

Research impetus towards biomolecular medicines development: An overview

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Abstract

Research towards molecular medicines development for non-infectious diseases such as cardio-vascular diseases, cancer, diabetes, neuro-degenerative diseases and musculo-skeletal joint inflammatory disorders (arthritis) needs a fresh impetus, in view of the developments in the assessment of the therapeutic proteins such as interferons, cytokines, interleukins, erythropoietin H, somatostatin, defensins, tumor necrosis factor, monoclonal antibodies, polyclonal antibodies, lectins etc. The new therapeutic strategies like gene therapy and cell based/stem cell therapy augment the efficacy of the various biomolecular proteins and their application towards clinical management of the diseases. The above research areas also imply a shift in the existing paradigm of both the clinical and therapeutic evaluation.

Key words: Gum arabic, agar gum, immobilization, α -amylase, reactor system.

Introduction

Among non-infectious diseases, Cardio-Vascular diseases (CVDs), cancer, diabetes, neuro-degenerative diseases and musculoskeletal joint inflammatory disorders (arthritis) have become a cause for concern and warrant new lines of research towards their amelioration. These apart, several hereditary diseases in various haplo group populations remain as a big hurdle to the dream of the famous Galtonian eugenic human society. The prevalence rates of these diseases, world over seems to be shooting up due to various factors such as environmental pollution, global warming, ozone depletion, life style changes, nutritional deficiencies due to famine or under productivity and affluence etc. Among non-infectious diseases, cancer seems to be a co-evolved disease in human population, as evidences are accumulating in recent years on the role of genes and genetic mutations culminating in cancers of various

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tissues/organs. The arthropathic manifestations which were once believed to be age-related debilitations with different manifesting profiles are now found to be afflicting all age groups with multiple etiology. Like arthritis, diabetes also is now realized as a scourge draining human economy. Diabetes leads to many overlapping human diseases and vice versa. Several genetic disorders of the nervous system are accompanied by diabetes including mitochondrial disorders, Friedrich's ataxia and the Wolfman's syndrome. As most of these non-infectious ailments lead to the loss of human life as well as lost productivity, an understanding of their etiopatho- genesis will provide more insights and impetus to new research and clinical approaches towards their management and alleviation. Therapeutic research in recent years centers upon three cardinal areas viz. proteomics, genomics and stem cells. The research outcome and output in these have been turned into biotechnological byproducts of both diagnostic and therapeutic values. With the completion and compilation of the Human Genome Project in 2003, and the insights derived out of karyological studies, we are now in a position to identify the specific chromosomes and the defined genomic loci for more than hundreds of hereditary diseases. The genetic technologies such as PCR, RTPCR, RFLP, SNP, gene sequencers have opened up a new avenue of Human gene therapeutics which has its divisions viz. ex vivo gene therapy, in vivo gene therapy and/or anti-sense therapy. Human gene therapy has now reached its critical phase and biotech and pharmaceutical companies are involved in clinical trials (Phase III) for most of the diseases. Next to proteomics and genomics, the stem cells research is gaining rapid momentum. The knowledge and availability of organ sources for the stem cells procurement (both embryonic and adult) and the technological feasibility of transforming stem cells into differentiated cells and/or tissues of varied categories have evolved new strategies in the clinical treatment modalities of human diseases. These strategies also promise that cell based/stem cell therapy may bring benefits to the patients who suffer from hereditary genetic diseases. Thus proteins, genes and cells may constitute the biomolecular medicines of the future years for which a research reappraisal is needed to validate the observations made previously in these areas to draw new propositions.

Interferons

Human diseases like auto immune disorders, cancers and neurodegenerative diseases are manifestations due either to up regulation and/or down regulation of cellular protein synthesis. Some of these proteins are serving as diagnostic indicators to monitor the disease progression. Hence either the substitution of such functional proteins and/or deletion of the same could ameliorate the diseases. Proteomics has led to the discovery of several such therapeutic proteins. The various therapeutic proteins include the interferons (α , β and γ), cytokines, interleukins, erythropoietin H, somatostatin, defensins, tumor necrosis factor, monoclonal antibodies and polyclonal antibodies.

The interferons are of three types of functionally related proteins or glycoproteins each consisting of a single polypeptide chain of molecular weight 16000 – 26000 daltons. They are IFN – α , IFN – β , IFN – γ . Macrophages and lympho-blastoid cells secrete IFN – γ while epithelial cells and fibroblasts synthesize IFN – β . IFN – γ are specifically produced by T – lymphocyte which were presensitized to antigens and mitogens. IFN – γ has been attributed with greater antitumour than antiviral activity as compared to other types. Several sources of interferon have been exploited for clinical use against cancer. Natural interferons are produced by tissue culture techniques while recombinant interferons are produced through genetic engineering and/or recombinant DNA technology. The tumouricidal activity of interferons has been ascribed by Toy as early as 1983. The various mechanisms conceived as antitumour are as follows: Interferons cause direct inhibition of cell division by slowing the cycling time. In terms of cell loss, interferons have greater effects on a rapidly proliferating tumour system. Interferons may also effect changes in cell metabolism and behaviour following membrane binding and even reversion of the transformed malignant cells to more normal phenotypes. (a phenomenon documented *in vitro*). Interferons also bring immuno-modulation in the host. The modulation that brings tumour regulation involves augmentation of cytotoxicity of NK cells against the malignant cells; increased macrophage phagocytosis and enhancement of cell mediated and humoral responses (antibodies) and IFNs may also affect the secretion of variety of agents viz., hormones, amines and prostaglandins which may be important in favoring tumour micro environment and their growth, differentiation and invasion.

