

The Government of India is promoting biotech industry and its products. Funds through Venture Capitalists (VCs) for biotech startups, rebate on R&D, 100% foreign direct investment, excise and customs duty waiver on certain products, etc. are some of the incentives introduced by the government. The Indian biotech industry today encompasses 325 companies. Some of the leading companies being Biocon, Serum Institute of India and Panacea Biotec which are alone contributing to 27% of revenues. Others include Nicholas Piramal, Wockhadrt Limited, Glaxo SmithKline, Bharat Serum Krebs Biochemicals & Industries Limited, Zydus Cadila and Indian Immunologicals. Indian biotech firms have started scaling up their capabilities to become global players and attracted overseas partners & investors from countries like Singapore, Japan, Taiwan, Korea and China.

***Biocon*** **:** Biocon is the India's first and presently the leading and largest biotech company in India established in 1978. It is a fully integrated healthcare company that delivers innovative biopharmaceutical solutions. Earlier Biocon was in manufacturing microbial enzymes and was the first India's biotechnology company which generated revenues by exported them to USA and Europe. In past few years, Biocon has transformed into drug firm and ventured into therapeutics drugs for the treatment of cancer, autoimmune and metabolic diseases. It is also the first Indian company to be approved by US FDA for the manufacture of lovastatin, a cholesterol-lowering molecule. Biocon is also the first company globally to manufacture first recombinant human insulin. The company has also launched India's first anti-cancer, therapeutic Monoclonal Antibody-based drug for treating solid tumors.

## Status of Biotechnology in Punjab – a leading destination for biotechnology

The Govt. of Punjab has recognized biotechnology as a sunrise area. In order to promote knowledge intensive sector of biotechnology for growth in agriculture and agri-processing, the State Govt. has taken major initiatives for creating world-class infrastructure to support R&D as well as industrial growth in the said areas with help of Department of Biotechnology, Govt. of India. The first state-of-the-art Agri-Food Biotech Cluster is being set up in Knowledge City, Mohali as a joint venture of GOP and DBT, GOI. The cluster is going to comprise of National Agri-food Biotechnology Institute (NABI), Bioprocessing Unit (BPU), Biotechnology Park and Incubator. NABI is being set up with focus on agriculture and agri-processing for value addition using biotechnological innovations; BPU for facilitation of the scale-up and process optimization of new technologies and Biotechnology Park (to be set up in public private partnership) to boost the biotech industry in the state. The setting up of NABI and BPU by Department of Biotechnology, Govt. of India at a cost of Rs. 380 crore has been approved by the Union Cabinet.

## Biotechnology in Health Care

*Biotechnology has made a huge difference in human health care and contributed significantly in the field of medicine. Due to understanding of the molecular basis of health and disease, scientists have led to improved methods of treating and preventing various diseases. The tools and techniques of biotechnology have opened up new doors and has enabled scientists in the following:*

- *Deeper insight into human body via Human Genome Project*
- *Development of diagnostic tools /kits for quicker and more accurate detection of disorders / diseases*
- *Advanced biotechnological products & therapies for cure of various human disorders*

### Human Genome Project (HGP)

HGP is one of the largest single investigational and international scientific research project which began in 1991 with an objective to understand the genetic makeup of the human species and primary goal to determine the sequence of chemical base pairs making up DNA and identification of genes of the human genome from both a physical and functional standpoint. The project was headed by Dr. Watson of U.S. National Insitutes of Health. A parallel project was conducted outside the government by the Celera Corporation. A working draft of the genome was released in 2000 and a complete one in 2003.

***Potential benefits of human genome project*** : Technology and resources promoted by (HGP) have profound impacts on biomedical research and promise to revolutionize the wider spectrum of biological research and clinical medicine. Knowledge about the effects of DNA variations among individuals has led to revolutionary new ways to diagnose, treat, and someday prevent the thousands of disorders that affect us. detailed genome maps have aided researchers seeking genes associated with dozens of genetic conditions, including myotonic dystrophy, fragile X syndrome, neurofibromatosis types 1 and 2, inherited colon cancer, Alzheimer's disease, and familial breast cancer. HGP has led to generation of the new era of molecular medicine with potential applications as listed below :

- Improved diagnosis of disease
- Earlier detection of genetic predispositions to disease
- Rational drug design
- Gene therapy and control systems for drugs
- Pharmacogenomics "custom drugs"

## Role of biotechnology in diagnosis of disease & disease susceptibility

The diagnosis of a disease and its treatment are the main aspects related to human health. A disease can be cured if diagnosed early. All diseases have genetic component whether inherited or resulting from the body's response to environmental stresses like viruses or toxins. The success of HGP have enabled researches to identify genes and the errors in genes that cause or contribute to disease production. With such information, biotechnology companies have designed and commercialized various diagnostic testing kits to detect errant genes in people suspected of having particular disease or of being at risk for developing them. For example, kits for diseases like HIV, filariasis, typhoid, hepatitis C & B, syphilis and other diseases are now available and are being used. These kits are easy, safe, sensitive, reproducible and eventually automated to facilitate the evaluation of large numbers of samples. Moreover, testing by these kits is quick and cheaper.

