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Stem Cell : Its Evolving role in Cancer Management and Research

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Summary : Hematopoietic stem cell transplantation has become established mode of treatment for various malignancies and hematological disorders. Continuous efforts are being made to improve the availability, applicability and survival by using different sources of stem cells (cord blood, fetal liver, partially matched/ mismatched donors), and reduced intensity conditioning regimens. During last few years there has been a growing interest in the area of stem cell research world wide. Apart from being implicated as a source of carcinogenesis, their self renewal, proliferative and differentiation potential is being utilized in treatment of various chronic ailments and congenital disorders (myocardial infarction, cardiomyopathy, cerebral palsy, muscular dystrophy and retinal degeneration). These are the areas of active research though the responses and underlying mechanism are still in their infancy and poorly understood.

Keywords : Hematopoietic stem cells, transplantation, fetal liver, stem cell therapy.

Introduction

The growing knowledge of biology has made it clear that stem cells have a key role not only in genesis and development of various organisms but also in tumorigenesis. Stem cells are present in almost every tissue of the body and they have the capability of self-

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renewal, trans-differentiation and extensive proliferation. These attributes if not regulated appropriately (genetic/ environmental factors) can result in development of malignant phenotype. These 'cancer stem cells' have been identified in various malignancies of hematopoietic system, breast and brain (1,2). Currently much of the research is being done to identify these stem cells and to target them (resistant to conventional treatment) to achieve complete cure.

In contrary to the cancer stem cell concept, the use of hematopoietic stem cells (HSC) in treating various malignant and non-malignant conditions is relatively well established. First successful transplant was done in 1968 in Minnesota (USA) in a child with severe combined immune deficiency (SCID) from an HLA matched sibling. Since then, over last 3-4 decades, HSC transplants have been used to treat cancers and hematological disorders such as aplastic anemia and hemoglobinopatheis. Hematopoietic stem cells are traditionally obtained from bone marrow. More recently they have been obtained from peripheral blood after mobilizing them from bone marrow with the help of growth factors and/or chemotherapy. Umbilical cord and fetal liver are other sources of hematopoietic stem cells.

In present article we will limit ourselves primarily to the HSC transplantation, and its current status and future prospects.

Fetal liver hematopoietic stem cells: Clinical and in vitro studies

Liver is the major site of hematopoiesis during second trimester of pregnancy (3). Due to paucity of lymphoid cells and weak expression of HLA antigens (3-5), fetal liver has the minimal potential to induce graft versus host disease (GVHD). Since GVHD is the major killer following bone marrow/ peripheral blood stem cell transplant, fetal liver appeared an attractive source for clinical use. More over, compared to adult marrow/peripheral blood, fetal liver hematopoietic stem cells have greater proliferative potential as revealed by the formation of more number of colonies, higher plating efficiency and larger colony size (6).

At AIIMS, in 1970's when we did not have the facilities for performing bone marrow or peripheral blood stem cell transplants, we treated aplastic anemia and acute myeloid leukemia (AML) patients following chemotherapy with the help of fetal liver infusion (FLI). Preconditioning was not utilized. Of the 41 patients of aplastic anemia treated with FLI, 40% responded in 10-30 days time. Median survival of the patients was better compared to that achieved with supportive care. However, since, preconditioning was not used; sustained engraftment could not be achieved. Engraftment demonstrable in some patients was temporary. It thus appeared that fetal liver infusion had induced

autologous hematologic recovery in 40% of the subjects (7-10).

Various in-vitro studies were carried out in our lab to find the mechanism of recovery. ELISA confirmed the presence of some colony simulating factors in fetal liver conditioned medium (FLCM). Stem cell factor (SCF) was found to be one of the candidate growth factor (11). Subsequent studies looked into the possibility - if additional factors like IL-6 and FLT-3 may also be contributing to autologous hematologic recovery. Indeed, apart from SCF which was present in about 55% of FLCM samples, IL-6 and Flt-3 was demonstrable in 80% or more of samples.

Bioneutralization assays with the use of antibodies revealed 40% suppression of Colony Forming Unit – Granulocyte Macrophage (CFU-GM) colonies with the addition of anti SCF antibodies, 42% suppression with anti IL-6 antibodies and 20% suppression with anti Flt-3 antibodies. This observation strongly supports the role of SCF, IL-6 and Flt-3 in hematopoiesis following FLI. Cytokine gene expression studies with the help of RT-PCR revealed the presence of genes in fetal liver for all the cytokines namely SCF, IL-6, GM-CSF, Flt-3 and EPO studied by us. It is noteworthy that the percentage of HSC is maximum in fetal liver compared to bone marrow, peripheral blood and cord blood (12).

Currently, we are studying the correlation of cytokine secretion and expression of genes with the fetal gestation period, if any and we are also studying the interaction of various cytokines. Due to limited availability of abortuses in mid gestation, we are also in the process of establishing fetal liver HSC lines so as to study further the potentials of fetal liver HSC.

Hematopoietic Stem Cell Transplantation (HSCT)

In 1950's Sir E. Donnal Thomas pioneered the application of early studies of transplantation in animals to the treatment of leukemia in humans (13). Since then over the years improvement in HLA typing, supportive care and graft versus host disease (GVHD) management has made the HSCT a relatively safer option with considerable cure rates. It is being done for various hematological and non-hematological malignancies. HSCT utilizes the self-renewal potential of stem cells after myeloablation (14). The myeloablative preparative regimens are aimed to eradicate the malignant cells, create space for the graft and to induce immunosuppression (in allogeneic transplantation). Various preparative regimens include alkylating agents, platinum with or without total body irradiation. Most of these regimens are too toxic (non hematological toxicity) and contribute toward high transplant related mortality (15). This has limited the use of conventional HSCT to the young patients with good performance status. Recently, the emerging concept of non-

myeloablative and reduced intensity transplantation has widened the application of HSCT to elderly and patients with poor performance status as the regimen related toxicity is less (16,17). It relies on the use of immunosuppressive drugs and graft versus malignancy effect where donor lymphocytes eradicate the malignant cells. Non-myeloablative transplants are also being used for solid tumors such as renal cell carcinoma (18).

HLA matching and haplo-identical stem cell transplantation- With the help of molecular techniques HLA matching has become more accurate. This has led to decrease in incidence of GVHD due to previously undetected mismatches (using serology and mixed lymphocyte culture techniques) (19). These advancements come at the cost of lesser availability of fully matched sibling or unrelated donors resulting in rise in the numbers of mismatched or haploidentical transplants. Various graft manipulation techniques such as T cell depletion (mechanically or with the help of monoclonal antibodies - Campath) are being used to decrease the high risk of GVHD in these settings. Still due to mismatches and graft manipulation these stem cell transplantation suffer the high incidence of graft failure (20).

Sources of stem cells - Conventionally stem cells are obtained from the bone marrow (donor or patient) under general anesthesia. More recently the use of peripheral blood stem cells has come in a big way. It involves mobilization of stem cells with the use of growth factors with or without chemotherapy. Apheresis is done with the use of cell separator over a period of 6-8 hours. The advantages over conventional bone marrow transplantation include early hematopoietic recovery for both platelets and neutrophils, safety for the donor and no requirement for general anesthesia (21).

Use of cord blood stem cells- As discussed above, due to the limited availability of suitable donors, umbilical cord blood is being used as a source of stem cells in various malignant and non-malignant disorders. It has the advantages of easy procurement, no risk to donors, acceptable partial mismatches and reduced risk of transmitting infections. It has certain limitations - limited cell dose leading to failure of engraftment, limited application in adults due to body size and lack of back up for stem cells in case of graft failure (22).

Graft versus host disease - The risk of severe acute and chronic GVHD has come down due to better HLA match, use of immunosuppressive drugs for the prophylaxis and treatment (23).

Cryopreservation of stem cells– Cryopreservation is required to preserve the viability of autologous stem cells, cord blood stem cell and as stem cell

back up (in allogeneic transplants). Depending on the temperature, the cells can be preserved for variable time periods. Cryopreservation can be done by dump freezing or rate controlled freezing.

Supportive care - In post transplant period the patient needs to be supported during the period of aplasia with packed red cells, platelets and antibiotics. Due to mucositis, patients may need parenteral nutrition and organ function should be monitored closely. Isolation nursing is required to decrease the chances of infection though in most cases it's the endogenous flora, which is responsible for infectious episodes.

Stem cell transplantation at IRCH

Two hundred and fifty two (252) transplants have been performed by us at IRCH; autologous - 170 and allogeneic-82. Autotransplants have been for multiple myeloma (95), lymphomas (35), acute leukemia (17), CML (5), and solid tumors (18). Allogeneic stem cell transplants have been performed for CML (40), CLL (1), severe aplastic anemia (19), acute leukemia (12), Hurler's syndrome (3), congenital erythropoietic porphyria (1) Beta thalassemia (2), Multiple myeloma (1), Myelodysplastic syndrome (2), and Hodgkin's disease (1).

Results following allogeneic as well as autologous transplants have been similar to those achieved at other Indian centers engaged in hematopoietic stem cell transplant programme. For instance, in CML-chronic phase, reported survival from CMC Vellore is 47% at a median follow-up of 30 months, 47% by Tata Memorial Hospital at a median follow up of 48 months and 65% at IRCH at a median follow up of 27 months. Similarly, in severe aplastic anemia, survival has been 16% at TMH, 32% at CMC and 21% at IRCH. Multiple myeloma has been gratifying to manage with autologous hematopoietic stem cell transplants as the survival at 5 years has been 52% which is much superior to that achieved with chemotherapy alone (24, 25, 26).

Stem Cell Therapy at AIIMS

More recently stem cells have achieved wider and even greater recognition due to their capacity to differentiate into variety of cell types namely, cardiac, neural, hepatic and muscular. This has opened up newer potentials for the use of stem cells to treat myocardial, neural, pancreatic and muscular diseases (27, 28).

AIIMS has taken a lead in the use of autologous stem cells in various cardiac, muscular, neurological & ocular degenerative disorders. The special advantage is that there are no rejection reactions, because the cells are from the same body i.e. autologous transplantation.

Stem cell therapy in myocardial infarction (MI):

Forty two patients of MI, underwent stem cell therapy at AIIMS during

coronary artery bypass graft (CABG). Control group had 10 patients who did not receive stem cell therapy. MI patients underwent stem cell therapy procedure in addition to routine CABG. None of the patients suffered any mortality and morbidity as a result of this therapy. The preliminary results showed no arrhythmia. There was improved ventricular function. There was improvement in New York Heart Association functional class (from 2.9 ± 0.7 to 1.25 ± 0.6) and left ventricular ejection fraction (from $32 \pm 12\%$ to $41 \pm 9\%$ $p = 0.04$). All Patients of MI had Transthoracic echocardiography (16 segment analysis); stress thallium (20 segment analysis), ventricular angiography (5 segment analysis) before surgery and on follow-up. Left ventricular dimensions remained stable with no progression to aneurysm formation in the stem cell group Vs control group. Not only the scar size reduced but also there was viability at the center of the scar in two patients. This will be confirmed once we get more information from PET scan, which has been installed, at our institute recently. The 10 control patients showed no change in left ventricular function and no change in the number of scarred segments. (30)

Stem cell therapy in Dilated cardiomyopathy (DCM):

We studied the effects of intracoronary autologous bone marrow stem cells (BMSC) implantation in patients with dilated cardiomyopathy (DCM). Twenty four patients with DCM with normal coronaries formed the study group while 20 patients who refused the stem cell therapy formed the control group. Injection of BMSC was made into the coronary arteries with percutaneous occlusion of the coronary sinus for 3 minutes. The patients were reevaluated at 3 months with echocardiography and endomyocardial biopsy.

There was improvement in the New York Heart Association (NYHA) Class (3.3 ± 0.5 to 2.4 ± 0.7, $p < 0.05$) in the treatment group. Left ventricular ejection fraction improved from $20 \pm 8.2\%$ to $27 \pm 13\%$ ($p < 0.05$) in the NYHA III patients while class IV patients showed no improvement in LV function. Out of 7 patients who were NYHA class IV, 4 expired. Endomyocardial biopsy showed evidence of increased vascularity with no evidence of any immature cells or any evidence of any adverse pathology (inflammation, infarction). There was evidence of cell proliferation (binucleate cells in 2 and Ki-67 positive cells in one). This is the first study to show the potential safety and efficacy of intracoronary transplantation of autologous bone marrow stem cells in patients of dilated cardiomyopathy. It demonstrates clinical and echocardiographic improvements in class III patients. Preliminary histopathological evidence points to a possible paracrine effect (30).

Although various studies have shown remarkable improvement in myocardium regeneration following stem cell therapy; the mechanism of this potential benefit is not clear. The various mechanisms postulated are generation of new myocytes, endothelial cells, and smooth muscle cells by transdifferentiation, cell fusion or paracrine effect. However there is a risk of tumor formation using these stem cells as suggested by some researchers. (31)

Stem cell biology still remains one of the most intriguing fields of scientific inquiry and holds long-term potential. There is still some unanswered questions regarding the optimum cell type, cell dose, in vivo delivery, efficiency of grafting, tracking of stem cells. There is a need to conduct studies with large number of patients, double-blind, randomized-controlled clinical trials to establish the effect of stem cell therapy. Our goal is to understand the mechanism/ basis of this potential therapy. (30)

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CANCER VACCINES WITH SPECIAL REFERENCE TO HUMAN PAPILLOMA VIRUS (HPV)

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Abstract

Despite various advances in diagnostics and therapeutics, cancer is the second most leading cause of death worldwide, accounting for almost 12.5% of deaths annually. Multiple factors like various mutagens, carcinogens, viral and bacterial infections individually or in combination are responsible for development of cancer. Infectious agents are responsible for about 15% of the cancers, such as cervical cancer, liver cancer, stomach cancer etc. The prophylaxis against these agents is an attractive strategy towards prevention of these cancers. This is evident from the successful Hepatitis B virus (HBV) vaccination to prevent hepatocellular cancer. Two prophylactic vaccines against HPV which have been shown to be highly immunogenic and successful in protecting from HPV infection are to be available for vaccinating females of age 9 to 26 years. Current research focuses on the development of cheaper second generation vaccines including DNA vaccines and therapeutic vaccines that can effectively curb the established cancers at their initial stages and are in the phase I/II clinical trials. Therefore prophylactic vaccine, early diagnosis and therapeutic vaccine certainly hold the promise of controlling the most dreaded disease of humankind.

Key Words : Cancer, Cervical Cancer, Human Papillomavirus, Vaccine, Cancer Prevention

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Introduction

Cancer is the leading cause of death worldwide with approximately 10.9 million new cases diagnosed each year and about 6.7 million deaths. Current estimates bring the grim scenario that the number of new cancer cases is expected to grow by 50% over the next 20 years to reach 15 million by 2020 and more than 70% of them will be from developing countries. There are currently more than 24.6 million persons living with cancer and are in need of life saving drugs.

Cancer is a multistep and multifactorial disease arising due to uncontrolled clonal growth of cells. This is due to alteration in the structure and / or function of the genes controlling cellular growth and proliferation that may be because of exposure to various mutagens, carcinogens and viral/bacterial infections. The infectious agents are responsible for more than 15% of the cancers and some of these infections at different organ sites may also cause benign tumors. Some important examples include cancer of the uterine cervix in women due to persistent infection with oncogenic Human Papillomavirus (HPV) types, liver cancers caused by infection of Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV). Recently, stomach cancer has also been found to be linked to the infection with the bacterial pathogen, *Helicobacter pylori*.

As most of the cancers are detected in late stage, the only option is to conduct chemotherapy, radiotherapy, surgery etc which will extend the life span but certainly no cure is possible. Recent developments in immunology, genetics and molecular biology have fostered development of vaccines for infectious diseases, cancer, allergies and auto immune diseases. As 15% of the cancers are caused due to infectious agents, the prophylactic vaccine against these pathogens is expected to reduce the burden of the cancer cases.

This article reviews the development of different cancer vaccines with a special emphasis on cervical cancer vaccines and the different approaches being adopted for their development.

Cancer and Cancer Vaccine

The extensive research in understanding the role of infectious agents in cancer causation has lead to unraveling of oncogenic mechanisms and pathogenesis of these infectious agents. Fruitfully this has lead to development of number of prevention strategies against cancer including vaccines. The best example is the prophylactic recombinant vaccine against HBV which is the first vaccine that has been shown to prevent cancer. Recently, the US-FDA has approved marketing of the virus like particle (VLP) based HPV vaccine which will be second such vaccine that can prevent cervical cancer. Taken together the widespread implementation of both these vaccines will reduce the cancer burden to a great extent. There are

several other viruses and bacteria that are shown to be causative agents for cancer and the vaccines against them are at different stages of development. The infectious agents, cancer and the vaccine status is summarized in Table-1.

Table-1 : Infectious agents causing cancer and status of cancer vaccine development

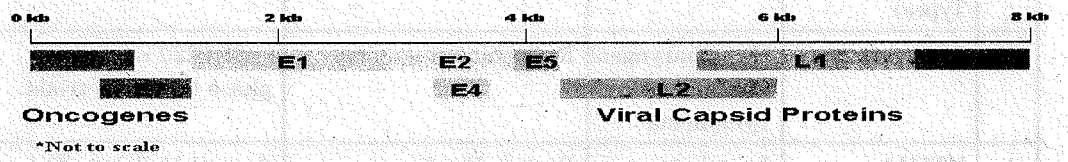
Sr. No.	Infectious Agent	Cancer	Vaccines Strategy	Current Status
1.	Hepatitis B Virus	Liver Cancer	Recombinant vaccine	HBsAg vaccine available
	Hepatitis C Virus	Liver Cancer	Recombinant vaccine/ Peptide vaccine	Phase II Clinical Trials Phase I clinical Trial
2.	Human Papillomavirus (High Risk Types)	Cervical Cancer	Recombinant vaccine	VLP based vaccine against types 16, 18 available
3.	EBV	Nasopharyngeal Carcinoma Lymphoma	Recombinant vaccine	gp350/220 vaccine in phase II clinical trials
4.	HTLV-1	Lymphoma	Peptide Vaccines	Animal Model studies.
5.	Helicobactor pylori	Stomach Cancer	Enzyme targeted/ Attenuated vaccine	Several Phase I clinical trials

Cervical Cancer and Human Papillomavirus

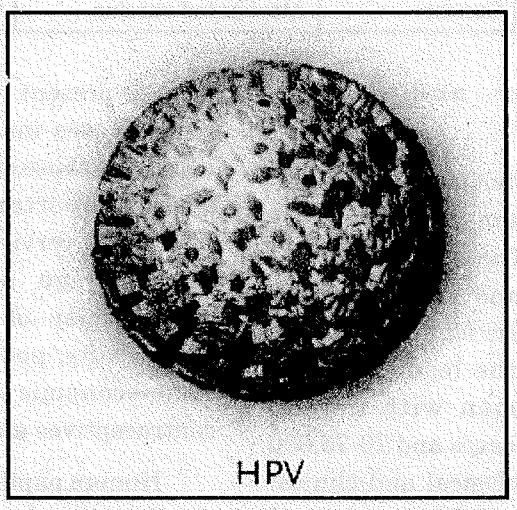
Cancer of the uterine cervix is the second-most common cancer among women worldwide. There are estimated 493,000 new cases and 274,000 deaths annually due to cervical cancer (1). Cervical cancer is the leading cancer among Indian women with annual incidence of 130,000 cases and 70-75,000 deaths (2). Epidemiological and clinical studies have confirmed that cervical cancer develops due to the persistent infection of High Risk HPV (HR-HPV) types. Recent studies show that HPV-DNA is present in 99.7% of the cervical cancer cases indicating that HPV is a necessary cause of cervical cancer (3). The other important risk factors for developing cervical cancer are early age of marriage or sexual exposure, multiplicity of sexual partners or promiscuity, poor genital hygiene, low socio-economic status, smoking, oral contraceptives and multiparity (3).