Tumorigenesis and interferons

The functional attributes of interferons viz the first line defense against viruses, enhancement of effector immune cells function and production of other lymphokines made them as potential anti cancer agents. As far as the cancer cells concerned, the failure of immune recognition of tumour can be correlated to the modulatory function of interferons. Often, the tumour cells exhibit unusually either high or low levels of surface class I antigens (Hart, 1985). In many cancers, surface MHC antigen has not been detected serologically. The above loss of surface antigen expression could allow the cancer cells to escape immune surveillance mediated by T cells. Mouse tumour cells were seen to express a variety of altered or anomalous MHC antigens (Invernizzi and Parmiani 1975; Bortin and Truitt 1981). Goodenow et al (1985) have revealed that the above alteration in mouse sarcoma has been brought by recombination mechanisms between MHC class I sequences. Natali et al (1983) and Doyle et al (1985) have suggested that expression of lower level of MHC antigens is related to the decreased immunological recognition of tumours. Direct evidence for the contribution of low histocompatibility antigen expression to the malignant and metastatic properties of tumours has also been obtained in mouse models (Eisenbach et al 1985, Tanakia et al 1984). Studies by Hui et

al (1984), Tanaka et al (1985); Wallich et al (1985) have construed that reduction of class I gene expression is directly involved in the multi step process which leads to tumour formation. Conversely raised levels of H – 2 class I antigen on the cell surface correlates with the loss of metastatic potential. Early embryonic stem cells derived from murine embryonal carcinoma (EC) cells do not transcribe H – 2 class I genes. (Rosenthal et al 1984). Yoshie et al (1982) have revealed that interferon raises the levels of surface H – 2 and/or HLA class I antigens. Cells treated with interferon become better targets for the Tc – cell lysis. Murine model studies have also revealed that oncogenic adenoviruses alter the transcriptional activity of class I genes which in turn causes the transformation of normal cells into neoplastic cells. (Brickeill et al 1983, 1985; Burgert and Kvist 1985) Such neoplastic transformation in human cells by the adenoviruses induced loss of surface class I expression has also been documented.

Erythropoietin

In hematopoiesis, the haemotopoietic stem cells in the bone marrow proliferate and differentiate so as to give rise to all the cellular components of blood and the lymphoid system. The normal proliferation and differentiation of erythroid progenitor cells require besides several essential nutrients such as iron, folate and B2, a growth factor called erythropoietin (EPO). EPO is a 30400 MW glycoprotein hormone produced mainly in kidney and secondarily in the liver. Chronic anemia is a common complication in patients with cancer especially those in advanced stages (III & IV) and/or those receiving aggressive chemotherapy. The erythropoietin (EPO) is in clinical use to relieve the anemic symptoms in chronic inflammations like rheumatoid arthritis, secondary anemia in cancer, primary bone marrow diseases, haemolytic diseases, and cytostatic bone marrow depression etc. (Means et al 1989; Pincus et al 1990; Gudbjornsson et al 1992; Abels, 1993; Hellstrom et al 1993; Klaesson et al 1994; Mitus et al 1994). Clinical research investigations like proliferation assay, tyrosine kinase assay and c-fos Northern blot have elucidated that erythropoietin in cancer cells acts as an inhibitor of their proliferation rate.

Lectins/ heamagglutinins

Lectins are proteins and/or glycoproteins with binding affinities to the sialic acid components of the cell surface complex carbohydrates viz glycocalyxes. Both plant lectins and animal lectins have been employed to map the cell surface carbohydrate stoichiometry. These are good molecular probes to determine the changes in the transformation of normal cells during tumorigenesis. Their ability to bind with sialoglycoconjugates entitle them the property as recognition molecules to detect malignant transformations. Various cellular functions attributed to lectins include cell adhesion of lymphocytes, potentiation of host immune response by means of complement mediated cytotoxic reactions and apoptosis, establishing intercellular contacts, activation differentiation and proliferation of B/T cells, triggering polyclonal activation of immune cells, opsonisation, mitogenecity, signal transduction etc. (Mody et al 1995, Renwartz et al 1993, Indra et al 2000; Chitra and Chowdhury 1991). Lectins which are polyreactive with different isoforms as well as lectins with identical subunits reacting with different monosaccharides have been reported. Lectin isoforms and isolectins with wide spectrum of affinity to different monosaccharides and lectins with narrow specificity are also present. Chitra and Chowdhury (1991), Indra et al (2000) have identified the lectins of narrow specificity in the serum of *Achatina fulica*. The snail's haemolymph lectin termed Achatinin H binds specifically to 9 – 0 – acetylsialic acid. This lectin has been demonstrated as an immune marker of transformed lymphoblasts in