### *Gene Testing*

Gene tests / DNA-based tests are the newest and most sophisticated tests being used for detection of genetic disorders. The techniques nvolve direct examination of the DNA molecule itself or biochemical testing of gene products i.e. enzymes / proteins. Application of genetic tests is for:

- Clarification of diagnosis of particular disease in human which could direct a physician towards appropriate treatments.

- Carrier screening for identification of people at high risk for conditions that may be preventable.
- Prenatal diagnostic testing for families to identify and avoid children predisposed of devastating diseases.
- Preimplantation genetic diagnosis (PGD) : PGD is a test that screens for genetic flaws among embryos used in in vitro fertilization. With PGD, DNA samples from embryos created in-vitro by the combination of a mother's egg and a father's sperm are analyzed for gene abnormalities that can cause disorders. Fertility specialists can use the results of this analysis to select only mutation-free embryos for implantation into the mother's uterus.
- For presymptomatic testing for predicting adult-onset disorders such as Huntington, Alzheimer disease and various cancers.
- Forensic/identity testing

***Biotechnology techniques and tools for diagnosis at gene level***

Detection of nucleic acids

1. Polymerase chain reaction (PCR) and real-time PCR
2. Diagnosis by DNA probes and DNA microarray technology

**1. Polymerase chain reaction (PCR)**

PCR exploits natural DNA replication mechanisms and results in the *in-vitro* production of large quantities of a desired sequence of DNA from a complex mixture of heterogeneous sequences. PCR can amplify aselected region of 50 to several thousand base pairs into billions of copies. The target DNA is first heat-denatured to separate the two complementary strands to provide a single-stranded template. Specific primers (short synthetic molecules of DNA complementary to both strands and flanking the target sequences) are then annealed to the single-stranded template at low temperature and extended with DNA polymerase at an intermediate temperature. Once the polymerase has synthesised a new strand of DNA, the product is separated from the template by heating to a higher temperature. These steps, referred to as cycles, are repeated 20–40 times, resulting in amplification of target DNA sequences. PCR is a highly sensitive procedure for detecting :

- Infectious agents and to differentiate between pathogenic & non-pathogenic species by virtue of specific genes in host tissues and vectors, even when only a small number of host cells are infected.
- Early diagnosis of malignant / cancer diseases such as leukemia and lymphomas. PCR assays can be performed directly on genomic DNA samples to detect malignant cells at a sensitivity which is at least 10,000 fold higher than other methods.
- Identification of non-cultivatable or slow-growing microorganisms such as mycobacteria, anaerobic bacteria, or viruses from tissue culture assays and animal models,

- Detection of viral DNA can likewise be also detected by PCR.

Classical PCR methods for diagnosis of pathogens, both bacterial and viral, are now being complemented and in some cases replaced with real-time PCR assays. Real-time PCR monitors the accumulation of PCR product during the amplification reaction, thus enabling identification of the cycles during which near-logarithmic PCR product generation occurs. The assay can be used to reliably quantify the DNA or RNA content in a given sample. The technique is highly sensitive and specific, thus retaining qualitative efficiency, and provides quantitative information.

**2. Diagnosis by DNA probes and DNA microarray technology** :

Conventional DNA probing and microarray analysis utilize the similar principle of hybridisation. Both processes involve binding (hybridisation) of DNA, derived from a sample suspected of containing a pathogen (the 'unknown'), with highly characterised DNA derived in advance from a pathogen of interest (the 'known' DNA). In conventional **DNA probing** the unknown DNA (or RNA), the target, is immobilised on a solid surface e.g. a filter. The known DNA, made into a probe by labelling or tagging it in some way, is in the liquid phase and is applied to the target which can be nucleic acids extracted from clinical material or cultured cells. **DNA microarray technology** offers a new way to clinical diagnostics and allows more rapid, accurate, and cost-effective detection of pathogens compared with traditional approaches. Humans have tens of thousands of genes, and the development of DNA microarrays made it possible to examine the expression of thousands of genes at once. A microarray is so-called because it can comprise 20,000 or more different known DNAs, each DNA being spotted onto glass slides, to form the array. Each spot is only around 10 µm in diameter, hence it is called microarray. DNAs complementary to parts of selected genes of pathogens can be used to make the arrays. In order to visualise a probe bound to its target, the probe is labelled with a radioactive nuclide or, more commonly and safely, 'tagged' non-radioactively with fluorescent molecules. Microarrays have been used for :

- Determination of genes expressed differently between normal and cancerous cells. Testing for elevated expression of certain genes can assist in predicting cancer outcomes and in assigning appropriate treatment programs.
- Genetic predisposition for certain diseases or conditions.
- Early detection of diseases like prenatal diagnosis of Thalassemia, Tuberculosis, Hepatitis.