Human papillomaviruses (HPVs) are small nonenveloped DNA viruses having approximately 8.0 kb double stranded circular genome that encodes L1, L2 structural proteins and several other

early proteins (E1-E7) responsible for replication, transcription and transformation (See Fig1). Till date, more than 100 genotypes of HPV have been described, among them about 30 are associated with ano-genital infections. The two most important oncogenic HPV types termed as 'high risk' HPVs (HR-HPV) responsible for cervical cancer are HPV types 16 and 18 (4, 5). There are at least twelve more HPV types also designated as high risk types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68 and 73). 'Low risk' HPVs such as HPV 6 and HPV 11 and others (40, 42, 43 and 44) are mainly associated with benign cervical lesions such as condylomata, accuminata and genital warts (6). More than 70-90% of the HPV infection shows natural clearance while about 10-30% cases show persistent infection with "high risk" types leading to malignant transformation and invasive cervical cancer. The virus has a long latent period and takes at least 10-15 years to develop cancer if persists after initial infection (6).



A. Human Papillomavirus Genome



B. Human Papillomavirus Particle

Fig. 1 : Schematic representation of (A) the linearised HPV genome and (B) the viral particle

HPV infects basal layer of epidermis in genital tract, anal, and perianal areas, oral cavity, esophagus and larynx where viral replication occurs. High risk HPV types responsible for cervical cancer infect the basal epithelial cells in the transformation zone between ectocervix and endocervix at the female genital tract. A persistent infection with HR-HPVs may lead to transformation due to loss of cell cycle control imparted by the viral oncoproteins E6 and E7 (7,8). This leads to different grades of cervical lesions that may eventually lead to invasiveness resulting in cervical cancer. Figure 2 and 3 shows the diagrammatic representation of the biology of HPV infection, its persistence, precancerous conditions and development of invasive carcinoma along with the factors contributing to the development of cervical cancer.

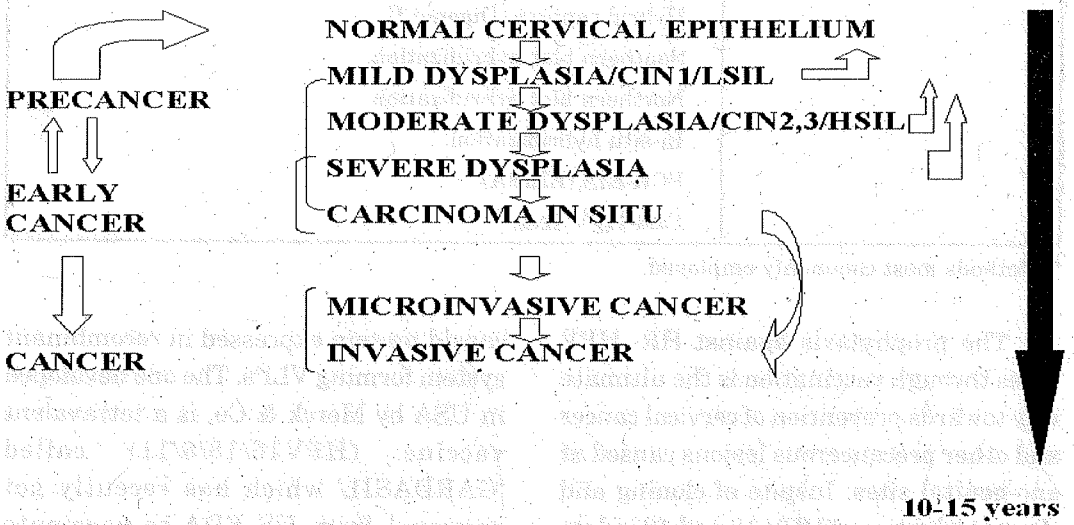


Fig. 2 : Diagrammatic presentation of different stages during progression of precancerous lesions to invasive cancer

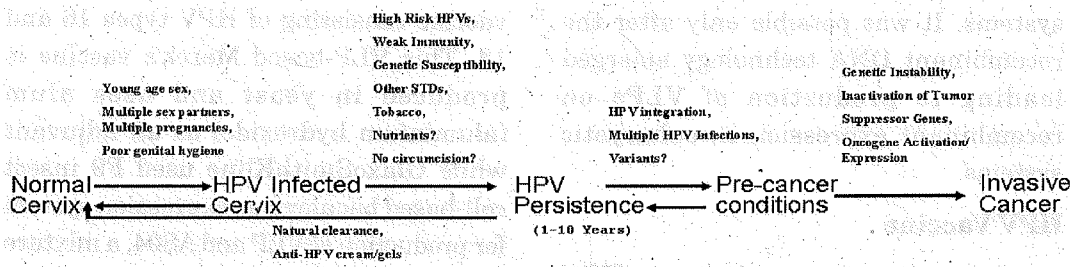


Fig. 3 : Biology of HPV infection and development of cerical cancer

Early diagnosis of the cervical lesions and their treatment helps in preventing development of invasive cancer. There are a number of cellular and molecular methods for diagnosis of HPV infection and cervical lesions at an early stage (See Table 2). Among them the most effective and widely employed method is the Pap smear test that is routinely followed in most of the developed countries. The availability of cytological Pap test has lowered the incidence of invasive cervical cancer by 70-75% in developed countries.

Table 2 : Cellular and molecular diagnostic methods for detection of lesions and HPV infection

Cytological Diagnosis	Papanicolaou-stained smear method (Pap Test)
HPV Diagnosis*	Polymerase Chain Reaction (PCR).
	Hybrid capture (Digene) II
	Southern blot hybridization.
	Northern blot hybridization.
	In-situ hybridization.
	PCR-EIA (ELISA).
	Fast-HPV Test.

* Methods most commonly employed.

The prophylaxis against HR- HPV types through vaccination is the ultimate way towards prevention of cervical cancer and other precancerous lesions caused at ano-genital sites. Inspite of cloning and characterization of HPV 16 and 18 in late seventies (9, 10), the development of HPV vaccine was delayed because of difficulty to propagate virus in tissue culture systems. It was possible only after the recombinant DNA technology emerged leading to production of VLPs on recombinant expression in eukaryotic systems.

HPV Vaccine

Recently, two prophylactic HPV vaccines have been developed using L1 capsid protein expressed in recombinant system forming VLPs. The one developed in USA by Merck & Co, is a tetravalent vaccine (HPV16/18/6/11) called 'GARDASIL' which has recently got approval from US FDA to vaccinate females of age 9 to 26 years (11). Another vaccine, 'CERVARIX' developed by GlaxoSmithKline in Belgium is a bivalent vaccine consisting of HPV types 16 and 18. This VLP-based Merck's vaccine is produced in yeast and uses alum (aluminium hydroxide) as an adjuvant while GlaxoSmithKline used F9 insect cell-based baculovirus expression system for production of VLP and AS04, a mixture of alum and monophosphoryl lipid A as adjuvant which provides more stability to

the vaccine. Both the vaccines have successfully undergone phase III clinical trials and are found to be well-tolerated, highly immunogenic and showed protection against persistent HPV infection for a period of 5 years (12,13).

Although prophylactic vaccination appears to be successful in young adolescents, it would take decades to perceive the ultimate benefits in reduction of cervical cancer cases. As the vaccine is not effective against already established HPV infection and there are estimated 5 million women worldwide already infected with HPV, development of therapeutic vaccine is an important aspect of current research. There are several therapeutic HPV vaccines in phase I and phase II clinical trials world over. Most of them target the HPV early proteins E6 and E7 or peptides derived from them largely because these are the transforming viral proteins that are expressed in cervical tumors. Frazer et al. 2004 showed in Phase I study of HPV 16-specific immunotherapy with E6E7 fusion protein and ISCOMATRIX ™ adjuvant in women with cervical intraepithelial neoplasia that this immunotherapy was well-tolerated and subjects developed HPV 16 E6E7 specific immunity (14). Some other approaches for development of therapeutic HPV vaccine uses autologous Dendritic Cell (DC) pulsed with full-length HPV 16 or 18 oncoproteins to induce HPV specific antitumor immune response (15).

DNA Vaccine

One of the second-generation vaccines that have great potential to address various limitations of recombinant protein-based vaccines is "Genetic vaccines" or "DNA vaccines". In last few years, DNA vaccines have been developed against different viral as well as bacterial and parasitic infections in animal models showing lasting immunity and protection. Clinical trials of DNA vaccines have been performed or are under way for various diseases, including cancer, influenza, hepatitis B, HIV, and malaria. Recently, first DNA vaccine against West Nile virus got approval from Department of Agriculture, United States (USDA) for commercial use in horse.

Since genetic vaccine is a plasmid-based vaccine, it is cost-effective to produce in large quantities. Robustness and stability of DNA vaccine even in higher temperature and single dose administration provide an edge over the other vaccines, particularly for distribution in the developing countries. Rocha-Zavaleta et al (2002) showed that parenteral and oral immunization with a plasmid DNA expressing HPV 16 L1 can induce systemic and mucosal antibody production together with cytotoxic T lymphocyte responses in animal models (16). The research towards development of chimeric DNA vaccines will be another milestone in vaccine research as it can serve as both prophylactic and therapeutic vaccine.

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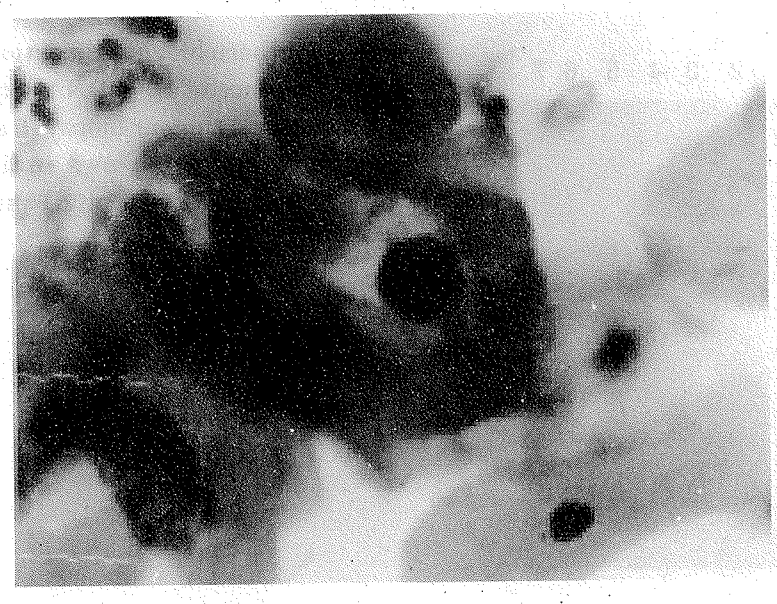
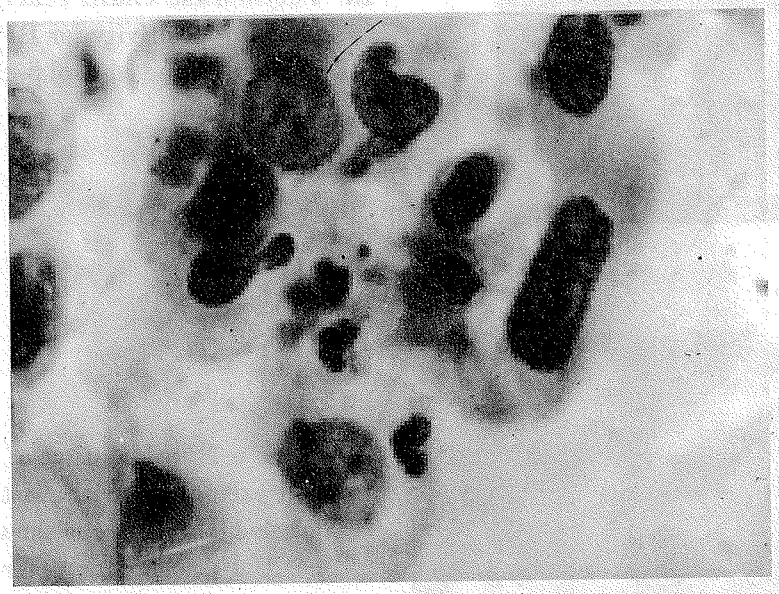
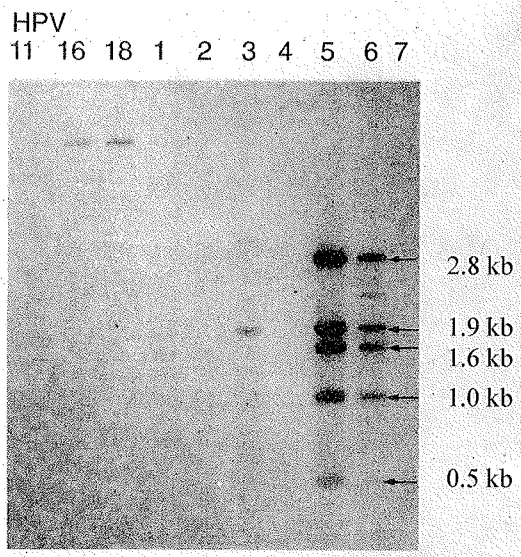


A. Kaliocytes showing infection of HPV at the LSIL stage of lesion

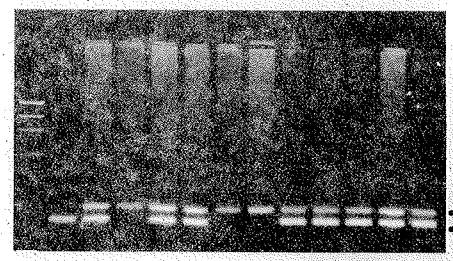


B. HSIL stage of cervical precancer lesion with or without HPV infection as revealed by Pap test during cytological screening

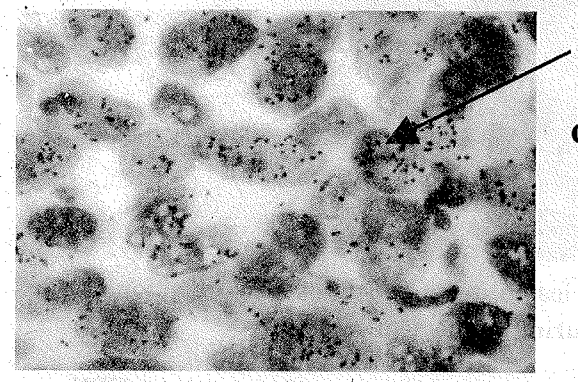
Fig. 4 : Different stages of precancerous cervical lesions with or without HPV infection as revealed by Pap test during cytological screening.



A. Southern blot hybridization showing presence of HPV DNA sequences in cervical tumour biopsy in samples 1, 3, 5 and 6. Arrows indicate PstI digested HPV-specific band pattern.



B. Polymerase chain reaction (PCR) showing positivity for HPV 16 DNA sequences and control β-globin gene.



C. In Situ Hybridization of squamous cell carcinoma with ^{3}H-thymidine-labeled HPV16 probe showing highly positive nuclei with silver gains.

Fig. 5 : Detection of HPV DNA in the cancerous tissues by different molecular approaches.

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GENETIC PREDISPOSITION TO CANCER

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Summary

Genetic factors are known to play a major role in the etiology of cancer. In hereditary cancers, germ line gene mutations have been identified which strongly predispose individuals to cancers. These gene mutations have a high penetrance conferring an elevated risk (>90%) of developing the disease. Although these cancers are rare they have been well studied. On the other hand, the more abundant sporadic cancers are caused due to interaction of low penetrance genes with the environment. There is a cumulative effect of several low penetrance genes which, in the presence of carcinogens, predispose the individual to cancer. As a consequence of the germ line gene mutations / polymorphisms there are further somatic mutations resulting in activation of oncogenes / loss of tumour suppressor genes leading to genetic instability which is the hallmark of cancer. Once initiated, the cancer accumulates further genetic lesions in the form of oncogenes and tumour suppressor genes which play a crucial role in the progression of the disease. Genetic predisposition to breast and colorectal cancers are discussed in the review.

Key words : low penetrance genes, mutations, polymorphisms, breast cancer, colorectal cancer

Introduction:

Cancer is a complex, multistep process involving changes in the genome. It was discovered in late 1980s that genes in their mutant forms were inherited in affected families (1). Gain of function mutations (oncogenes) or loss of function mutations (tumour suppressor genes) led

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to the initiation of cancer. This formed the genetic basis for cancer predisposition. Many cancers arise from germ line mutations in proto-oncogenes and tumour suppressor genes which regulate cell proliferation and apoptosis such as p53 associated with Li Fraumeni syndrome (2,3) and RB1 associated with childhood retinoblastoma (4). There can also be mutations in genes responsible for maintaining genetic stability which includes DNA repair genes such as BRCA1 (5) and BRCA2 (6,7) both associated with breast and ovarian cancers and Mismatch Repair (MMR) genes associated with hereditary non-polyposis colorectal cancer (8,9). Following these inherited or spontaneous mutations, there is a sequential accumulation of mutations in the oncogenes and tumour suppressor genes leading to genetic instability and cancer. However, individual susceptibility is determined by a complex interaction between the germ line genetic variation, which constitute low penetrance genes, and exposure to environmental carcinogens. Mutations disrupt several molecular pathways in the cell and lead to self-sufficiency in growth signals, insensitivity to growth-control signals, evasion of apoptosis, limitless replicative potential, sustained angiogenesis, invasion and metastasis (10,11). Some of these genetic lesions, which are high penetrance genes, are present in the germ line and predispose individuals to cancer.

Low penetrance genes are associated with sporadic cancers and are more difficult to study than high penetrance genes. To gain sufficient evidence to prove that a gene is involved in cancer predisposition, it is probably necessary for multiple, adequately-powered studies to demonstrate an association with the disease, especially if the allelic variants have only a small differential effect on risk. It may also be possible to show how genes interact with each other and the environment, although this will be even more difficult (12). We and others have proposed that there is a cumulative effect of a number of low penetrance genes which together confer high risk for certain cancers (13).

Genetic predisposition to breast cancer by rare, high penetrance alleles:

Familial clustering of breast cancer has been known for decades although the genetic basis was proven only recently. Familial breast cancer constitutes only 5-10% of all breast cancers out of which only ~20% are due to the well studied, high penetrance, BRCA1/2 genes (11). Almost 25% of familial breast cancers are due to unknown familial predisposing genes. BRCA proteins function in damaged DNA repair. Inherited mutations in BRCA1/2 genes confer high risk of breast, ovarian, prostate and colorectal cancers. However, unlike BRCA1 although BRCA2 confers a high risk to breast cancer it does not confer a high risk to ovarian cancer (6). Germ line mutations of p53 gene strongly predispose individuals to Li Fraumeni Syndrome which includes breast cancer

and other neoplasms (14). Individuals carrying BRCA1 and BRCA2 gene mutations are at an increased risk of breast cancer if exposed to X-rays since BRCA protein plays a role in DNA repair (15). Selecting for breast cancer cases with a strong familial background not accounted for by BRCA1 or BRCA2 show a strong association with mutation in a cell cycle check point kinase CHEK2*1100delC (16).

Low penetrance genes in sporadic breast cancer

A large proportion of breast cancers have unknown polygenic predisposing genes along with varying environmental factors (11). The identification of susceptibility factors that predispose individuals to breast cancer could give further insight into the etiology of this malignancy and provide targets for the future development of therapeutics. The candidate low penetrance genes with a variety of functions including carcinogen metabolism, DNA repair, steroid hormone metabolism, signal transduction, and cell cycle control need to be studied (17).

Female breast cancers are known to be associated with prolonged exposure to estrogens. Estrogens are known to influence breast cancer risk by interacting with estrogen receptors. Inter-individual variability due to polymorphisms in DNA sequence, associated with prolonged exposure to increased levels of estrogen, may define a sub-set of women with breast cancer (18-20). Oxidative metabolites of estrogen are known to cause DNA damage (20). Polymorphisms in genes involved in estrogen biosynthesis, and the conversion of estrogen metabolites and their by products could be the low penetrance genes conferring risk in the etiology of sporadic breast cancers. $Leu^{84}Phe$ polymorphism affects the capacity of O-Methyl Guanine Methyl Transferase (MGMT) to inhibit estrogen receptor-mediated cell proliferation and is associated with breast cancer risk (21). All these low penetrance genes could play a role either alone or in combination on exposure to exogenous or endogenous estrogens, in predisposing women to cancer.

Increased expression of Transforming Growth Factor β (TGFβ) plays a role in breast cancer progression. A functional polymorphism in the promoter region of TGFβ gene has been shown to increase breast tumour progression and metastasis (22). aThere are reports that DNA repair gene polymorphisms are important biomarkers for sporadic as well as familial breast cancer susceptibility (23). It has also been reported that RAD51 polymorphism in carriers of BRCA2 mutations are at a significantly elevated risk for breast cancer (24). In addition to endogenous factors, life-style factors such as smoking and alcohol use could contribute greatly to sporadic breast cancers.

Genetic predisposition to colorectal cancer by rare, high penetrance alleles

The incidence of colorectal cancer (CRC) in India is low compared to western

countries (25), although there are a large number of patients seen. The lifetime risk of colorectal cancer (CRC) in the general population is ~5%. Of all the CRCs ~5% are hereditary cancers. Germ line mutations in the DNA mismatch repair genes are known to be associated with genetic instability leading to Hereditary Non-Polyposis Colorectal Cancer (HNPCC) also known as Lynch Syndrome (~2-4% of all CRC) (26). In individuals with HNPCC mutations the risk is greater than 70%. Similarly in individuals with mutation in the gene Adenomatous Polyposis Coli (APC, ~1% of all CRCs) the risk of developing Familial Adenomatous Polyposis (FAP) is greater than 70%. The risk of CRC increases if there are affected members in the family. Mutations in APC are not only responsible for FAP but also play a rate-limiting role in sporadic CRC (27). Genetic testing is routinely being used in the west to detect HNPCC and FAP. Microsatellite Instability (MSI) testing detects alterations in the genome and is characteristic of HNPCC although it is also seen in 10-15% of sporadic CRC. In families with a moderate history of cancer, the presence of MSI indicates the likelihood of HNPCC. There is also a commercially available test which determines whether or not a person has a mutation in the MMR genes MLH1 and MSH2 gene.

Low penetrance genes in CRC

A large number of studies have reported that common genetic variations in low penetrance genes confer risk of CRC. Inherited predisposition to CRC is in part mediated through polymorphic variation (28). Houlston and Tomilson (29) carried out a metaanalysis of 50 studies where polymorphisms in 13 genes had been studied and concluded that only 3 - APC-I1307K, HRAS1-VNTR and MTHFR variants represented the strongest candidates for low penetrance susceptibility alleles for CRC (29). APC-I1307K – a germ line missense mutation, is common in Jews from Eastern Europe and has been reported to confer a two-fold increased risk to CRC (30). So also, biallelic mutations in MYH gene are associated with an attenuated FAP phenotype (30). Low-penetrance genes such as TGFBR16A may account for a sizable proportion of familial colorectal cancer occurrences (31).

Diet in association with genetic variations, has been reported to play a major role in CRC. Polymorphism in the gene CD36 which plays a role in metabolism of oxidized low density lipoprotein and long chain fatty acids is positively associated with CRC in individuals with moderate-high meat consumption (32). Folate metabolism supports the synthesis of nucleotides as well as the transfer of methyl groups. Polymorphisms in folate-metabolizing enzymes have been shown to affect risk of colorectal neoplasia and other malignancies (33).

Hereditary breast and colorectal cancer (HBCC):

HBCC has been observed in a subset of breast cancer patients. The

1100delC variant in the cell cycle check point kinase gene CHEK2 was found to be present in 18% of 55 of HBCC patients as compared to 4% of 380 non-HBCC families (34). Although it is not the major predisposing factor for the HBCC phenotype, it appeared to act in synergy with other, as-yet-unknown susceptibility gene(s) (34,35). Another low penetrance gene, STK15 (Aurora-A) which is a serine/threonine kinase and involved in mitotic chromosomal segregation has been associated with breas and colon cancer. A genetic variant in STK15 T+91A (resulting in the amino acid substitution F31I) shows an increased risk of colon as well as breast cancer (36).

Conclusion:

A large number of tests are available for genetic screening and genetic screening has tremendous implications in patient management. Gene-environment interactions have a major role in susceptibility to majority of sporadic cancers. Our current understanding of these interactions is limited, and concerted research efforts in this area will be important for a full understanding of predisposition to cancer (26). When mutations are detected, specific cancer screening and follow up programs should be offered to mutation carriers, which could prove to be profitable in terms of cost-effectiveness when compared to standard care.

Individuals found to be carriers can be offered:

- counselling to avoid environmental exposures that further elevate risk,
- intensive medical surveillance for early detection,
- participation in chemoprevention trials, and
- prophylactic surgery to remove at-risk tissues.

Clinical cancer genetics should become an integral part of cancer management.

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TUMOR BIOMARKERS

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Biomarkers are biochemical or molecular parameters associated with the presence and severity of specific disease states. Biomarkers are detectable and measurable by a variety of methods including various laboratory assays and medical imaging systems like computed tomography (CT), magnetic resonance imaging (MRI), or nuclear medicine techniques such as positron emission tomography (PET). In addition to these established techniques, there are a variety of imaging methods in various stages of development that hold great promise in the imaging of biomarkers, such as molecular imaging, a method that allows detection of specific molecules in living organisms. Tumor biomarkers are substances, usually proteins that are produced by the body in response to cancer growth or by the cancer tissue itself. Some tumor biomarkers are specific, while others are seen in several cancer types. Many of the well-known biomarkers are also seen in non-cancerous conditions. Consequently, these tumor biomarkers are not diagnostic for cancer.

There are only few well-established tumor biomarkers that are being routinely used by physicians. Many other potential biomarkers are still being researched. Some biomarker tests cause great excitement when they are first discovered but, upon further investigation, prove to be no more useful than biomarkers already in use. The goal is to be able to screen for and diagnose cancer early, when it is the most treatable and before it has had a chance to grow and metastasize. So far, very few tumor biomarkers have gained wide acceptance as a general screen. Other biomarkers are either not specific enough with high false positives, leading to expensive and unnecessary follow-up testing or they are not elevated early enough in the disease process. Some people are at a higher risk for particular cancers because they have inherited a genetic mutation. While not considered tumor makers, there are tests

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that look for these mutations in order to estimate the risk of developing a particular type of cancer. BRCA1 and BRCA2 are examples of gene mutations related to an inherited risk of breast cancer and ovarian cancer.

Examples of biomarkers include genetic biomarkers (e.g., nuclear aberrations [such as micronuclei], gene amplification, and mutation), cellular biomarkers (e.g., differentiation biomarkers and measures of proliferation, such as thymidine labeling index), histologic biomarkers (e.g., premalignant lesions, such as leukoplakia and colonic polyps), and biochemical and pharmacologic biomarkers.

Tumor biomarkers are not diagnostic in themselves. A definitive diagnosis of cancer is made by looking at biopsy specimens (e.g., of tissue) under a microscope. However, tumor biomarkers provide information that can be used for:

Screening: Some biomarkers may be suited for general screening in those with a strong family history of a particular cancer. In the case of genetic biomarkers, they may be used to help predict risk in family members, e.g. PSA for Prostate cancer.

Diagnosis: In the diagnosis of cancer tumor biomarkers can be used as an aid. A patient presenting with a symptoms, tumor biomarkers may be used to help identify the source of the cancer, such as CA-125 for ovarian cancer, and to help differentiate it from other conditions.

Guiding Treatment: Some tumor biomarkers will give doctors information about what treatments their patients may respond to e.g. Her2/neu positive breast cancer and respond to Herceptin treatment.

Monitoring Treatment: Tumor biomarkers can be used to monitor the effectiveness of treatment, especially in advanced cancers. If the biomarker level drops, the treatment is working; if it stays elevated, adjustments are needed e.g. CEA in colorectal cancer.

Determining recurrence: Currently, one of the biggest uses for tumor biomarkers is to monitor for cancer recurrence. If a tumor biomarker is elevated before treatment, low after treatment, and then begins to rise over time, then it is likely that the cancer is returning. (If it remains elevated after surgery, then chances are that not all of the tumor was removed.)

A list of commonly used tumor biomarkers is given in Table 1 and tumor biomarkers being evaluated is listed in Table 2.

Genetic Alterations in Tumors as Biomarkers

The post human genome project era, and the advances in biotechnology have generated many candidate biomarkers in cancers with potential clinical value. These biomarkers can be a signature for cancer staging at the time of diagnosis and/or personalization of therapy which

Table 1 : Common Tumor biomarkers Currently in Use

Tumor Biomarkers	Tumors	Applications	Sample
AFP (Alpha-feto protein)	Liver, germ cell cancer of ovaries or testes	Diagnose, monitor treatment and determine recurrence	Blood
B2M (Beta2 microglobulin)	Multiple myeloma and lymphomas	Determine prognosis	Blood
BTA (Bladder tumor antigen)	Bladder Cancer	Diagnose and determine recurrence	Urine
CA-125 (Cancer antigen 125)	Ovarian Cancer	Diagnose, monitor treatment and determine recurrence	Blood
CEA (Carcino-embryonic antigen)	Colorectal, lung, breast, thyroid, pancreatic, liver, cervix, and bladder	Monitor treatment and determine recurrence	Blood
CA 19-9 (Cancer antigen 19-9)	Pancreatic, sometimes colorectal and bile ducts	Stage disease, monitor treatment and determine recurrence	Blood
CA 15-3 (Cancer antigen 15-3)	Breast and others including lung, ovarian	Stage disease, monitor treatment and determine recurrence	Blood
hCG (Human chorionic gonadotropin)	Testicular and trophoblastic	Help diagnose, monitor treatment and determine recurrence	Blood, Urine
Estrogen receptors and Her-2/neu	Breast cancer	Determine prognosis and guide treatment (Hormones and Herceptin)	Tissue
Monoclonal immunoglobulins	Multiple myeloma and Waldenstrom's macroglobulinemia	Help diagnose, monitor treatment, and determine recurrence	Blood, Urine
NSE (Neuron-specific enolase)	Neuroblastoma, small cell lung cancer	Monitor treatment	Blood
PSA (Prostate specific antigen)	Prostate cancer	Screening, diagnose, monitor treatment, and determine recurrence	Blood
Thyroglobulin	Thyroid cancer	Determine recurrence	Blood

Table 2 : Tumor biomarkers being evaluated

Tumor Biomarkers	Tumors	Applications	Sample
Cytokeratin 18 (CK18), bladder tumor fibronectin (BTF), Bladder tumor antigen (BTA)	Bladder cancer	Help diagnose and determine recurrence	
TA-90	Metastatic melanoma	Help diagnosis	
CA242, CAM 17.1 and Tissue polypeptide specific antigen (TPS)	Pancreatic cancer -	Help diagnosis	

could improve patient care. Assessment of genotypes at single locus may contribute to evaluation of cancer risk, diagnosis and prediction of disease progression. In case of sporadic cancers we are at a stage where there is a lot of information and research which is being translated to the clinic. There are molecular biomarkers which are clinically applied in selecting the patients for specific therapy and to monitor the prognosis. The HER2 gene is over expressed in ~30% of breast cancers, increasing the aggressiveness of the tumor. Herceptin (or trastuzumab), recombinant monoclonal antibody against HER2, is now part of the ideal management in patients who over express this receptor. This was first conclusively shown in a phase 3 clinical trial in women with metastatic breast cancer who over expressed HER2. When added to conventional chemotherapy the antibody resulted in a significantly longer time to disease progression, a higher rate of response, a longer duration of response, and improved overall survival (1). In oligodendroglioma, several studies have shown that 1p and 19q loss are 100% responsive to PCV (Procarbazine, Carbamazipine and Vincristine) therapy and have an average survival of 12 years instead of 2 years before the era of PCV. It also helps avoid patients with no loss of 1p/19q, who are not responsive to PCV, from unnecessary risk of chemotherapy. In tumor monitoring and in determining the response of hematological

malignancies, molecular typing is increasingly used. The BCR-ABL fusion protein of its translocation can be determined by PCR for monitoring CML. N-myc amplification correlates with poor prognosis in neuroblastoma patients. Amplified N-myc confers resistance to certain drugs like cisplatin used in the therapy of the disease. There is a vast body of literature on p53 that has yet to be transformed to clinical practice. For example in prostate cancer, mutations in the *p53* gene are associated with metastasis and may serve as a biomarker for progression (2).

Hereditary cancers

In the case of hereditary cancers, assessing the molecular alteration may contribute to evaluation of cancer risk and early diagnosis. The linkage of mutations in the BRCA1 and BRCA2 genes to susceptibility to both breast and ovarian cancers is now used in clinical practice. Given this risk, otherwise healthy individuals who test positive for BRCA1/2 mutations may opt to undergo prophylactic bilateral mastectomy and oophorectomy (3). The same reasoning can be used for screening for p53 mutations in detecting 'at risk individuals' in families of Li Fraumeni syndrome. Similar mutation in APC gene contribute both to hereditary and sporadic colorectal cancers, making screening for mutations in this gene a possible component of colorectal cancer risk assessment and clinical management (4). HNPCC (hereditary nonpolyposis colon cancer) is a hereditary syndrome that is caused when a person inherits a mutation in different mismatch repair genes, mutation in two of the gene MLH1 and MSH2 account for the vast majority of detectable mutations. Members of families with an error in one of the genes associated with HNPCC should strongly consider some special colon cancer screening and prevention options.

Biomarker discovery in post genomic era : Molecular profiling of cancers

Biomedical research in general is in the midst of an informational and technological revolution. Over the past decade, molecular profiling has emerged as a dynamic new discipline, capable of generating a global view of DNA alterations, mRNA and protein patterns in various cell types and disease processes by integrating the expanding genetic databases from the Human Genome Project with newly developed expression analysis technologies. It helps in explaining the relationship between genotype and phenotype in humans, which is still largely unknown. It also provide with the identification of new molecules for development of new diagnostic and therapeutic targets for clinical intervention.

The underlying cause of each patient's disease is typically unique to the individual. Molecular profiling provides advanced analysis and tools to better help the physician determine the molecular characteristics of each patient's disease so that they may better modify the

medical strategy specific to the individual. Hence molecular profiling with Genomic-based & Proteomic-based approaches and tissue arrays is being used to discover next generation biomarkers which help to obtain more global views on cancer genes. These include:

DNA-based biomarkers - Genetic changes and mutations in oncogenes, tumor suppressor genes and genes involved in maintaining the cellular integrity.

Mitochondrial DNA based biomarkers.

Pattern-based RNA expression analysis - Demonstrated in a number of tumor types.

Epigenetic biomarkers - Potential to guide treatment decisions for a number of marketed and developmental agents.

Specific proteomic patterns associated with disease.

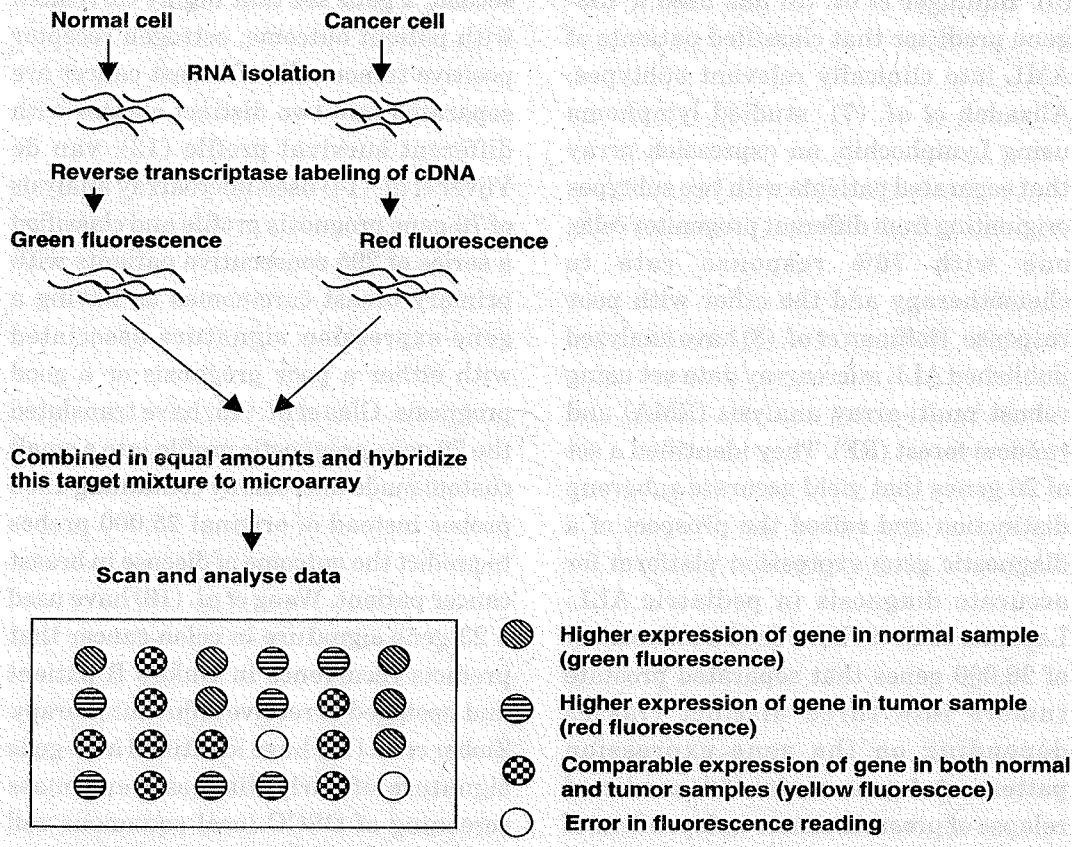
Genomic based approaches: Genomics involves the study of complex set of genes, their expression and interaction with each other using microarray system. The cancer development is dependent on many different genes, their expression and interaction with each other to create a favorable environment for disease to develop. With the advancement in the study of genomic analysis, the identification of a set of genes (rather than single genes) expressed in the tumor is possible that may provide far more specific and reliable information regarding the tumors.

DNA microarrays: These are the cornerstone of genomic analysis. They comprise a number of (often thousands) of genes spotted onto a glass slide in a precise manner. There are two types of DNA microarrays cDNA array and oligonucleotide array. cDNA array is used mostly for gene expression profiling. RNA isolated from the tumor tissues are labeled and then hybridized to the microarray (chip) which contain known DNA molecules (~100 nts) immobilized on a solid surface (Figure1). An array can accommodate ~20 thousand specific sequences on a single chip, either chosen randomly or deliberately biased to represent collection of genes typically expressed in a cell type of interest. Oligonucleotide array contain short nucleic acids upto 25 nts immobilized on a glass slide surface. They are used to screen for sequence variations (mutations) of specific genes and for genotyping where labeled DNA from an individual is tested for genetic markers like single nucleotide polymorphisms (SNPs), microsatellite markers to yield a fingerprint, which in turn may be linked to the risk of developing diseases

In cancer DNA microarray can be applied to

- Study the global gene expression pattern in tumors contributing to malignancy i.e. snapshots of genes either up- or down-regulated in tumors.

Figure1: Schematic representation of Microarray analysis



The intensity and color of each spot encode information on a specific gene from the tested samples.