## Role of biotechnology in therapeutics

### *Biotechnology derived products*

Through genetic engineering, scientists have been able to isolate specific genes i.e. genes of interest and insert them into DNA of certain microbes, plant / animal or mammalian cells, with the result the microbes / cells have become living factories and are mass producing the desired protein / product of interest. The scientists have led to mass production of various biotechnology based therapeutics products which have been commercialized by various biopharma companies. In India products which have been approved for marketing include Insulin, Alpha Interferon, Hepatitis B surface antigen based vaccine, GM-CSF, G-CSF, Blood clotting factor VIII, Human growth hormone, Erythropoietin, Streptokinase, Follicle stimulating hormone etc. Approx. 418 products and vaccines have been developed through biotechnology are being used to treat more than 100 diseases:

- 210 medicines to treat cancer
- 50 to treat infectious disease
- 44 to treat autoimmune disorders
- 22 to treat HIV Infection and related-conditions
- 22 to treat cardiovascular diseases

Besides, there are range of monoclonal antibodies targeting asthma, lupus and various types of cancer.

***Recombinant vaccine***

Recently, recombinant DNA technology has helped to develop new generation vaccines i.e. recombinant vaccine which are cheaper, safer and more effective with less side effects. Recombinant vaccines are created by utilizing bacteria or yeast to produce large quantities of a single viral or bacterial protein. This protein is then purified and injected into the patient, and the patient's immune system makes antibodies to the disease agent's protein, protecting the patient from natural disease. The vaccine based on recombinant proteins are also called sub-unit vaccines. Advantages of the recombinant vaccine technology are that there is virtually no chance of the host becoming ill from the agent, since it is just a single protein, not the organism itself. Traditional vaccine risks come from the organism not being totally weakened (attenuated) or a reversion to a virulent (disease causing) form. Another advantage of a recombinant vaccine is that it does not need an adjuvant (agent that stimulates (irritates) the immune system to find and react to the vaccine). Some adjuvants have been implicated in causing cancer in some animals over time. The world's first genetically engineered vaccine against a human disease--Hepatitis B--is considered one of biotechnology's greatest triumphs. It is the only recombinant vaccine currently in use in humans is the Hepatitis B Virus (HBV) vaccine, which is a recombinant subunit vaccine. Hepatitis B surface antigen is produced from a gene transfected into yeast cells and purified for injection as a subunit vaccine. This is much safer than using attenuated HBV, which could cause lethal hepatitis or liver cancer if it reverted to its virulent phenotype.

The other important developments in therapeutics include :

## Stem cell therapies

Stem cells are unspecialized cells that can potentially reproduced themselves and generate more specialized cells indefinitely. Stem cells occur naturally in the human body and have the ability to differentiate themselves into many different cell types; for example, a stem cell found in a hair follicle can be coaxed to turn into a heart muscle cell or a nerve cell depending on the chemical environment in which it finds itself. A specific type of stem cell that is found early in fetal development is called the embryonic stem cell which is characterized by an extreme degree of ‘‘plasticity,’’ namely, the ability to change or differentiate into any type of tissue. Fetal stem cells are found in two main varieties: totipotent and pluripotent stem cells. Totipotent stem cells are found in the embryo immediately after fertilization. Because some people feel that as soon as the embryo starts to divide it constitutes a human being, it is sacrosanct and must not be destroyed. A little further along the line of cell division come the pluripotent stem cells. These cells are not as plastic as the totipotent stem cells and cannot form every cell type, at least not with today's technologies, so they are not as valuable to scientists as the embryonic stem cells. However, even the totipotent stem cells can be encouraged to differentiate into many different cell types under the influence of specific growth factors. In the near future, it is not unrealistic to envision a scenario in which a patient suffers, say, a myocardial infarction and receives a transplant of cloned heart muscle cells generated from his own stem cells to replace the region of infracted myocardium.
The research is going on the usage of stem cells for treatment of Parkinson's disease & other neuro degenerative disorders viz. Alzheimers etc. Patients with spinal cord injuries or a history of cerebrovascular accident may soon be able to receive stem cell implants to regenerate damaged tissues and restore function. A small number of stem cells persist into adult life. Most adult tissues contain few stem cells in tissues including blood, intestine, skin, muscles, liver & brain which are important in healing and relative little cell division required in adult tissue.