- Molecular classification of neoplasms by gene expression signatures
- Discovery of new prognostic or predictive indicators and biomarkers of therapeutic response
- Identification and validation of new molecular targets for drug development
- Identification of genes conferring drug resistance
- Prediction or selection of patients most likely to benefit from, or suffer from particular side effects of drugs (pharmacogenomics)

There are reports in the literature where microarray analyses was done to classify and to predict the clinical outcome of cancers. In case of acute myeloid leukemia a 13,000-gene array was used that separated acute myeloid leukemia patients into classes, including one class

with a particularly poor clinical outcome (5). Bullinger *et al.* (6) has used a 133-gene predictor that classified patients of AML into clinically relevant subtypes. Alizadeh *et al.* (7) studied lymphoma using Lymphochip, an expression array that separated patients with two subtypes originating from different progenitor cells, one with 76% response rate to chemotherapy and the other with poor response. Hoffman *et al.* (8) have analyzed published ALL microarray data set using robust multi-array analysis (RMA) and random forest (RF). They identified a set of 26 genes that yield accurate subgroup distinction and raised the prospect of a diagnostic gene expression platform for accurate diagnosis in pediatric ALL. Lapointe *et al.* (9) have used a microarray of 26,000 genes that separated prostate tumors into three distinct classes depending on the gene expression pattern. A 4-gene model (10) predicted relapse of prostate cancer independent of stage and grade. Biopsy specimen of lung cancer was analyzed (11) using a 99-gene profile and a 42-gene profile associated with increased risk of death. A review article (12) has discussed the molecular profiling of non-small cell lung cancer, the progress made thus far in relation to its etiology, pathogenesis, molecular classification and potential biomarkers that may be of use in diagnosis, screening, and assessing the effectiveness of therapy. By molecular profiling of breast cancer using two different gene sets: first, a set of 456 cDNA clones that reflect the intrinsic properties of the tumors and, second, a gene set that highly correlated with patient outcome, estrogen receptor positive tumor cells of breast cancer are separated into two distinct classes with different survival profile (13). van de Vijver *et al.* (14) used microarray analysis of 70-gene prognosis profile and classified a series of 295 consecutive patients with primary breast carcinomas as having a gene-expression signature associated with either a poor prognosis or a good prognosis. Glas *et al.* (15) have translated the 70 gene prognostic profile into a small custom made microarray containing 1900 probes instead of original 25,000 probes to predict the outcome of disease in breast cancer patient. Wang *et al.* (16) have used a 23-gene signature in colon cancer that predicts recurrence in Duke's B patient and upstaged to receive adjuvant therapy. Ziober *et al.* (17) have identified a 25-gene signature of early diagnosis and mass screening of OSCC (oral squamous cell carcinomas). This gene signature was found to be 96% accurate on cross validation. These arrays are measuring up well when compared with conventional classification and prognostication methods

Arrays are also used to study the drug response in different malignancies. A 31 gene profile distinguishes individual with CML that achieved major cytogenetic response treated with imatinib (tyrosine kinase inhibitor) (18). A 95 gene profile was identified that predicted imatinib sensitivity in ALL (19). Gene expression

pattern in childhood ALL and response to drugs like prednisolone (33 genes), vincristine (40 genes) L-asparaginase (35 genes) or daunorubicin (20 genes) was studied by (19). Xu *et al.* (20) did pharmacogenomic profiling of PI3K/PTEN-AKT-mTOR pathway in many solid tumors and identified variable expression of all the signaling protein in this pathway and predicted sensitivity to mTOR inhibitor (rapamycin). Potti *et al.* (21) have developed gene expression signatures that predict sensitivity to individual chemotherapeutic drugs and also to multidrug regimens in malignancies of different tissue origin.

After the discovery stage has been completed using microarrays, a smaller subset of targets is spotted on a 'macroarray' which can be used more conveniently. The differences between microarray and macroarray are given in Table 3.

Table 3: Microarray Vs Macroarray

Microarray	Macroarray
Molecular portrait of the whole genome	Molecular portrait of the biological pathways
DNA/Oligo's spot size smaller than 200 microns in diameter	Spot size over 300 micron in diameter
Contain thousands of spots per array	Fewer spots are present usually hundreds or less per array
Capture a molecular portrait of the living cell or tissue	Molecular signature obtained by parallel comparison of the portrait among samples of different physiological and pathological origin
Done for discovering or for research purpose	Clinical application, for tumor classification, prognostication and therapeutic response

Proteomics based approach

Proteomics: The goal of Proteomics is to obtain a more global and integrated view of biology by studying all the proteins of a cell rather than each one individually. The growth of proteomics is a direct result of advances made in large scale nucleotide sequencing of expressed sequence tags (ESTs) and genomic DNA as without this information protein could not be identified. Proteomics is important as many type of information cannot be obtained from the study of gene alone. Protein expression and function are subject to modulation through transcription as well as through posttranscriptional and posttranslational events. More than one RNA can result from one gene through a process of differential splicing. Additionally, there

are more than 200 post translation modifications that proteins could undergo that affect function, protein-protein and nucleotide-protein interaction, stability, targeting, half-life etc., all contributing to a potentially large number of protein products from one gene. Proteins are responsible for the phenotypes of cells, and it is only through the study of proteins can protein modification be characterized and the targets of drugs identified. Depending on the type of study different approaches are applied in proteomics like

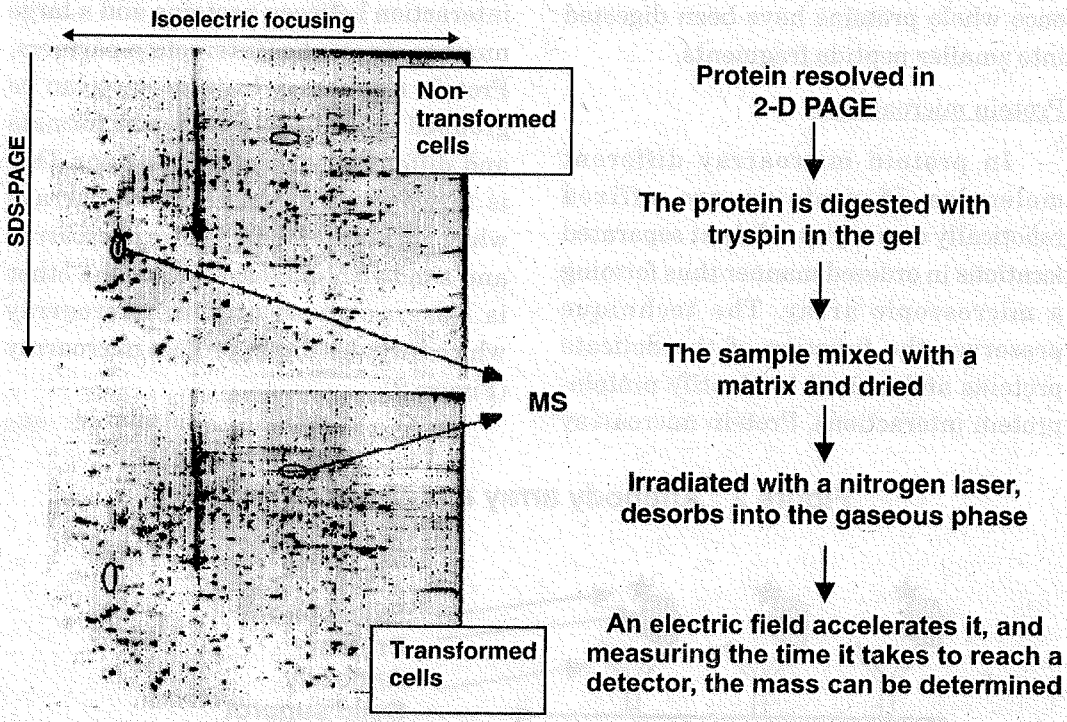
Two-dimensional Gel electrophoresis (2-DE): 2-DE is done for separation and isolation of different proteins in a sample. 2-DE is able to resolve proteins that have undergone some form of posttranslational modification and/or different forms of proteins that arise from alternative mRNA splicing or proteolytic processing. A number of improvements have been made in 2-DE over the years like introduction of immobilized pH gradients (improved the reproducibility of 2-DE), the use of fluorescent dyes (improved the sensitivity of protein detection), specialized pH gradients (able to resolve more proteins) and automation of the process of 2-DE from gel running to image analysis and spot picking.

Mass spectrometry (MS): MS is an important emerging method for the characterization of proteins and acquisition of protein structure information. MS can give sequence data from any Coomassie stained band or gel spot. The protein is digested with trypsin in the gel, peptides eluted and fractionated by reverse phase chromatography and introduced into the mass spectrometer. The mass spectrometer determines the mass of the peptides and the sequence (by collisionally induced dissociation). From the masses of the peptide fragments, sequence data is determined by comparison with known sequences or by manual interpretation. The methods for ionization of whole proteins are electrospray ionization (ESI), matrix-assisted laser desorption/ ionization (MALDI) and surface enhanced laser desorption ionization (SELDI).

ESI: Samples are analyzed by loading into an ion trap mass spectrometer using an ElectroSpray Ionization (ESI). Peptides require some form of purification after in gel digestion and manual loading of the samples in individual microcapillary tubes. It is tedious and slow.

MALDI: Most often, masses are determined by Matrix Assisted Laser Desorption Ionization-Time of Flight mass spectrometry (MALDI-TOF) (Figure 3). In this technique, the sample is mixed with a matrix, commonly cyanohydroxycinnamic acid for peptides or sinapinic acid for proteins and dried. When irradiated with a nitrogen laser, the matrix adsorbs energy, which is transferred to the peptide molecule, which desorbs into the gaseous phase. An electric field accelerates it, and by

Figure 3 : Protein separation by 2DE and protein identification by MALDI (Matrix assisted laser Desorption ionization).



measuring the time it takes to reach a detector, the mass can be determined. Sample application can be performed by a robot, the entire process including data collection and analysis can be automated and the samples can often be used directly without any purification after in-gel digestion (Qin *et al* 1997).

SELDI: SELDI overcomes many of the problems associated with sample preparation inherent with MALDI-MS. The underlying principle in SELDI is surface enhanced affinity capture through the use of specific probe surface or chips. A 2-DE analysis separation is not necessary because it can bind protein molecules on the basis of its defined chip surfaces. Chips with broad binding properties, including immobilized metal affinity capture, and with biochemically characterized surfaces such as antibodies and receptors, form core of SELDI (22). This MS technology enables both biomarker discovery and protein profiling from the sample source without preprocessing.

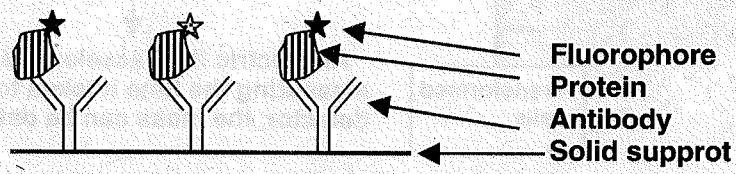
Mass analysis of proteolytic peptides is a much more popular method of protein

characterization, as cheaper instrument designs can be used for characterization. Additionally, sample preparation is easier once whole proteins have been digested into smaller peptide fragments.

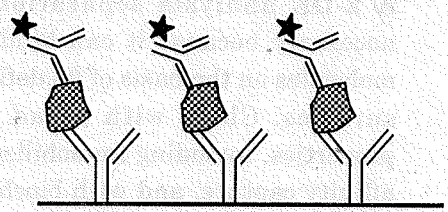
Protein microarrays:

In protein microarray different molecules of proteins are affixed robotically over a glass slide at separated locations in ordered manner thus forming a microscopic array. The technique preserves the function of the delicate proteins and is used to identify protein-protein interactions. Protein microarray technology has the potential to monitor complex intracellular protein expression and to simultaneously study the interaction between proteins and a large number of potential interaction partners. Protein microarray based assays can be grouped according to different formats and different types of applications. One is forward phase protein microarrays which include protein capture microarray and sandwich microarray and the other is Reverse phase protein microarray which includes direct protein microarray (Figure 2).

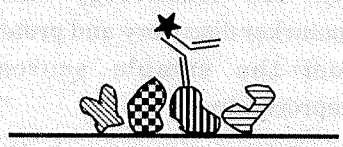
Figure 2 : Antibody array and protein array



Protein capture microarray (Forward phase protein microarray)



Sandwich microarray (Forward phase protein microarray)



Direct protein microarray (Forward phase protein microarray)

Protein expression proteomics:

The quantitative study of protein expression between samples that differ by some variable is known as expression proteomics. In this approach, protein expression of the entire proteome or of subproteomes between samples can be compared. Information from this approach can identify novel proteins in signal transduction or identify disease-specific proteins.

Structural proteomics:

Structural proteomics attempts to identify all the proteins within a protein complex or organelle, determine where they are located, and characterize all protein-protein interactions. An example of structural Proteomics was the recent analysis of the nuclear pore complex. Isolation of specific subcellular organelles or protein complexes by purification can greatly simplify the Proteomic analysis. This information will help piece together the overall architecture of cells and explain how expression of certain proteins gives a cell its unique characteristics.

Functional proteomics:

"Functional proteomics" is a broad term for many specific, directed proteomics approaches. This could include the isolation of protein complexes or the use of protein ligands to isolate specific types of proteins. This approach allows a selected group of proteins to be studied and characterized and can provide important information about protein signaling, disease mechanisms or protein-drug interactions.

Application of proteomics in cancer

New proteomic technology allows us to gain an overview of thousands of proteins simultaneously as a proteomic pattern, analyze the individual protein signaling pathways being utilized by neoplastic cells, characterize the neoplasm biologically, and select specific targeted treatment modalities, known as "personalized" molecular medicine.

Several studies have already shown that protein fingerprints can reproducibly distinguish between normal and tumor samples e.g. Breast cancer (23, 24) and bladder cancer (25). Moreover, protein profiling facilitates the differentiation between samples (including cytological material) obtained from histologically different types of tumor like non-small-cell lung cancer (26), leukemias (27). Refining diagnosis at the molecular level would obviously greatly improve the classification of tumors, ultimately ameliorating our ability to predict clinical outcome and to identify individuals at a higher risk of disease recurrence.

The potential utility of chip-based proteomics in the early detection of cancer has been demonstrated by (28 a,b), who have identified a serum proteomic pattern that accurately distinguishes individuals with ovarian and prostates cancer from control subjects. Valerio *et al.* (29) have applied proteomic analysis of serum samples to the differential diagnosis of tumors from tumor-like diseases, such as pancreatic carcinoma and chronic pancreatitis. Sera from individuals with

melanoma and healthy volunteers have been analyzed by MALDI-MS (Matrix Assisted Laser Desorption Ionization-Mass Spectrometer) This (30) showed the presence and abundance of proteins with molecular weights of 2500–3500 Da, which were completely absent in control subjects, indicating the potential of protein profiling not only in early diagnosis but also in disease prognosis. de Noo *et al.* (31) have used MALDI-TOF to assess the feasibility of this approach for the detection of breast cancer. Preoperative serum samples were obtained from 78 breast cancer patients and 29 controls. High sensitivity (100%) and specificity (97%) were shown for the detection of breast cancer and indicated the potential usefulness of serum protein profile in breast cancer detection. Miguet *et al.* (32) have used SELDI-TOF-MS technology to discover and identify potential biomarkers for accurate diagnosis of the different forms of chronic mature B-cell lymphomas. New biomarkers were observed in 3 mass ranges (m/z = 13000, m/z = 9000, m/z = <2000). These markers were observed in 38% of the patient's sera but in none of the control sera.

Besides providing new insights into cancer pathogenesis, proteomics could have an unprecedented impact on some vital areas of cancer patient management, such as

- Early detection of disease, by using proteomic patterns of body fluid samples (33, 34)
- Cancer diagnosis and/or prognosis, based on proteomic signatures of tumor samples as a complement to histopathological evaluation (26, 35, 25.)
- The development of new disease and/or patient-specific therapeutic strategies after the identification of differential display between normal tissue and tumor tissue (36,37) and
- A rational modulation of therapy according to changes in protein profiles associated with drug resistance (38, 39, 40).

Tissue microarrays

Compared with the high-throughput techniques of genomics and proteomics, most tissue based molecular analyses are slow, cumbersome and require extensive manual interaction. Using conventional molecular pathology technique only single sample can be process, by cutting thin 5μm section and analyzing the specimen by immunostaining or in-situ hybridization, and it is cumbersome and slow. To overcome these limitations of conventional technique and to enable genome scale molecular pathology studies, Tissue microarray (TMA) technology was developed. TMAs facilitate the analysis of molecular alterations in thousands of tissue specimens in a massively parallel fashion. Construction of TMAs is achieved by acquiring cylindrical core specimen from up to 1000 fixed and paraffin embedded tissue specimens and arraying them at high density into a recipient TMA block.

The composite array block is sliced into sections that are placed on a glass slide. The slide now contains hundreds of tumor samples. Immunohistochemical staining or *in situ* hybridization can be used to query the array for specific molecules such as insulin-like growth factor binding protein (e.g. IGFBP1), apoptosis related proteins (e.g. BCL2), heat-shock proteins (e.g. HSPs) and a transcription factors (e.g.GATA2).

Studies are done using tissue array method to look for the increased expression of various proteins in cancer tissues. Singer *et al.* (41) have used tissue arrays to investigate the expression pattern of transglutaminase-2 in 57 invasive breast cancer biopsies and 62 ovarian cancers. Transglutaminase-2 has a role in cell growth and is also known to be associated with cell adhesion, metastasis and extracellular matrix modulation. Increased protein expression was seen in 84% of breast tumors and 58% of ovarian tumors. Ortiz-Rev *et al.* (42) have investigated the frequency and pattern of expression of CD10 and renal cell carcinoma (RCC) marker in 40 clear cell renal cell carcinomas using two tissue arrays prepared from paraffin blocks. They suggested that CD10 and RCC were often expressed by clear cell renal cell carcinomas and they may be useful markers to suggest the renal origin of carcinomas.

Conclusions

Recent technological advances in genomics and proteomics could revolutionize the way of doing research in medicine by providing researchers with a formidable high-throughput laboratory tool to study gene and protein expression profiles and functions in health and disease. New diagnostics and therapeutics biomarkers will also be discovered using methods that provide global views of cellular function. The greatest potential in the care of cancer patients could lie in the personalization of diagnosis and treatment which could be possible using molecular profiling that provides the global gene/protein information. Despite the promise demonstrated with molecular profiling, several barriers must be overcome prior to routine diagnostic implementation for patient intervention. One barrier is the cost of microarray technology for determining the molecular profile of the tumor. This technology is expensive and requires special handling procedures. Efforts are underway to reduce the problems associated with molecular profiling in order to bring this technology from bench to bedside. Small number of genes selected from a larger expression data set can be tested for clinical relevance. In case of B-cell lymphoma clinically relevant outcomes have been predicted using biomarkers of a set of 6-gene reverse transcription (RT)-PCR that can be easily standardized in a clinical laboratory (43). And biotechnology companies are trying to commercialize the array chips for different cancers.

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MOLECULAR TARGETS FOR CANCER PREVENTION AND THERAPY

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Summary

Cancer is caused by accumulation of genetic damage in a susceptible cell. Majority of cancer causing mutations affect proteins that regulate essential cellular functions such as cell proliferation, cell cycle, differentiation, angiogenesis and apoptosis. Dominant mutations convert proto-oncogenes to oncogenes, and recessive mutations inactivate tumor suppressor genes. Many of the genes that are altered in human cancer define molecular pathways that are central to the control of the above processes. These pathways are not strictly linear but, rather, constitute molecular networks. The pathway of growth factors and cell cycle include Tyrosine Kinase receptors and downstream signaling via phospholipase C, Ras, PI-3 kinase, cdks. The dysregulation of these signals in tumor cell leads to multiple cellular changes. Therapeutic approaches include monoclonal antibodies against growth factors receptors, antisense oligos against key target proteins, enzyme inhibitors, antiangiogenic therapy, apoptotic triggers/ activating death signaling pathways. Nutrients such as Vitamin D, Selenium, folate, curcumin, flavonoids, etc. also influence the above molecular targets and can help in cancer prevention.

Keywords: Cancer prevention, cancer therapy, molecular targets, signaling

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Introduction

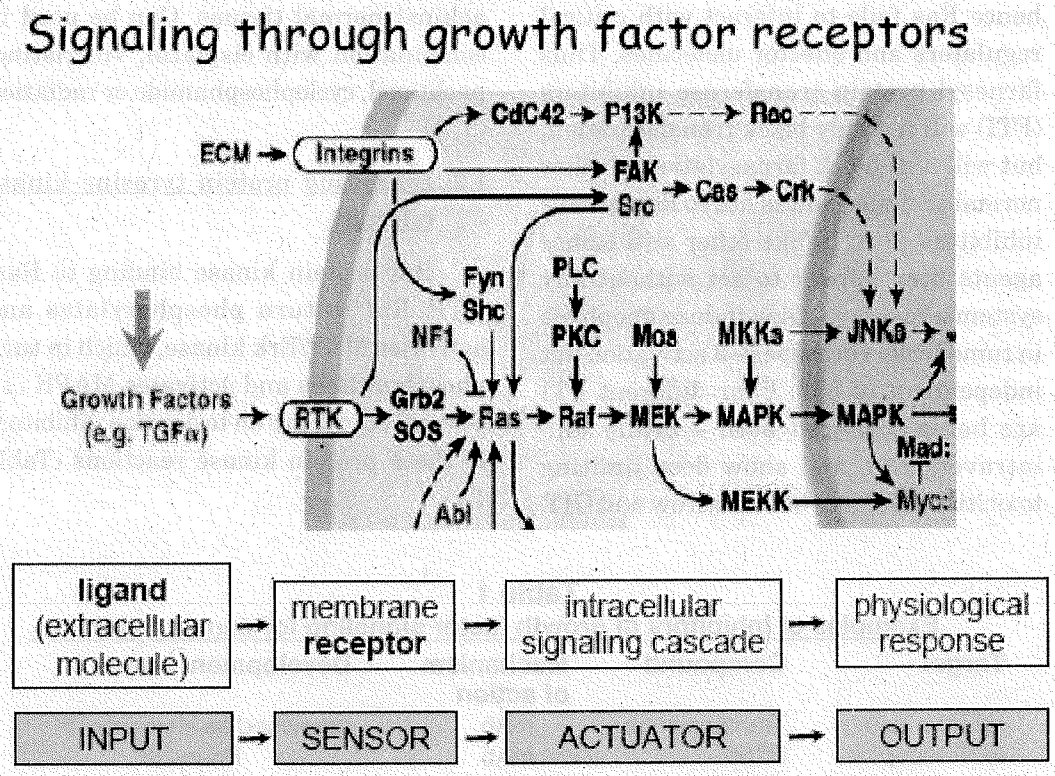
Cancer is a disease of multiple and changing genetic alterations that must be attacked with therapies having different mechanisms of action. Rational molecular approaches for anticancer therapies must be developed to control cell proliferation, cell cycle, angiogenesis, apoptosis, but these therapies/agents should have lethality to tumors without overt systemic toxicity. Signaling pathways that drive cell proliferations are closely related with tumor malignancy. Components of these pathways are encoded by oncogenes such as PDGF-like ligand Sis, Tyr kinase Src and HER-2/C-Neu and GTP-binding switch Ras. Mutations in key components lead to constitutive activation of these pathways and to proliferation.

I. Growth factor signaling

Signaling pathways are initiated with the binding of a ligand such as PDGF, EGF, EGF-like ligand (TGF-α) or IGF to its transmembrane receptor. Ligand binding induces the dimerization of receptor subunits promoting autophosphorylation of the receptor and recruiting a variety of intracellular docking proteins such as Grb2, Shc to the plasma membrane. From these docking proteins subsequent signals emanate. Ras serves as a molecular switch in the plasma membrane that alternates between the inactive GDP-bound state and an active GTP-bound state. Normally Ras is bound to GDP, but on recruitment of Sos to the plasma membrane, Sos binds Ras-GDP and facilitates release of GDP. GTP which is highly abundant in cells and binds to Ras. Ras-GTP adopts a conformation that permits interaction with downstream effector molecules such as Raf, which activates MAP kinase cascade (1). The dysregulation of signals in tumor cells leads to multiple cellular changes including alterations in DNA synthesis, lipid metabolism, cellular morphology, cell adhesion properties and gene expression (Figure 1).

The signaling mechanism has given therapeutic agents in the treatment of cancer which include anti-estrogens, anti-androgens, agonists of gonadotropin releasing hormones and stem cell growth factors. Therapeutic approaches include molecular antibodies (mAbs) against extracellular domain of receptors, antisense oligonucelotides to key target proteins and enzyme inhibitors (Table 1). Inhibition of HER-2 yielded the first cancer therapeutic agent based on growth factor signaling. Unlike other members of EGF – receptor family, HER-2 has no known ligand, its expression is upregulated in 25-30% of human breast cancer. This upregulation promotes HER-2 heterodimerization with other members of EGF receptor family, as well as HER-2 homodimerization, resulting in constitutionally active tyrosine kinase. The mAb against HER-2, Transtuzumab is directed against extracellular domain of HER-2. It inhibits proliferation by several mechanisms. The mAb upregulates P27 Kip inhibitor of some

Figure 1



cyclin-dependent kinases, it also accelerates the internalization and degradation of HER-2, reducing cellular level of tyrosine protein kinase. It includes immune mediated effects such as cell mediated cytotoxicity and complement fixation. Along with cisplatin, doxorubicin, paclitaxel it shows enhanced anti tumor activity but with doxorubicin, may have higher cardiotoxicity. Therapeutic antibodies have also been developed against EGF receptor for eg. C-225 and E7.6.3, which are being clinically evaluated. Inhibitors of the intracellular tyrosine kinase for eg. ZD-1839, a competitive inhibitor of ATP are in clinical trials (2).

Drug Targets include the following

I.1. GTPase switch

Mutated forms of Ras have been found in many solid tumors as well as leukemia. The agents in clinical trials are based on regulating Ras gene expression or by inhibiting protein farnesylation. Ras proteins carry an essential lipid moiety – a farnesyl group at carboxy termini. Inhibitor of Ras farnesylation blocks Ras

localization to the plasma membrane and hence Ras fails to interact with critical regulators and effector molecules. Thus farnesyl protein transferase inhibitors (FTI) will not only block transformation, but will also block farnesylation of other normal proteins, and so not so Ras specific inhibitors. But, unlike other anti tumor agents they appear to act without any systemic toxicity – they induce apoptosis in tumor cells via caspase-3 activation but independent of p53. Four different FTI are being explored both – orally and intravenously, and show dose limiting toxicities involving bone marrow and GIT. FTIs can have antiproliferative effect against normal tissues. Can be used in combination with cisplatin, vincristine, paclitaxel, cyclophosphamide or radiation (3, 4).

I.2. Inhibiting protein tyrosine kinase effectors

Raf protein kinase binding to Ras-GTP. Raf in turn phosphorylates and activates MAP/Erk kinase, which in turn phosphorylates and activates MAPK (1). Several efforts are on to develop inhibitors of these protein kinase reactions (Table 1)

Table 1
Examples of inhibitors of growth factor signaling for cancer treatment

Target	Compound	Mechanism of action	Development status
HER2/c-neu	Transtuzumab	mAb	Launched as Herceptin™
EGF receptor	C225	mAb	Phase III
	E7.6.3	mAb	Preclinical
	ZD-1839	Kinase inhibitor	Phase II
	CP-358,774	Kinase inhibitor	Phase II
	PD-168,393	Kinase inhibitor	Preclinical
PDGF receptor	SU-101	Kinase inhibitor	Phase III
IGFR	AS ODN	Antisense	Preclinical
Ras	ISIS-2503	Antisense	Phase II
	R115777	FTI	Phase II
	SCH66336	FTI	Phase II
	L-778,123	FTI	Phase I
	BMS-214662	FTI	Phase I
Raf	ISIS-5132	Antisense	Phase II
	ZM336372	Kinase inhibitor	Preclinical
	L-779,450	Kinase inhibitor	Preclinical
MEK	PD-184352	Kinase inhibitor	Preclinical
	U0126	Kinase inhibitor	Preclinical
PKC	ISIS-3521	Antisense	Phase II
	CGP41251	Kinase inhibitor	Phase II
	UCN-01	Kinase inhibitor	Phase I
PI3'-Kinase	LY294002	Kinase inhibitor	Preclinical

I.3. Blocking lipid mediated signaling

Activation of GF receptor is also associated with changes in phospholipid metabolism. The phosphorylated residues on the intracellular domain of these receptors bind phospholipase C which then cleaves membrane phospholipids. One of these breakdown products, diacylglycerol activates PKC-α, which has been implicated in cell proliferation and tumorigenesis (5). PKC-α has been found to be increased in breast tumor. Both antisense inhibitors of PKC- α and inhibitors of PKC kinase activity are in clinical trials. The kinase inhibitors are derivatives of staurosporine. Therapies developed against growth factor regulated proliferation pathways are reaching the clinic to be tested. Most of these compounds do not act solely on tumor tissue, and hence their toxicities must be managed.

II. Cell cycle and checkpoint controls

Components of cell cycle machinery are frequently altered in human cancer. Central players are cell cycle dependent kinases (cdks) which help in cell cycle phases. Cdks are controlled by their association with cyclins and cdk inhibitors, by their state of phosphorylation and by ubiquitin mediated proteolysis. As malignant cells evolve both genetic and epigenetic mechanisms affect the expression of cell cycle regulator proteins causing overexpression of cyclins and loss of expression of cdk inhibitors – major consequence is deregulation of cdk activity providing cells with a selective growth advantage. Specific kinase inhibitors can block cell cycle progression and induce growth arrest (6). Failure of cell cycle arrest or apoptotic responses in malignant cells in response to cellular damage and ensuing instability may lead to emergence of malignant clone. Many anticancer agents act at multiple steps in the cell cycle and their effects may be cytostatic or cytotoxic depending on status of cell cyle of the target cell.

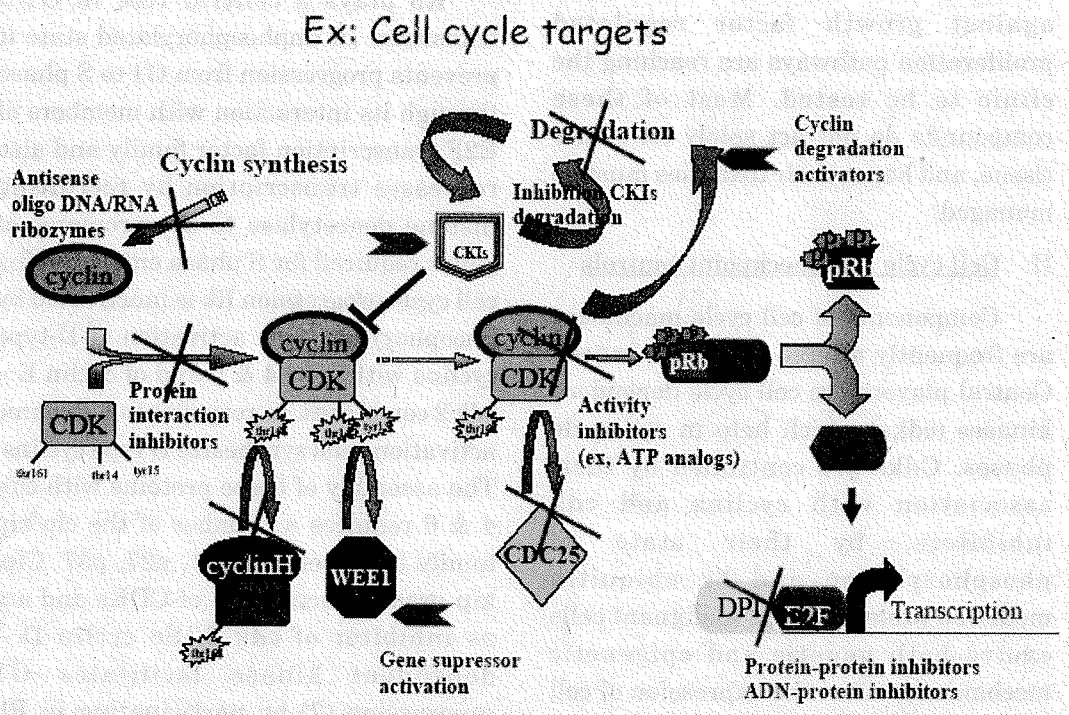
II.1. Drug targets – inhibition of cdk activity during the G1 phase.

Rb plays a central role in G1/S transition. In unphosphorylated state it prevents progression from G1 to S phase through its interaction with members of E2F transcription factor family and also represses transcription by recruiting histone deacetylase to the promoter of genes required for S phase entry. During cell cycle progression Rb is inactivated by phosphorylation by activation of D-type cyclins with cdks 4 & 6 and of cyclin E – cdk 2 complexes. In response to mitogenic activation cells synthesize D type cyclins. The assembly of these proteins with cdk 4 & 6 requires a member of the cip/kip family of proteins – p21, p27, p57. Cip/kip promote activation of CDKs and act as inhibitor of cdk 2. So cyclin D – dependent kinase facilitates G1 progression (7) by participating in Rb phosphorylation which relieves transcriptional repression by the Rb-E2F

complex sequestering cip/kip protein which facilitates the activation of cyclin E – cdk 2 (8). Cyclin E – cdk 2 – mediated Rb phosphorylation disrupts the binding of Rb to E2F, allowing E2F activation and transcription of genes necessary for S phase entry and progression. Rb is the primary target of cyclin D-dependent kinases. G1 progression is also regulated by INK4 family which acts as specific inhibitors of cdks 4 & 6. Most common alterations in tumors having wild type Rb is the inactivation of p16 INK4A by gene deletion, point mutation and transcriptional silencing by methylation (Figure 2). An inhibitor which blocks the ATP binding site of cdk4 maintains Rb-E2F as an active transcriptional repressor and promotes G1 arrest, but not in tumors lacking Rb?

The cdk inhibitors include several classes of drugs, all derived from microbial and plant sources. Of these flavopiridol and UCN-01 (7-hydroxystaurosporine) are in clinical

Figure 2



Flavopiridol: CDK inhibitor resulting in cell cycle arrest at G1/S and G2/M
Bryostatin-1: CDK2 inhibitor resulting in cell cycle arrest at G2

trials. They both bind to the ATP-binding pocket of the kinase and block cell cycle progression at G1 phase and change the levels and distribution of endogenous cdk inhibitors, independent of p53. The cdk inhibitor purvalanol B is more potent and shows high degree of selectivity for cdks. Short peptides are being investigated that block the interaction of cyclin A – cdk2 with substrates such as E2F1. They induce S phase arrest and cause abrupt apoptosis and are selective for transformed cells. Drugs that facilitate mitotic entry following DNA damage – G2 checkpoint abrogators therefore sensitize cells to chemotherapy and radiation and this sensitization is selective for p53 – deficient cells making them attractive as novel antineoplastics.

The development of drugs that target specific cellular pathways can improve efficacy, lower toxicity and lower costs – personalized medicine. Strategies designed to reduce cdk4 activity that would also cause cip/kip protein to redistribute into complexes with cyclin E and cdk 2 may be preferable to the use of drugs that simply block the ATP-binding site of cdk4. Such strategies include altering cdk4 stability, reducing cyclin D levels, replacing p16 INK4a expression using adenovirus vectors or reactivating methylated p16 INK4a. (Figure 2)

III. Angiogenesis

It is the process of *de novo* formation of vasculature that nourishes the growing tumor with oxygen and nutrients. Sprouting angiogenesis involves multiple linked and sequential steps that include endothelial cell proliferation, migration, invasion, survival and capillary tube formation, mediated by multiple factors. The strategy of antiangiogenesis therapy provides an alternate to controlling the tumor. All solid tumors show angiogenesis. The targeted vascular endothelial cells are normal, genetically stable cells and therefore less likely than tumor cells to become drug resistant. Thus antiangiogenic therapy must be endothelial cell-specific and must distinguish between tumor and normal vasculatures. Tumor vessels are poorly structured and immature i.e. have incomplete coating with periendothelial cells. Immature vessels depend on survival factor. There are certain cell surface proteins on angiogenic tumor vessels, for eg. VEGF receptors and $\alpha_V \beta_3$ and $\alpha_V \beta_5$ integrins are relatively weakly expressed in normal endothelium and might provide useful targets for inhibition of tumor vessel angiogenesis. Angiogenesis is activated by perturbation in oxygen homeostasis and hormones. Both pro and anti angiogenic factors co-exist within the same tissue and the process of angiogenesis is mediated by specific interaction between them, at multiple levels i.e. between protein, including ligand-receptor, extracellular matrix, and antiangiogenic factor interactions. Thus identifying peptide domains that are specifically involved in tumor angiogenesis i.e. isolation of peptides from phage display libraries that bind to proteins that are preferentially

expressed on tumor blood vessels, has proved helpful in uncovering receptor selective expression on tumor vasculature. A chimeric peptide containing

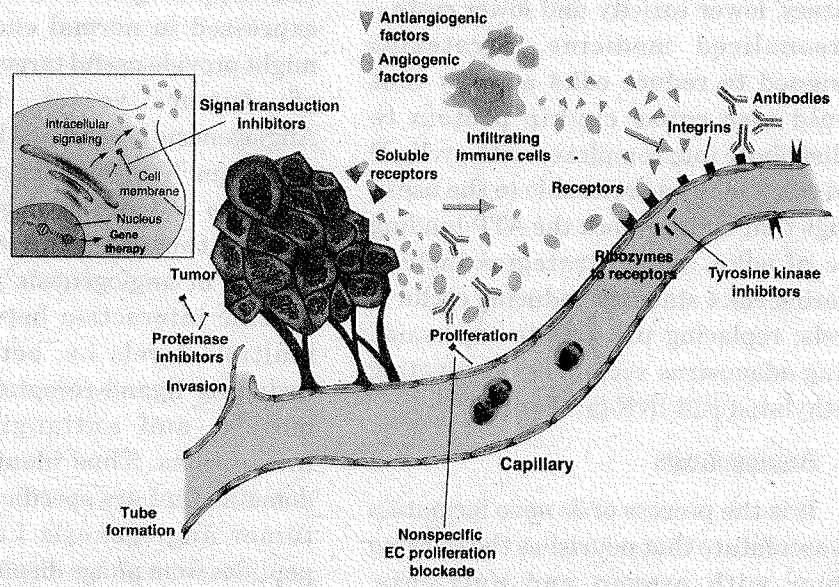
a tumor blood vessel homing motif and a pro-apoptotic peptide was selectively toxic to angiogenic endothelial cells and showed anticancer activity in mice (9).

III.1. Antagonizing proangiogenic activities

Angiogenic stimulus comprises of a series of steps which include local degradation of bone marrow, directional migration of the underlying endothelial cells, invasion of the surrounding stroma, endothelial cell proliferation, capillary tube morphogenesis, coalescence of capillaries into large vessels, vascular pruning and acquisition of a periendothelial cell coating. Thus, tumor neovascularization could be inhibited at each of these strategic junctions VEGF family i.e –A, -C, R1, R2 are upregulated in angiogenesis (Figure 3). VEGF plays a role in both vasculogenesis and angiogenesis and is an embryonic cardio vascular morphogen. It is tightly

Figure 3

Strategies to Inhibit Tumor Angiogenesis



Adapted with permission from Fidler et al. In DeVita et al. *Cancer: Principles and Practice of Oncology*. 6th ed. 2001:137.

regulated during development. Different classes of VEGF antagonists include anti VEGF neutralizing antibodies, inhibitors of VEGF-R2 tyrosine kinase, soluble versions of VEGF-R1 & R2.

VEGF is strongly induced by hypoxia and hypoglycemia. It functions as a survival factor for immature blood vessels which become VEGF independent only upon their maturation and engagement with periendothelial cells. For eg. early regression of blood vessels on androgen ablation in prostate cancer is due to suppression of androgen-regulated VEGF production. VEGF has dual role as an angiogenic factor and a vascular survival factor. In vicinity of primary tumor, pro-angiogenic signals override anti angiogenic, resulting in growth, but in remote sites the balance tilts in other direction because pro-angiogenic factors have short half life, whereas antiangiogenic factors in circulation last longer creating a net inhibitory effect.