## Cloning

There are two types of cloning: reproductive cloning and therapeutic cloning. In reproductive cloning, you recreate an entirely genetically identical organism. Reproductive cloning has already been used in animals, but due to the thorny moral issues involved, has not been used in humans. ‘‘Dolly the sheep’’ was the first cloned animal, and there have been many additional animal species that had been cloned. Genetics Savings & Clone, Inc., for example, is a private company that is in business of cloning beloved pets for their owners. This is to be differentiated from therapeutic cloning in which you create individual tissues, not entire organisms. Therapeutic cloning uses germ cell lines before they are implanted and causes them to differentiate and develop into specific tissues or organs. Currently, researchers are growing simpler organs such as corneas and urinary bladders, and soon should be able to grow skin, blood vessels and other complex tissues and organs by cloning them from germ cells.

## Gene therapies

Genes, which are carried on chromosomes, are the basic physical and functional units of heredity. Genes are specific sequences of bases that encode instructions on how to make proteins. When genes are altered so that the encoded proteins are unable to carry out their normal functions, genetic disorders can result. Gene therapy is a technique for correcting defective genes responsible for disease development. Researchers may use one of several approaches for correcting faulty genes:

- A normal gene may be inserted into a nonspecific location within the genome to replace a nonfunctional gene. This approach is most common.
- An abnormal gene could be swapped for a normal gene through homologous recombination.
- The abnormal gene could be repaired through selective reverse mutation, which returns the gene to its normal function.
- The regulation (the degree to which a gene is turned on or off) of a particular gene could be altered.

*How does gene therapy work?*

In most gene therapy studies, a "normal" gene is inserted into the genome to replace an "abnormal," disease-causing gene. A carrier molecule called a vector must be used to deliver the therapeutic gene to the patient's target cells. Currently, the most common vector is a virus that has been genetically altered to carry normal human DNA. Viruses have evolved a way of encapsulating and delivering their genes to human cells in a pathogenic manner. Scientists have tried to take advantage of this capability and manipulate the virus genome to remove disease-causing genes and insert therapeutic genes.

Target cells such as the patient's liver or lung cells are infected with the viral vector. The vector then unloads its genetic material containing the therapeutic human gene into the target cell. The generation of a functional protein product from the therapeutic gene restores the target cell to a normal state. Some of the different types of viruses used as gene therapy vectors include Retroviruses, Adenoviruses, Adeno-associated viruses, Herpes simplex viruses.

Besides virus-mediated gene-delivery systems, there are several nonviral options for gene delivery. The simplest method is the direct introduction of therapeutic DNA into target cells. This approach is limited in its application because it can be used only with certain tissues and requires large amounts of DNA. Another nonviral approach involves the creation of an artificial lipid sphere with an aqueous core. This liposome, which carries the therapeutic DNA, is capable of passing the DNA through the target cell's membrane.

*Current status of gene therapy research*

The Food and Drug Administration (FDA) has not yet approved any human gene therapy product for sale. Current gene therapy is experimental and has not proven very successful in clinical trials. Little progress has been made since the first gene therapy clinical trial began in 1990. In 1999, gene therapy suffered a major setback with the death of 18-year-old Jesse Gelsinger. Jesse was participating in a gene therapy trial for ornithine transcarboxylase deficiency (OTCD). He died from multiple organ failures 4 days after starting the treatment. His death is believed to have been triggered by a severe immune response to the adenovirus carrier.

Another major blow came in January 2003, when the FDA placed a temporary halt on all gene therapy trials using retroviral vectors in blood stem cells. FDA took this action after it learned that a second child treated in a French gene therapy trial had developed a leukemia-like condition. In April of 2003 the FDA eased the ban on gene therapy trials using retroviral vectors in blood stem cells.

***Recent developments in gene therapy research***

- Successful world's first gene therapy for inherited blindness showing sight improvement (April 2008) by UK based researchers from the UCL Institute of Ophthalmology and Moorfields Eye Hospital NIHR Biomedical Research Centre.
- Use of combination of tumor suppressing genes delivered in lipid-based nanoparticles drastically reduced the number and size of human lung cancer tumors in mice during trials (January, 2007).
- Successful usage of gene therapy for treatment of cancer in human by researchers at the National Cancer Institute (NCI) by successfully reengineering immune cells i.e. lymphocytes, to target and attack cancer cells in patients with advanced metastatic melanoma (August, 2006).
- Usage of Gene therapy for cure of diseases of the myeloid system (including a variety of bone marrow failure syndromes, such as acute myeloid leukemia) (March, 2006).
- Usage of Gene Therapy for cure of deafness in guinea pigs. (February, 2005).
- RNA interference or gene silencing may be a new way to treat Huntington's. Short pieces of double-stranded RNA (short, interfering RNAs or siRNAs) are used by cells to degrade RNA of a particular sequence. If a siRNA is designed to match the RNA copied from a faulty gene, then the abnormal protein product of that gene will not be produced. (March, 2003).

## Selected References


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