III.2. Antiangiogenic therapy

Angiostatin is an antiangiogenic protein, a 38 KD internal fragment of the serum protein plasminogen. Another protein endostatin is a terminal fragment of collagen XVII a component of blood vessel wall. Both proteins inhibit VEGF activity, are non toxic, non immunogenic and natural body proteins. Introduction of DNA that codes for antiangiogenic factor, circumvents the need for daily injection. Another strategy is that of inducing the release of antioangiogenic peptides from their endogenous precursor proteins, e.g. conversion of plasminogen to angiostatin using tissue specific plasminogen activator or streptokinase (10).

III.3. Modulation of endothelial cell-ECM interactions

The extracellular matrix (ECM) has a profound effect on the angiogenic phenotype through multiple interactions with endothelial cells and transduction of signals by cross-linking integrin receptors on endothelial cells. In addition MMP (matrix metallo proteases) appears to be specific for angiogenesis. Antiangiogenic therapeutic approaches target MMP activity (11). Integrin mediates adhesion of endothelial cells to ECM components. Antagonists of α integrin are in phase II clinical trials. α_v p_3 mAb (vitaxin) is in trials. The identification of receptors for newly discovered antiangiogenic proteins might open new routes for therapeutics with small molecules.

IV. Apoptosis

In cancer the goal is to trigger tumor selective cell death. Biology of neoplasia has expanded to incorporate not only lesions that cause dysregulation of growth but also those that lead to inefficient cell death. Apoptosis represents an efficient cellular suicide pathway. Molecular pathways whose end point is death/ apoptosis coincide with the goal of successful treatment. Expression of apoptotic modulators within a tumor appear to correlate with its sensitivity to traditional cancer therapies. A drug that activates apoptosis might achieve a

therapeutic index in several ways – (1) activate a death cascade via a drug target uniquely expressed in a cancer cell. (2) it might be delivered to the target tissue in a manner that is selective for the cancer cell. (3) most promising strategy is to exploit a pathway that is activated by oncogenes, in order to provoke apoptosis selectively in cancer cells. Oncoproteins can interact with apoptosis regulatory pathways. Thus overexpression of Myc sensitizes cells to a wide assortment of apoptotic trigger, probably reflecting the role of apoptosis in the intracellular immunity that prevents normal cells from persisting in the body once they acquire cancer causing genetic defects. Thus oncogenes can sensitize cells to pro apoptotic treatment (12).

Apoptotic cell death is triggered by intracellular cues such as DNA damage and osmotic stress and extracellular cues including GF withdrawal, matrix detachment and direct cytokine mediated killing. Two central pathways are involved in the process of apoptotic cell death, one involving the activation of caspase proteases and a second the mitochondrial pathway. Caspase activation is regulated by adaptor molecules (FADD) that promote or inhibit caspase activation (IAPs). A number of death pathways converge on caspase cascade. Some of these begin when a death ligand such as TNF or Fas L interacts with its cognate receptor. TNF-R or Fas (CD95) induces the trimerization of the receptors. These adaptor molecules then activate caspase-8.

The second, mitochondrial pathway to apoptosis is the likely province of the Bcl-2 family of proteins which contain both pro apoptotic factosr such as Bax, Bak, Bcl-XS and anti apoptotic factors such as Bcl-2, Bcl-XL. In response to apoptosis signals, pro-apoptotic Bcl-2 family members translocate to and alter the permeability of the mitochondrial membrane by forming channels, leading to changes in mitochondrial membrane potential, cytochrome C release and the production of reactive oxygen species. Antiapoptotic members of Bcl-2 family reside in the outer mitochondrial membrane and counter these effects. The mitochondria and caspase apoptotic pathways are intimately connected, leading to activation of caspase 9 and 3.

IV.1. Drug targets in apoptosis

1. Inhibition of Bcl-2 by antisense oligos, antisense mRNA, ribozyme constructs, peptides that mimic the domain of Bax which mediates binding to Bcl-2 would disrupt the interaction of Bcl-2 with Apaf-1, which suppresses caspase-9 activity.
2. Activating death pathway directly using soluble death ligands
3. NF-κB is a transcription factor composed of dimers of the NF-κB/ Rel family of proteins. Its activity is induced by a number of stimuli, including cytokines TNF-α, IL-1 and viral infection. It mediates a critical anti apoptotic signal, and so inhibition of NF-κB appears to lead to cell death. Targets of NF-κB

regulation include the IAPs, TRAF-1 & -2 which are thought to suppress caspase-8 activation. Multiple human tumors (Hodgkins) and an avian retrovirus have evolved mechanism for dysregulating the NF-κB pathway, suggesting that NF-κB participates in cellular transformation. Drug targets in NF-κB pathway include therapeutic inhibitors of NF-κB activation, IKK-2 inhibitor as IKK-2 is a regulator of IκB whose inhibition should block the disruption of the IκB / NF-κB complex, adenoviral mediated delivery of IκB α targeting NF-κB at the level of transcriptional activation. NF-κB activates transcription through the p300/CBP family of co-activators, so molecules that disrupt this complex could prove therapeutic.

4. P53 is the most commonly mutated gene in human cancer. It regulates cell cycle and apoptosis. Restoration of p53 pathway in p53-deficient cells is a promising approach for controlling tumors by gene transfer vectors, targeted delivery of transgenes – to enhance apoptosis only in tumors in which the p53-apoptotic pathway is otherwise intact.

5. P13K/AKT pathway is a potent mediator of cell survival signals. Extracellular survival signals delivered as soluble factors or through cell attachment can inhibit apoptosis by activating this pathway. PI3K acts on membrane phosphoionosides to generate PI 3, 4 bis PO4 and PIP3, which serve as foci for recruiting and activating a number of signaling proteins to the membrane. Among these proteins are AKT family. Overexpression of AKT can confer cell survival. AKT phosphorylates and inactivates a number of substrates that are connected with apoptosis such as caspase 9, BAD. AKT can also inhibit cytochrome C release and thus affect apoptosis at multiple points. PI3K/AKT pathway is attractive target for therapeutic intervention.

V. Anticancer drug targets - Oncogenes and tumor suppressor genes

Among the genetic damage seen in cancer are dominantly acting mutations that convert proto-oncogenes to oncogenes as well as recessive mutations that inactivate tumor suppressor genes. Majority of hereditary cancer syndromes are due to germ line mutations in tumor suppressor genes. Most human cancers harbour mutation that directly or indirectly inactivate the Rb protein e.g. mutations affecting genes such as p16/INK4A, cdk4 and cyclin D1 which lead to phosphorylation and functional inactive of pRB. Altered growth factor receptors, as well as oncogenic Ras, likely, also impinge on this pRB pathway. pRB is an important negative regulator of cell cycle progression and serves to integrate positively and negatively acting mitogenic signals. Loss of pRB leads to mitogen independence of cancer cells. Likewise, p53 is mutated in 50% of human cancers

and many tumors that retain wild type p53 allele harbour mutations in other genes that regulate p53. For eg. ARF negatively regulates p53. Downstream target of p53 is apoptosis inducer Bax, the function of which is antagonized by Bcl-2, which is overexpressed in a variety of tumors including nodular lymphomas.

The importance of p53 and pRB in carcinogenesis is underscored by the fact that a variety of unrelated DNA tumor viruses have independently evolved the means to inactivate both pRB and p53, for eg. the oncogenic HPV product E7 and E6 proteins that inactivate pRB and p53 respectively. Thus it may be possible to choose the most suitable drug target from a variety of structurally unrelated molecules that all function in the same signaling pathway. These pathways are not strictly linear but, rather, constitute molecular networks. For eg. loss of pRB leads to derepression of the E2F transcription factor family, which in turn, leads to the induction of ARF and subsequent activation of p53.

Thus the potential for molecular cross – talk between pathways has implications for the development of drugs that specifically target tumor cells while sparing normal cells (13). Not all the abnormalities in a cancer cell would need to be corrected to have a therapeutic efficacy. For e.g. restoring p53 function in p53 defective tumor cells is sufficient to induce cell death, to induce a cell-cycle block, or, in some to restore sensitivity to conventional chemotherapy. Most cancer causing mutations induce a loss, rather than gain of function. Loss of function of one molecule is often similar in consequence to a gain of function of another for eg. loss of cdk inhibitor p16/INK4A might be viewed as a gain of cdk 4 and cdk 6 (14). Genes are actually members of gene families eg. p53 homologs are p63 and p73 and these are rarely mutated in human cancers, but, they can substitute for p53 to induce apoptosis in p53 defective tumors. So, p53 function can be restored by using small molecules that can activate p73 or p63. Thus, one can exploit mutations that were selected because they were essential for the survival of the cancer cell.

VI. Molecular targets for nutrients involved with cancer prevention

Discovery of molecular pathways critical to carcinogenesis is revolutionizing the treatment and prevention of cancer. Compelling experimental epidemiology and clinical evidence indicates that many cancers are preventable, especially because diet and nutrition are key factors in the modulation of cancer risk. The road to nutritional intervention in cancer prevention has led to successful trials as well as trials that did not reach their intended endpoints (15). Nutrition is intimately involved in cancer prevention and use of food stuffs represents a cost effective and noninvasive strategy for reducing cancer risk. Dietary nutrients can influence cancer risk by inhibiting or enhancing carcinogenesis through diverse mechanisms and molecular interactions. Transforming nutrition and cancer

research from an observational to a molecular approach offers potential of identifying individuals who will and will not benefit from dietary intervention strategies. Genetic polymorphisms can also influence the dynamics between nutrients and molecular targets.

We need to embody new and innovative preclinical and clinical approaches to nutrition and genetics. Numerous dietary components such as essential nutrients, phyto or allelo chemicals in plants, carotenoids, flavonoids, indoles, isothiocyanates, etc. can potentially alter genetic and epigenetic events and gene-regulated metabolic pathways through interactions with specific molecular targets. The molecular targets may be individual genes, molecules that result from gene expression or are affected by gene expression. The processes influenced by nutrients are differentiation, cell cycle, cell signaling, apoptosis, carcinogen metabolism. The areas of interest are (1) characterizing molecular events that govern, the ability of specific nutrients to alter cell cycle checkpoints, (2) credentialing of target receptors for cancer prevention that are modified by dietary constituents (3) methylation patterns that are influenced by dietary manipulations that influence gene expression and cellular phenotypes and (4) use of natural genetic variations to elucidate how nutrient exposures are linked to phenotype (5) signaling pathways that regulate cancer growth, development, differentiation and apoptosis as regulated by dietary components. Vitamin D, calcium, folate, genistain, selenium, reservatrol, curcumin are being investigated in chemoprevention trials (16). (Table 2) lists some of the nutrients that modify cancer risk.

Table 2

Partial list of Nutrients that may modify cancer risk

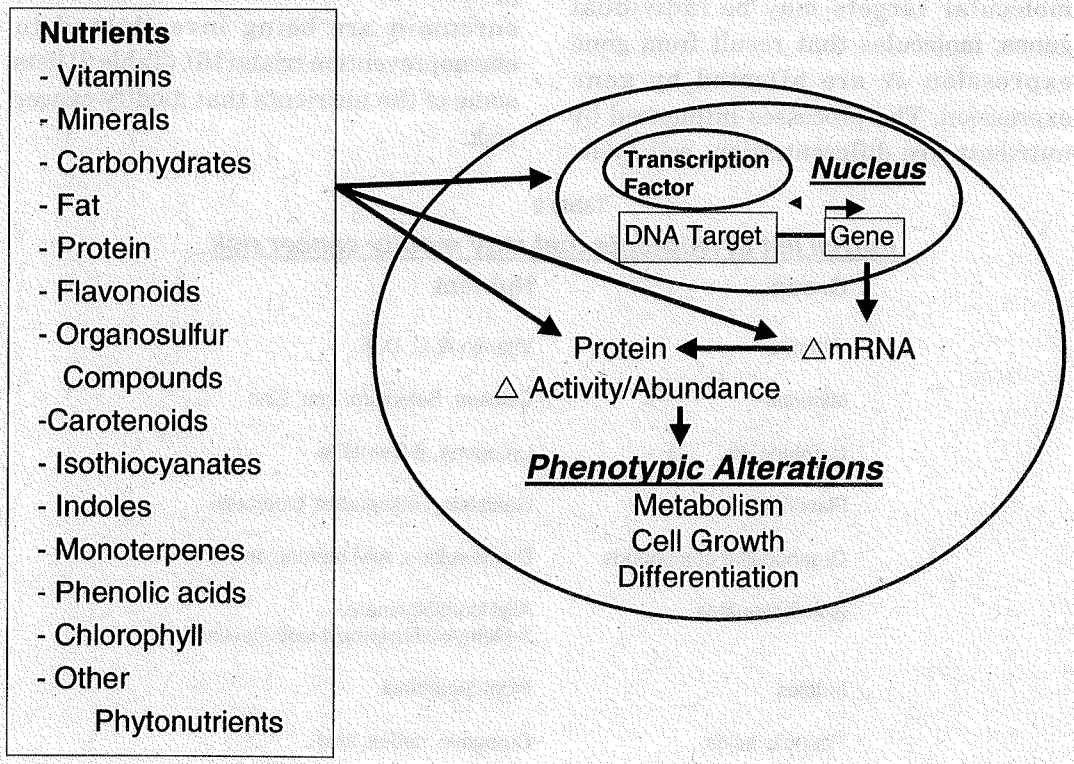
Group	Nutrient
Vitamins	Vitamin A, C, D, E
Minerals	Calcium, Selenium, Iron, Zinc
Carotenoids	Lycopene, β-Carotene
Flavonoids	Genistein, Resveratrol, Quercetin
Organosulfur compounds	Diallyl sulfide, Allyl mercaptan
Isothiocyanates	Allyl isothiocyanate, 3-Methylsulfinylpropyl isothiocyanate
Indoles	Indol-3-carbinol
Phenolic acids	Curcumin, caffeic acid

VI.1. Specific nutrients and their molecular targets

Nutrients act within numerous biochemical and molecular cascades involving nuclear and cytoplasmic events that regulate the amount and activity of specific proteins (Figure 4), which in turn influence cell proliferation, apoptosis and differentiation. Vitamin D and its metabolites act through a variety of molecular targets to inhibit carcinogenesis. Vitamin D binds to EGF receptor, reduces availability of EGF, with subsequent inhibition of growth and increased differentiation in normal and malignant cells. Vitamin D receptor binds to DNA adjacent to the jun-fos gene complex, inactivating jun, which is an activating gene for transcription. This results in repression of normal transcription and finally inhibition of cell proliferation. It also downregulates c-myc. Folate functions as a coenzyme in one-carbon transfer reactions in the metabolism of nucleic and amino acids. Folate deficiency results in altered DNA methylation, disruption of DNA integrity, and disruption of DNA repair and thus

Figure 4

Selected nutrients as regulators of gene expression



leads to increased risk for carcinogenesis especially of colorectal cancer. Cancer cells often show global hypomethylation which promotes genetic instability due to overexpression of oncogenes. It is also suggested that hypomethylation and DNA strand breaks resulting from folate deficiency might enhance the incorporation of tumorigenic viruses such as HPV into human DNA. Molecular targets for selenium have supported that besides its antioxidant property selenium has diverse biological functions for e.g. inhibition of proliferation, enhancement of immune response, alteration of metabolism of carcinogens and induction of apoptosis. Selenium plays a role in the thioredoxin system. The activity of thioredoxin reductase, a selenoenzyme has been linked to NF-κβ activation. NF-κβ is an inducible oncogenic nuclear transcription factor that responds to the redox state of the cells and has pivotal role in inducing genes involved in a number of physiological processes, including those associated with cytokines, GFs, adhesion molecules and immuno receptors. Selenium availability can also alter DNA methylation. Essential and nonessential nutrients cannot work in isolation but rather work together. Several factors might account for variability in response to dietary selenium. Vitamin C has been reported to reduce the effectiveness of selenium against chemically induced colon cancer, but selenium has been shown to enhance the ability of garlic to inhibit chemically induced mammary cancer in animals.

Certain classes of phytonutrients such as flavonoids, carotenoids, organosulfur compounds, terpenes and isothiocyanates have a range of molecular targets. Genistein a phytoestrogen present in soyabean can bind to estrogen receptor and exhibit estrogen like biological activity. It decreases the risk for certain hormone related cancers such as breast, endometrial and prostate. Genistein scavenges free radicals and is an antioxidant. It induces cell cycle arrest, inhibits PTK inhibitor, angiogenesis and metastasis and stimulates apoptosis by inhibiting antiapoptotic transcription factor NF-κβ.

Similarly resveratrol found in grapes and red wine is a good antioxidant. It inhibits ornithine decarboxylase a key enzyme which is enhanced in cancer cell proliferation. It inhibits Cox-2 activity through inhibition of PKC signal transduction pathways, influences the expression of Bax and p21 genes involved in the regulation of cell proliferation and apoptosis. It induces apoptosis through activation of p53 dependent transcription and caspase activation, and inhibition of NF-κβ.

Genetic differences also play a role in the ability of individuals to withstand exposure to exogenous carcinogens or to inhibit initiation, promotion or proliferation in carcinogenesis (17). Prevalence of polymorphisms in genes coding for activation of enzymes of cytochrome P450, detoxification enzymes glutathione-s-transferase may account for inter individual variation in cancer susceptibility and cancer prevention.

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EARLY DETECTION OF GYNAECOLOGICAL MALIGNANCIES - REFRESHING AWARENESS FOR MEDICAL FRATERNITY

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Summary

- Regardless of the frequency of cervical screening, an annual gynaecologic examination including pelvic examination is recommended.
- A dedicated team of cytopathologists for the study of PAP smears is essential for the success of any screening program for cancer cervix. However for visual inspection of cervix only the performer of the test should be trained.
- The latest guidelines given by American Cancer Society for screening of cancer cervix seem to be very thought out strategy to curb the tendency to over investigate the population.
- The use of cytobrush along with the conventional Ayre's spatula has reduced drastically the number of unsatisfactory PAP smears.
- Combined use of PAP smear and HPV DNA testing should be offered for suspicious patients with bad cervical erosions.
- Postmenopausal endometrial hyperplasias and endometrial polyps must be followed up with hysteroscopic directed biopsy.
- Lactating breasts, dense breasts and the malignant breasts will greatly benefit from frequent yearly Ultrasonographic evaluation.
- Mammograms must be interpreted by BIRADS classification.
- Ultrasound Breast should be advised more often than Mammography uptill the age of 50 yrs in general population.

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- Ultrasound Pelvis and CA 125 are two good modalities to diagnose and interpret the morphology of adnexal masses.
- Since majority of ovarian cysts are benign, a mature decision for futher evaluation of cysts by way of specific tumor marker evaluation, excision etc. must be made as per the morphology of the cysts.
- A thorough history taking, examination of breasts, inspection of cervix and biomanual examination of pelvis of all females should be a must in all women irrespective of the systemic disease she is suffering from. This will go a long way off in prevention, early detection and treatment of gynaecological malignancies.

Key Words: Gynaecological malignancy, Screening, PAP smear, Ultrasound pelvis, Mammography, Cancer cervix, Endometrial Cancer, Ovarian tumor, Breast Cancer.

Introduction

Cancer is increasingly recognized as a global problem and not limited to developed world. The incidence of all cancers varies from 90-120/100,000 populations (1). In India gynaecological cancer account for 55 to 60% of all cancers in woman. Cervical and breast cancers together account for 40% of all female cancers. Although ovarian cancer is the third common cancer of women in India, still more women die of it than any other cancers as ovarian malignancies present at advanced stages (2). (Fig 1)

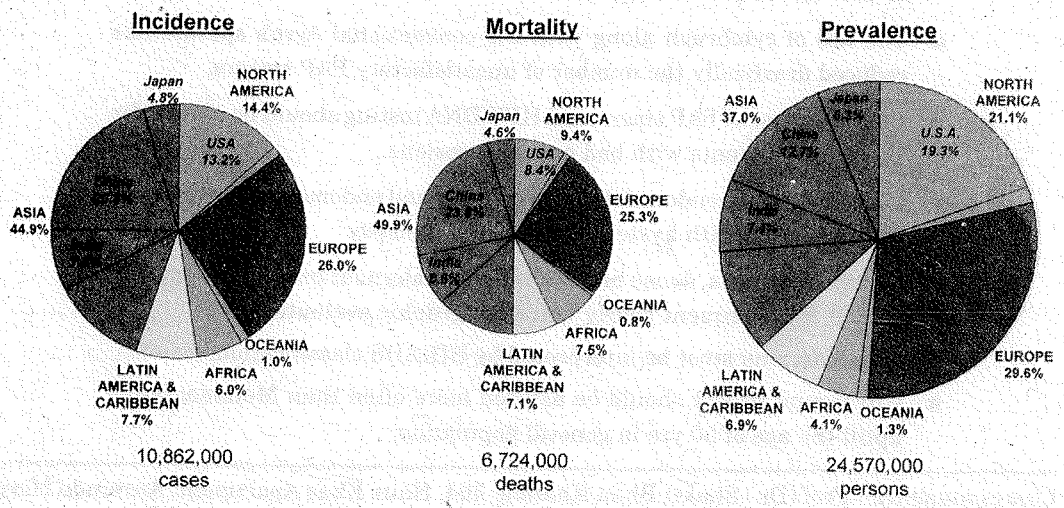

Fig 1. Global Cancer Statistics Incidence, Mortality, and Prevalence by Location (3)

One may think as why to talk about cancer in women in India, where more women die from malnourishment, childbirth trauma, infection, anemia etc than cancer, and very few reach the ages where cancer is prevalent. Very true! But if we aim for positive health in a community, we have to also inculcate amongst medical fraternity the concept of prevention of the disease, and the aim of today's presentation is to sensitize the medical professionals about the concept of preventive gynaecological oncology. The main thrust of the talk will be on cancer cervix which we know is a preventable cancer. One does not need the expensive infrastructure, for its screening. It is only the awareness of the basic facts about the etiology of the disease which all of us irrespective of the speciality to which we may belong, have to keep in mind, while dealing with a woman patient who may not have come for any Gynae complaint. A simple examination of breast and inspection & feel of the cervix is all what is needed to have the suspicion of the cancer of breast and cervix after a few leading questions have been asked from the woman. Prevention and early detection of cancer is also extremely critical in a country like India, where in case of late detection, cost of treatment is very high. Advanced stages of cancer call for expensive modalities of treatment. So cancer prevention and early detection becomes a medico-social responsibility and an economic necessity.

According to WHO "about half to one-third cancers can be prevented (tobacco & alcohol related diet modification and immunization against Hepatitis B & Human Papilloma viruses); about one-third can be prevented by early detection with current knowledge (breast and cervical cancers) and in about one-third cases palliation can improve the quality of life for incurable cancers. The primary prevention is possible if the causative organism is known and Human Papilloma Virus (HPV) is recognized to play a significant role in etiology of cancer cervix (4).

Role of History Taking

Currently, it appears that the best way to detect any early cancer is for both the patient and her clinician to have a high index of suspicion of the diagnosis in the asymptomatic woman. There is a definite role of detailed and appropriate history taking and also making patients aware of the risk of cancer as a strategy for preventing cancer. Advising high risk patients for surveillance and follow up and counseling them to get their peers for preventive health check is one of the important components of advice given to women after undergoing screening programme. Teaching the proper techniques of monthly self examination of breasts and counseling the women about its importance is another important aspect of the preventive gynaecological oncology. Therefore, under this scenario, there is a definite role of a general physician/generalist gynaecologist as a primary health care provider in

implementing the screening programme for cancer detection in the community.

WHO criteria for screening programmes (5) are:

- The condition sought should be an important health problem.
- There should be an accepted treatment for patients with recognized disease.
- Facilities for diagnosis and treatment should be available.
- There should be a recognisable or early symptomatic stage.
- There should be a suitable test for examination with high sensitivity and specificity.

Well Woman check (6)

Keeping the concept of preventive oncology in mind, a health package by the name of "Well Woman Check" is offered in the Indraprastha Apollo Hospital, New Delhi. A prospective study for 9½ years from December 1997 uptill August 2006 was done for all patients who came for this check. During this period, total O.P.D. attendance for the hospital was 1026,143 out of which 1190,76 patients (11.6%) were examined in preventive health checks. 47,630 (40%of all health checks) were females. 4295 women (11.08% of all female patients) had Well Woman Check done. This check is performed by the gynaecologist. It includes a detailed relevant history taking, general physical and systemic examination, and examination of relevant organs i.e., breast, abdomen and female genital organs by way of speculum examination and bimanual examination. The investigations performed under this check include complete blood counts, fasting and post prandial blood sugar, blood grouping and Rh typing, routine and microscopic urine and stool examination, X-ray chest PA view, PAP smear, Ultra sound Pelvis – trans abdominal and trans vaginal , Bilateral Mammogram and Ultra sound Breast.

It will be seen from Table I that 64% of the ladies were above 40 yrs of age. 70% were in the upper middle class and none from the lower socio-economic status.

Cervical Cancer

Cancer cervix is an important public health problem. It is the third cancer in frequency world wide and most or second most common cancer in women in developing countries (Fig 2). PAP smear

Table-1 : Clinical profile of Study Group (Well Woman Check)

Age	Number	(%)	Socio-Economic Class	Number	(%)
18-40yrs	1549	36	Lower Middle and <	0	0
40-60yrs	2199	51	Middle	1288	30
>60yrs.	0547	13	Upper middle and>	3007	70

has been the hallmark for screening of cervical cancer and it has been proved beyond doubt since 1960's that cancer cervix can be prevented by the detection and treatment of precancerous lesions in the Cervix by cytology.

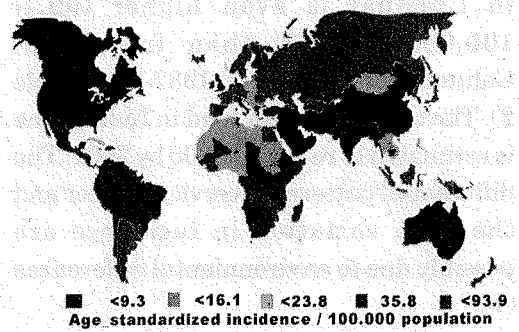

Fig. 2 The global burden of cervical cancer

Some facts about cancer cervix are:

- Cervical cancer is preventable
- Most patients are asymptomatic.
- Patients may present with postcoital bleeding, intermenstrual bleeding and an abnormal vaginal discharge.
- Risk factors for selective and opportunistic screening are:-
  - Multiple partners, S.T.D.s
  - Early onset of sexual activity.
  - HPV infection
  - High parity
  - Immunosuppresion and HIV infection.
  - Oral Contraceptive users have a 4 times increased risk in HPV poitive cases perhaps by decreasing folate levels or by activation of metaplastic cells.
  - Smoking
  - Husband with previous wife having cervical cancer.
  - Low socio-economic status.
  - Poor nutrition and poor hygiene.

During the last 20 years the understanding of the etiological agents for Cancer Cervix has improved a lot. The detection of high risk oncogenic Human Pε pilloma Virus (HPV) type 16 & 18, as the main etiological agent for occurrence of cancer Cervix has been established beyond doubt(4). It seems as if HPV induced Cervical cancer is an anomaly in the otherwise elusive search for the cause of human cancers, as almost no other cancer has a single exposure agent which is a necessary cause of the cancer. The detection of HPV viruses on cervix as a primary screening modality or as an adjunct to cervical cytology is no doubt one of the best screening methods for cancer Cervix, but the availability of the infrastructure and the health care resources is a very important impediment in their usage for mass screening programmes. This cancer being a disease of the poor, and developing nations, the low cost, low technology screening modalities have been evolved lately as an alternative to the cytology.

WHO criteria for screening programme have been fulfilled by cervical screening and the mortality from cervical

cancer is falling in systematically screened population. For cancer Cx, a long latent period of premalignant stage, HPV as a definitive agent, easy and direct access of uterine Cx for examination and sampling and effective treatment for pre malignant changes, make this cancer probably the only gynaecological cancer to satisfy all these criteria.

In India, cervical cancer is the commonest malignancy in the females and accounts for 85% of all gynaecological cancer and 25% of all female cancer. About 18% of all cervical cancer patients are in India (2). Ethnic variations in India reveal varied incidence rate for different communities i.e. 28/100,000 female population for Hindus, 18 for Christians, 15 for Muslims and 4 for Parsis (2). The truncated rate in the age group 35-64 yrs in Chennai is even higher (99.1/100,000;1982-95) than from Cali, Columbia (77.4/100,000;1987-91), (Table 2). The cervical cancer load in India alone is estimated to reach 100,000 by 2001. The differential patterns of cervical cancer and the wide variation in incidence are possibly due to environmental differences (7).

Table-2 : Incidence Rates of Cancer Cervix in India

Registry	Age Adjusted Rate		
	1982	1987	1991
Bombay	17.8	16.1	18.6
Bangalore	34.1	24.2	27.5
Madras	40.6	41.3	38.5

Screening tests for Cervical Carcinoma

1. Conventional cervical PAP smear.
2. Liquid based cervical cytology
3. Visual inspection
   a) Unaided visual inspection (VI)
   b) Visual inspection using Acitic acid (VIA)
   c) Visual inspection using Lugol's iodine (VILI)
4. Colposcopy
5. Cervicography
6. HPV, DNA testing
7. Other emerging techniques
   a) Computer assisted reading of cervical smears.
   b) Use of physical real time devices.
   c) Detection of molecular surrogate markers of cancer progression.

Cervical cytology and visual inspection are the main modalities for early detection of cancer and they will be further discussed here.

Cytology: - Cervical cytology is the gold standard for screening. For the simple and easy test of cytology, one needs a Cuscus speculum, an Ayres spatula and cytobrush. The sensitivity of the conventional Pap test is of only 55-60% with reported false negative rate varying from 25-50% and false positive rates from 15-20%. Sensitivity & specifity of the test is improved by taking smear form ectocervix by Ayres spatula and from endocervix by cytobrush. The fluid sampling techniques have further improved the results of the cytology. It is recommended that Colposcopy and colpomicroscopy directed cervical biopsies should be performed in abnormal PAP smear and appropriate treatment instituted.

VIA & VILI :- These are the low cost techniques for cervical cancer screening which have been extensively investigated in India. Visual inspection is now regarded as the best option for proposed cancer control programme in India. It needs short training course and para medical staff can be trained easily.

In VIA (Fig 3, Fig 4), the cervix is exposed by bivalve speculum and 50% Acetic acid solution is applied on it. After two minutes, the cervix is inspected for the presence of aceto-white areas when test is called positive for VIA. In VILI 50% Lugol's iodine is applied instead of acetic acid. Non uptake of iodine dye by the cervix qualifies for positive test (Fig5, Fig 6). The patients for positive cytology or VIA and VILI are further evaluated by colposcopy and biopsy. The patients with negative tests can be reassured and can have screening done after 5 years (8). The VIA has 90% sensitivity and 92% specificity with 17% the positive predictive value and 97% negative predictive value.

Fig . 3 Normal cervix – VIA negative

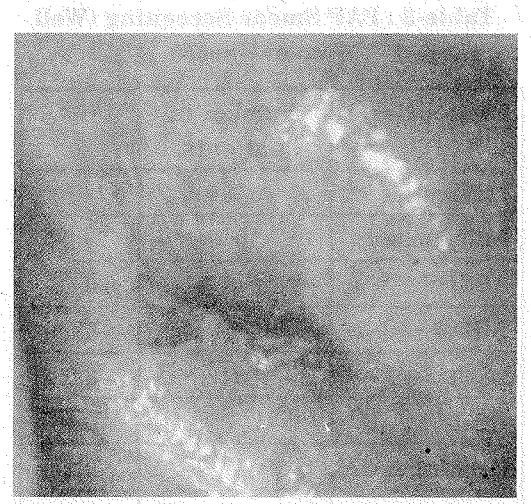

Fig. 4 Ecto Cervix - VIA Positive

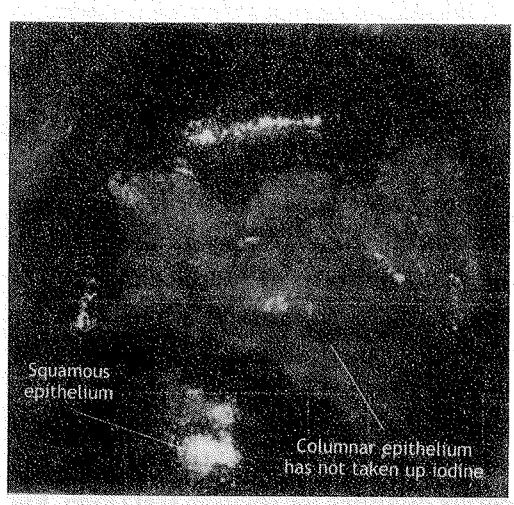

Fig. 5 Ecto Cervix - VILI negative

Fig. 6 Ecto Cervix - VILI positive

Table 3 shows Pap smear screening in well woman check. There were only 30 suspicious PAP smears out of 4295 smears taken. Frank cancer was detected in 5 patients. All the patients in the study group had two smears taken, one from ectocervix by Ayres spatula and the other from endocervix by cytobrush. 74.3% of PAP smears were normal, 24.40% were inflammatory, 0.18% Atypical squamous cells of undetermined significance (ASCUS), 0.09% Atypical Glandular cells of undetermined significance (AGUS), 0.2% low grade squamous intraepithelial lesion (LSIL) and 0.16% were high grade squamous intraepithelial lesions (HSIL). In other words, 30 suspicious smears (0.7%) were positive PAP smears for screening purpose which needed further evaluation. Non inclusion of low socio-economic status women accounted for such low incidence of positive PAP smears.

Table-3 : PAP Smear Screening (Well Woman Check)

Diagnosis	Number of patients	(%)
Normal	3194	74.03
Inflammatory	1049	24.04
ASCUS	8	0.18
AGUS	4	0.09
LSIL	11	0.02
HSIL	7	0.16
Cancer Cervix	5	0.11
Not done	17	0.39

There is no definite protocol for the cervical cancer screening. Where as in U.K.(9), it starts at 25 yrs of age, with 3 yearly interval for the age group 25-49 years, 5 yearly interval from 50-64 years and no screening from the age of 65 years on wards, the American Cancer Society

Guidelines (ASCG) (10) are slightly different.

- All women should begin cervical cancer screening about 3 years after they begin having vaginal intercourse, but no later than when they are 21 years old. Screening should be done every year with the regular Pap test or very 2 years using the newer liquid-based Pap test.
- Beginning at age 30, women who have had 3 normal Pap test results in a row may get screened every 2 to 3 years. Another reasonable option for women over 30 is to get screened every 3 years (but not more frequently) with either the conventional or liquid-based Pap test, plus the HPV DNA test. Women who have certain risk factors such as diethylstilbestrol (DES) exposure before birth, HIV infection, or a weakened immune system due to organ transplant, chemotherapy, or chronic steroid use should continue to be screened annually.
- Women 70 years of age or older who have had 3 or more normal Pap tests in row and no abnormal Pap test results in the last 10 years may choose to stop having cervical cancer screening. Women with a history of cervical cancer, DES exposure before birth, HIV infection or weakened immune system should continue to have screening as long as they are in good health.

Even though, there is no organised screening programme in India, even then 54.4% of women between 21 to 65 years of age have had a smear and 44.30% had it within the proceeding three years. The ACS guidelines cannot be recommended in India due to lack of infrastructure and resource crunch. The WHO recommendation of once a life time PAP smear between the ages of 35-40 years for Indian scenario is a good compromise.

Ovarian Cancer (Fig 7)

Ovarian cancer (204,000 cases and 125,000 deaths) is the sixth most common cancer and the seventh cause of death

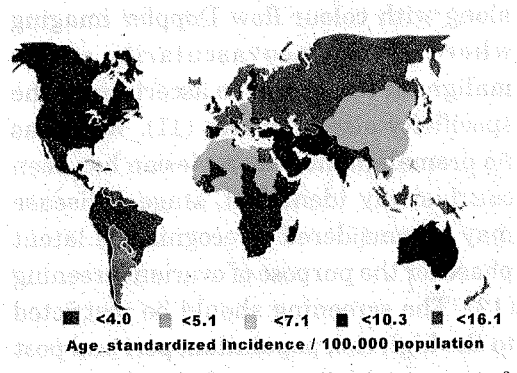

Fig. 7 The global incidence of ovarian cancer[3]

from cancer in women (4.0% of cases and 4.2% deaths). Incidence rates are highest in developed countries with rates in these areas exceeding 9 per 100,000, except for Japan (6.4 per 100,000). Incidence in South America (7.7 per 100,000) is relatively high. Incidence rates have been slowly increasing in many Western countries and Japan. The risk of ovarian cancer is reduced by high parity and use

of oral contraceptives. In India, the incidence is 4.65% of all female cancers (2).

CA 125 and Transvaginal ultrasonography (TVS) are the two extensively investigated diagnostic modalities for detection of ovarian cancer. Although, CA 125 is not a specific tumor marker for diagnosing ovarian malignancy, the rising levels even within the normal range should raise the suspicion of an occult ovarian or primary peritoneal cancer. Since 90% of ovarian tumors are epithelial tumors only, CA 125 is considered a screening marker. TVS has high sensitivity for ovarian cancer, and along with colour flow Doppler imaging where by the neovascularity of the malignant tumor can be ascertained, the specificity also increases (11). Where as no premalignant ovarian lesion has been conclusively identified, stage I disease may be considered a recognisable latent phase for the purpose of ovarian screening (12). The screening should be restricted to the high risk population, peri and post menopausal women with family history of ovarian, breast, endometrial or colon malignancy; late age at first pregnancy, non users of oral contraceptive pills and detection of 5cm or more sized cyst in post menopausal woman. The multilocular ovarian cysts with solid and cystic components are the suspicious cysts for malignancy.

Currently it appears that a high index of suspicion for the disease in the high risk patient, is the best way to detect early ovarian cancer. Routine bimaunal pelvic examination, thorough investigation of adnexal masses and impressing on follow up for patients with positive findings are the methods for early detection.

In the well women check study (Table 4), 8.6% of patient had ovarian cysts detected on TVS. No case of early ovarian cancer was detected in the study. Patients were advised to repeat pelvic ultrasound after 6-12 weeks if the size of the cyst was less than 5 cm with normal Colour Doppler studies. On the other hand, any mass with abnormal vascularity and suspicious of malignancy and all masses >5cm in size need surgical evaluation.

Table-4 : Ultrasound Pelvis - Adenexal lesions (Well Woman Check)

Diagnosis	Number	(%)
Normal	3788	88.01
Ovarian Cysts	371	8.06
Polycystic	119	2.07
Para ovarian cyst	4	0.03
Hydrosalpinx	13	0.03

Endometrial cancer (Fig 8)

Cancer of the endometrium has a rather similar geographic distribution to ovarian cancer. However, it appears more important as a cause of new cases (199,000 or 3.9% of cancers in women) than in terms of mortality (50,000 deaths or 1.7% of cancer deaths in women) because of the much more favorable

prognosis. It is a cancer of postmenopausal women; worldwide, 91% of cases occur in women aged 50 and older. Survival is rather good and similar to that of breast cancer-86% in the US SEER

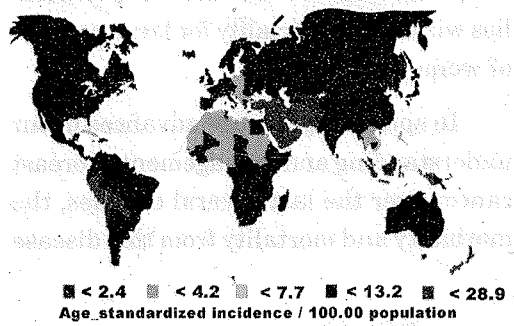

Fig. 8 The global incidence of endometrial cancer[3]

registries and 78% in European registries. The proportion of these cases surviving up to five years in developing countries is greater than the corresponding proportion of breast cancers. The highest incidences are in North America (22.0) and Europe (11.8 to 12.5). Rates are low in southern and eastern Asia (including Japan) and most of Africa (less than 3.5 per 100,000).

The American Cancer Society (10) recommends that at the time of menopause, all women should be informed about the risks and symptoms of endometrial cancer, and strongly encouraged to report any unexpected bleeding or spotting to their doctors. For women with or at high risk for hereditary non-polyposis colon cancer, annual screening should be offered for endometrial cancer beginning at 35 years of age.

U.S.G determined endometrial thickness is used as a screening method for endometrial cancer. A 5 mm thickness of endometrium is generally accepted as normal and anything above it warrants further evaluation of endometrium by aspiration cytology or endometrial biopsy. At an endometrial thickness threshold value of 5mm, TVS has a +ve predictive value of 9% for detecting any abnormality. The sensitivity is 90% and specificity of 48% with negative predictive value of 99%. Diagnosis of endometrial hyperplasia with atypia is considered as a precancerous lesion of endometrium (13). However, the routine screening for endometrial carcinoma is currently not justified.

3.7% of women in well woman check study (Table 5) had Postmenopausal Endometrial Hyperplasia, 1.6% had endometrial polyps and 0.7% had fluid in

Table-5 : Ultrasound Pelvis – Uterine Lesions (Well Woman Check)

Diagnosis	Number	(%)
Normal	2513	58.05
Fibroid Uterus	865	20.01
Adenomyosis	627	14.06
Postmenopausal Endometrial Hyperplasia	159	3.07
Fluid Polyps	30	0.07
Endometrial Polyps	72	1.06
Post Hysterectomy	27	0.06

endometrial cavity detected by TVS. Histopathological evaluation of all the patients was done by hyteroscopic directed biopsies. 4 cases of endometrial cancer from thick endometrial group and two from endometrial polyp group were detected by Histopathology.

Breast cancer (Fig 9)

In India, breast cancer accounts for 22.3 % of all female cancers, second only to the cervical cancer(14). Despite some arguments to the contrary, obstetricians and gynaecologists function as primary care physicians for women upto pre-menopause. Therefore, the diagnosis of breast carcinoma in its most curable form lies within this speciality for large number of women.

In spite of significant advances in our understanding and management of breast cancer over the last several decades, the morbidity and mortality from this disease

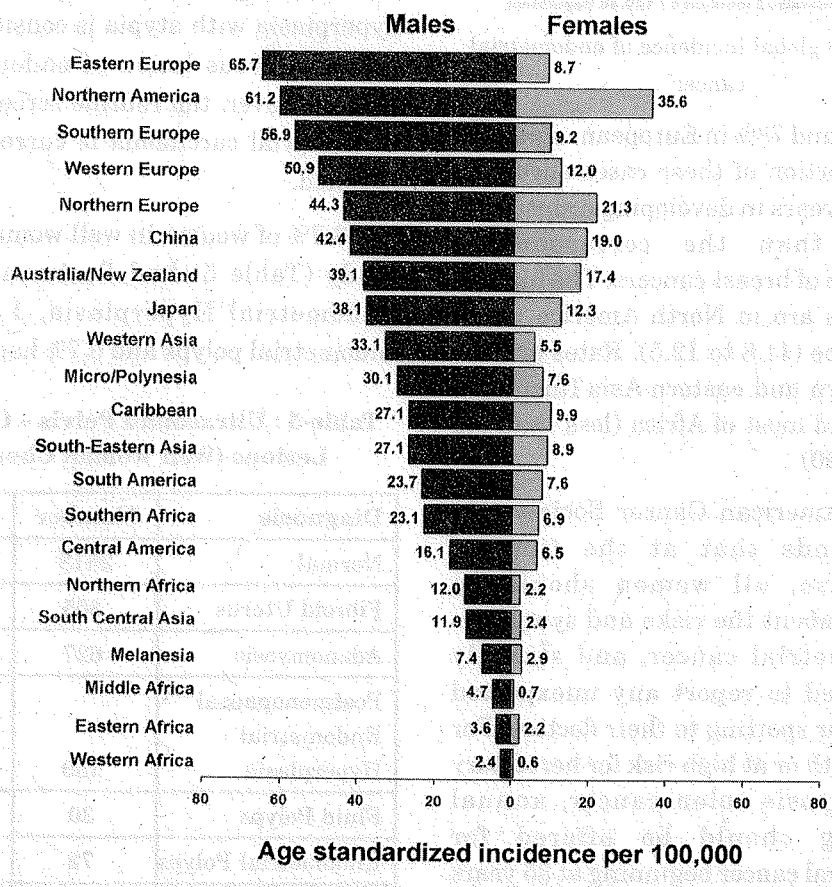

Fig 9. Global incidence of ovarian cancer[3]

remain high. Therefore, prevention of this disease has become one of the most important challenges for the medical community. It is estimated that several billion dollars per year could be saved if its development were prevented.

The ability to identify individuals or populations at risk for breast cancer is an integral part of effective preventive strategies, but until recently we have not been able to accomplish this task with any degree of certainty. Other than gender and age, fewer than 50 % of cases occur in women with other risk factors, and 85% are diagnosed in women without any family history. Recent advances in our understanding of the molecular biology of breast cancer have led to the identification of specific mutations that may help identify women with a hereditary predisposition to developing breast cancer, as well as to predict who will respond to adjuvant therapy (15).

The causes of carcinoma breast seem to be multifactorial and so are the risk factors. These risk factors can be identified and evaluated by good history taking and thorough examination of breasts. Finding a breast lump and evaluation of the nature of the lump is screening for and diagnosis of carcinoma breast. 90 % of breast lumps can be detected by clinical examination alone. Mammography and Ultrasound breast help in detection of rest of the 10 % of the non palpable lumps.

Mammogram and Ultrasound breast is advised to all patients above 40 yrs of age (Table 6, Table 7) Patients who are less than 40 years of age and have low risk for cancer breast are screened for breast lesion by Ultrasound. BIRADS-3 lesions need re-evaluation after about six months where as BIRADS 4 and 5 lesions need FNAC and biopsy from the lesions. In the present study no case of carcinoma breast was detected.

Table-6 : Evaluation of Mammograms (Well Woman Check)

Diagnosis	Number	(%)
BIRADS 0	149	3.04
BIRADS 1	3019	70.02
BIRADS 2	798	18.05
BIRADS 3	243	9.07
BIRADS 4	15	0.03
BIRADS 5	-	-
Not done	71	1.06

Table-7 : Ultrasound Screening for Breast (Well Woman Check)

Diagnosis	Number	(%)
Normal	2882	67.01
Fibroadenosis	827	19.02
Breasts cysts	230	5.03
Fibrocystic disease	226	3.09
Ductal Dilatation	38	0.08
Fibroadenoma	88	2.00
Cancer	4	0.09

Prevention of breast cancer is aimed at detecting pre-invasive lesions, such as ductal carcinoma *in situ* and lobular carcinoma *in situ* or early stage invasive breast cancers that have the potential to be cured with limited treatment. Screening tests aimed at breast cancer prevention include the Breast Self Examination (BSE), clinical breast examination by health care providers, Mammography and Ultrasonography of breast. Monthly self examination of breasts is a recommendation followed for quite some time now, but it has been seen that this has not appreciably decreased the overall mortality rates due mainly to the small number of women who actually perform these examinations. Extensive use of Mammography and Ultrasound screening of breasts have declined the overall mortality rate of breast cancer by 5% in U.S. women

The American Cancer Society guidelines for early detection of cancer breast are as follows:

- Yearly mammograms are recommended starting at age 40 and continuing for as long as woman is in good health.
- Clinical breast exam (CBE) should be part of a periodic health exam, about every 3 years for women in their 20s and 30s and every year for women 40 and over.
- Women should know how their breasts normally feel and report any breast change promptly to their health care providers. Breast self-exam (BSE) is an option for women starting in their 20s.
- Women at increased risk (for example, family history, genetic tendency, past breast cancer) should talk with their doctors about the benefits and limitations of starting mammography screening earlier, having additional tests (for example, breast ultrasound or MRI), or having more frequent examinations.

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BIOLOGY OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA IN INDIAN PATIENTS IS DIFFERENT FROM THE WEST

Biology of Childhood ALL

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Summary

The treatment outcome in childhood ALL in Indian patients has been reported to be poor as compared to 70 percent cure rate in the West. While this could be multifactorial in origin, one aspect could be a different biology of the disease affecting the treatment outcome. Collaborative studies between NCI, NIH, USA and three Indian centers, All India Institute of Medical Sciences, Tata Memorial Hospital and Cancer Hospital Chennai, revealed significant phenotypic and genotypic differences from the West. Thus, there was a high relative incidence of T Cell ALL, paucity of common ALL and absence of an early age peak. Molecularly, the frequency of chromosomal translocations studied by real time PCR revealed lower frequency of t (12; 21) which is associated with a good prognosis and more frequent translocations t (1;19) and t (9; 22) which are associated with a poor prognosis. It appears, therefore, that a different biology of childhood ALL in Indian patients contributes, at least in part, to the poor treatment outcome seen.

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Population based data from various Indian Cancer registries suggest that approximately 10,000 new cases of Acute Lymphoblastic Leukemia (ALL) occur each year. Most Indian publications have shown poor treatment outcome in these patients (1, 2, 3). This contrasts with present cure rates of over 70% in Western countries. This could be due to multiple factors in our region such as paucity of cancer treatment facilities, poor availability of drugs, poor compliance, lack of supportive therapy or poor hygienic conditions leading to infections and death. There is, however, reason to believe that the large difference in environments and in population genetics between developing countries and affluent nations would have corresponding differences in both the biology of ALL (phenotype and genotype) as well as in the patients themselves, e.g. with respect to drug metabolism, nutritional status and the presence of significant co-morbities such as hepatitis. These factors could potentially influence treatment efficiency and toxicity and, therefore, survival rates.

This article describes not only differences in patient characteristics from Western series but also the limited value of most widely used risk factors such as age and WBC count at presentation. It further describes the results of the immunophenotypic and molecular studies undertaken to characterize the biology of the disease in these patients.

The studies were initiated in an Indo-US collaborative project between the National Cancer Institute, NIH and three Indian institutions; All India Institute of Medical Sciences (AIIMS), Tata Memorial Hospital (TMH) and Cancer Institute, Chennai (CI) over several years.

Table-1 shows the outcome of treatment at the three Indian centers on a common therapy protocol (4). The protocol was based on standard treatment principles with a four drug induction (vincristine, prednisone, daunorubicin and asparaginase) followed by CNS prophylaxis with cranial radiation and intrathecal therapy. The study was open to patients aged 1 to 24 years old. Event Free Survival (EFS) was chosen as the

Table-1 : Outcome of Treatment at Three Indian Centers, 1990-1997

Centre	No. Patients	CR (%)	Toxic Deaths (%)	Relapses (%)	EFS at 4 yrs
TMH	652	94.8	10.6	28.8	60%
AIIMS	228	83.3	22.8	30.5	41%
CI	168	86.9	16.7	41.1	43%

most satisfactory primary endpoint to use as an overall indicator of efficacy and toxicity of treatment, since events included all deaths whether due to toxicity or disease progression.

Table-2 shows the patient characteristics at the three Indian centres between 1990 – 1997 and Table 3 the univariate and multivariate analysis of risk factors at AIIMS(4). Age could not be identified as a risk factor but WBC count was significatnt. Table 4 shows a comparison of WBCs in various series in India and the USA or Europe (4)

Immunophenotypic Studies

Immunophenotyping was done in all the three participating institutions. A much higher proportion of T cell cases was uniformly reported from all the three centers as compared to the Western figure of 15%. Cancer Institute, Chennai, reported 44% cases to express T cell markers (5). At AIIMS, it was 31.8% (6) and at TMH 21% (7). Interestingly, a similarly high percentage of T cell lineage ALLs (50%) in both children and adults, had also been reported from Egypt (8).

Molecular Studies

The four major chromosomal translocations observed in pre B-ALL in children include the t (12; 21), that results in the fusion of the TEL (ETV 6) and AML1 genes, t (1; 19), resulting in a chimeric protein of E2A and PB X 1, t (9;

Table-2 : Patient Characteristics at Three Indian Centers, 1990-1997

Characteristic	TMH	AIIMS	CI	P value
No Patients	652	228	168	-
Median Age	7.2	7.6	10	0.007
Pre B-ALL	75.2%	59.5%	45.4%	<0.0001
Pre T-ALL	20.7%	31.8%	43.1%	<0.0001
Other cell	4.1%	8.7%	11.5%	n/a
WBC[e] < 10,000/mm^3	44.0%	37.3%	39.9%	0.18
WBC 10 – 50,000[g]/mm^3	31.4%	31.6%	25.6%	
WBC 50 – 100,000/mm^3	10.0%	13.2%	11.3%	0.035
WBC > 100,000/mm^3	14.6%	18.0%	23.2%	0.023
Lymphadenopathy	74.2%	83.8%	72.0%	0.006
Hepatomegaly/splenomegaly	76.0%	89.5%	85.1%	<0.0001
Mediastinal mass, all patients	1.8%	7.8%	2.4%	0.0041
Mediastinal mass, pre-T cell patients	33.6%	46.8%	35.7%	0.21

Table-3 : Univariate and Multivariate Analysis of Risk Factors at AIIMS

	Univariate P-value			Multivariate P-values
Characteristic, Phenotype, or risk factor	**All Patients**	**Pre B-ALLb**	**Pre T-ALLc**	**All Patients**
Age	0.10	0.21	0.28	0.20
Sex	0.22	0.64	0.32	0.45
WBC count	0.0011	0.0025	0.95	0.0005
Blast count	0.073 n=218	0.052 n=113	0.45 n=59	0.39 n=208
Platelet count	0.041 n=218	0.0047 n=113	0.88 n=59	0.025
Phenotype	0.83 n=195	n/a	n/a	0.99 n=198
Hemoglobin	0.64	0.69	0.28	0.94
Lymphadenopathy	0.62	0.34	0.94	0.66
Hepatosplenomegaly	0.80	0.98	0.42	0.58
Mediastinal mass	0.93	0.84	0.53	0.32
Year of accrual	0.62	0.93	0.90	0.077
Median height for age	0.90	0.49	0.061	0.65
Median weight for age	0.99	0.92	0.22	0.70
Median height and weight for age	0.89	0.83	0.35	0.79

22), yielding a BCR-ABL fusion and t (4; 11), which juxtaposes MLL to AF4. These translocations define clinicopathological entities that have also been used in risk stratification for treatment purposes. An analysis of the presence or absence of the more common chromosomal translocations was, therefore, undertaken. This revealed significant differences from published Western series.

More than 250 newly diagnosed cases of pre B-ALL were analysed by real time multiplex RT – PCR for the four leukemia specific translocations mentioned above (12)). The frequency of t (12; 21) which is associated with a good prognosis was significantly lower in Indian series (7%) than in the USA (22%) or Europe (23%) ($P<0.005$). In contrast t (1, 19) and t (9; 22), the latter generally having a poor

Table-4 : Comparison of WBCs in Various Series in India and the USA or Europe

Institution	<50,000 per cu mm	>100,000 per cu mm	EFS (4-5yr)
CI (Chennai, India)	65.5%	23.2%	43%
AIIMS (Delhi, India)	68.9%	18.0%	41%
TMH (Mumbai, India)	75.4%	14.6%	60%
UK ALL XI (1990-1997) [15]	77.9%	12.0%	63%
POG AlinC14 and 15 Studies [16]	85%	6.6%	66.6%
ALL-BMF 83 [17]	80.2%	11.3%	64.3%
ALL-BMF 90 [17]	77.7%	12.4%	78.0%
St Jude 1988-91[18]	77.7%	13.8%	67.6%
St Jude 1991-94 [18]	73.3%	14.5%	76.9%
Dana Farber CI Study (USA) [19]	81.7%	10.9%	83%

[15]Eden OB et al; Leukemia 14; 2307-2320, 2000.
[16]Maloney et al; Leukemia 14; 2276-2285, 2000.
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prognosis, appear to be more commonly seen in the Indian series (7% and 5% respectively) than in western series (< 3.8% and <2.2%).

Immunoglobulin and T cell receptor (TCR) gene rearrangement in childhood ALL (Ref. 9)

Briefly, the molecular rearrangements observed were different from the West in terms of higher incidence of TCR-beta rearrangement, invariable deletion of Cgamma 1 and only monoallelic rearrangement of TCR-delta locus. Further, TCR beta arrangement in B cell precursor ALL was associated with a higher mean age at presentation, lower mean platelet count and a poorer disease free survival (% cumulative survival 0 versus 88.9±10.5, p=0.004)

Incidence, clinical characteristics and early treatment outcome in Indian patients of childhood ALL with ALL-1 gene rearrangement (Ref. 10)

Briefly, in 185 patients with median age of 7 years, the incidence of ALL-1 gene rearrangement was found to be 11.4%, conforming to the Western pattern. It was

associated with significantly high WBC count (p=0.01) and CD10 negativity (p=0.00000001). Complete remission and relapse rates in 98 patients evaluable for response to therapy on a uniform therapy protocol were independent of ALL-1 gene status.

Detection of BCR-ABL transcripts in acute lymphoblastic leukemia in Indian patients (Ref. 11)

A semi-nested cDNA-PCR was employed to detect the presence of BCR-ABL chimeric transcripts in 33 patients of ALL. They were found in 24% of children and 19% adults which is in sharp contrast to the published reports from the West where the presence of BCR-ABL has been reported in only 2-5% children and 35% adults. The significance of these results is that the BCR-ABL fusion transcript which is an indicator of poor prognosis may contribute to chemo-incurability in young Indian patients

Significance of MDR1, MRP1, GST pi and GST mu mRNA expression in Acute Lymphoblastic Leukemia in Indian patients (Ref.13)

Semi-quantitative RT-PCR in 167 patients of ALL showed significantly higher MDR 1 expression with age more than 15 years and higher MRP1, GST pi and GST mu expression with WBC counts more than 100 x 10^9/L. Inability to achieve complete remission was associated with a significantly higher MDRI expression in patients less than 25 years of age.

Significance of expression of MDR1, MRP, BCL2, BAX mRNA and BCL2/BAX ratio in ALL in children and young adults (Ref.14)

Using the cDNA-PCR approach in 57 samples drawn from ALL patients with a median age of 9 years, the MDR1 and MRP mRNA levels did not differ in patients having unfavourable treatment outcome. However, higher BCL2 values and higher BCL2/BAX ratios were associated with unfavourable treatment outcome. Thus the disease free survival was significantly better in patients having very low (less than the 30^{th} percentile) BCL2 mRNA levels and significantly poor for patients having BCL2/BAX ratio above the 80^{th} percentile. Thus BCL2 and BCL2/BAX ratio may significantly influence prognosis in denovo ALL in this age group.

Generally speaking, age, an important prognostic factor in Western series, was not associated with outcome. Age is, however, merely a surrogate marker and probably reflects differences in a number of other factors, such as the specific molecular subtype of leukemia (which tend to be age associated), and perhaps immunological and other factors. It is possible that the lack of an association of age with outcome in India is indicative, at least in part, of differences in the pattern of molecular subtypes.

Further, delayed diagnosis and a higher WBC at presentation probably accounts for at least some of the generally worse outcome in Indian patients.

While general principles learned in Western series provide the present foundation for treatment strategies, differences in the population treated, differences in environment and genetics, differences in tumor biology and in the quality of care received are likely to give rise to differences in the results achieved with the same treatment protocols. Thus, the criteria used for risk stratification in Western studies may not be appropriate for use in Indian patients. In addition, it is likely that the molecular study of tumors, including gene expression profiling of tumors, will provide new insights into genetic and environmental factors that determine development of ALL and into factors which influence the outcome of treatment.

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