childhood acute lymphoblastic leukemia. (Chitra et al 1997). By virtue of their binding specificities, lectins are considered as biochemical, cytochemical and histochemical probes in the study of subtle differences in the cell surface glyco-conjugates of non-malignant and malignant cells which are otherwise non-detectable even by monoclonal antibodies. In cancer research, lectins as probes could differentiate normal versus tumour tissues, between different types of tumours and even within a single class of tumours (Gablius 1990). In this context, Indra and Ramalingam (personal observations) have demonstrated the cytotoxic and cytopathic effects of Achatinin H over Herpes virus and virus infected vero cells (Fig 1).

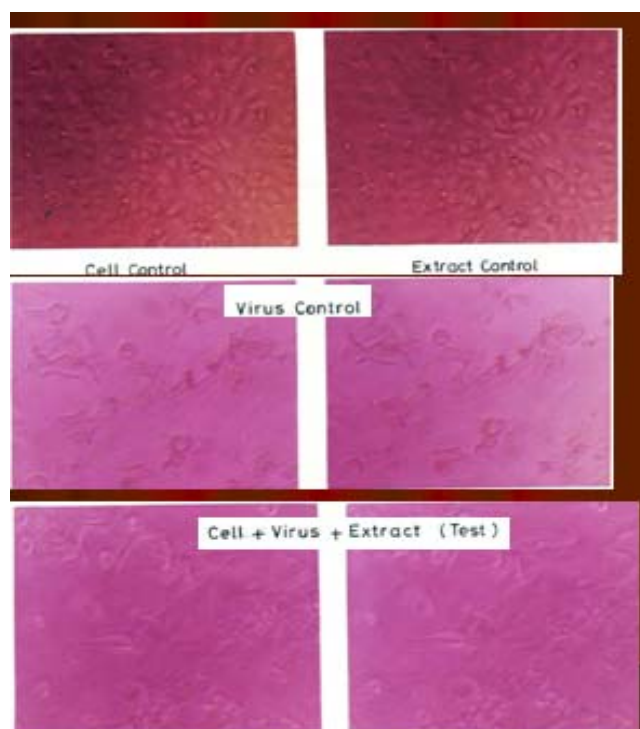


Figure 1. cytopathic effects of Achatinin H over Herpes virus and virus infected vero cells

The delineation of the β - pleated protein structure of the haemolymph lectin of *Achatina fulica* (Achatinin H) and the factors that cause conformational changes and its activation have been elucidated through such techniques as U.V absorption spectra and fluorescence absorption spectra by Indra et al (2000). The cytotoxicity and inhibitory effect on tumour marker enzymes viz acid and alkaline phosphatase, Lactate dehydrogenase, aspartate aminotransferase and alanine aminotransferase and the enhancing influence over free radical scavenging enzymes such as LPO, SOD, and CAT and ultimately the antimitotic/anti cancer/ pro apoptotic effect of Achatinin H over MCF human mammary carcinoma cells have been demonstrated in vitro recently by Indra et al (2007) through SDS PAGE electrophoresis, western blot, MTT assay and FACS assay etc.

Many different types of lectins prevail in animals, each with specificity to binding to a carbohydrate component of the cell surface antigenic epitope. Among them, Mannose Building Lectin (MBL) represents an innate immune serum protein which recognizes wide range of micro organisms including gram +ve, gram -ve bacteria, yeast, fungi, parasites and certain viruses such as HIV, influenza virus, respiratory syncytial virus and herpes simplex

virus etc. It is thought of as an auto - antibody, as it elicits the immune microbicidal response in the first few minutes to few hours (24 - 36hrs) after exposure to an infectious agent. MBL gene is hand wired in the genome and the serum concentrations of it is determined by the single nucleotide polymorphisms in the exon region and the regulatory segment of the MBL gene. The experimental evidences adduce that MBL is a disease modifier and its threshold concentration determines the disease severity and reduction of life expectancy in cancer patients. Cancer often accompanies anaemia and due to chemotherapy, it also results in suppression of immunity. The future therapy may therefore need to screen the MBL haplo typing in patients and warrant MBL replacement therapy.

Monoclonal antibodies

Antibodies are immunoglobulins of five different types viz IgG, IgA, IgM, IgE and IgD which play a crucial role in the humoral mediated immunity and effect their defensive functions either directly or in association with the cell mediated counter part of adaptive immunity. The immune functions of these natural antibodies against the non-self antigens have divulged the difference between the in vivo polyclonal antibodies and the hybridoma derived specific monoclonal antibodies. The later category of mABs are the ingenious invention of Kohler and Milstein (1975) who fused the myeloma cells and spleenocytes derived from an antigenically hyperimmunized animal to derive clones or cell lines (Hybridomas) for the permanent production of antigenic epitopes specific immunoglobulins. Their discovery could be acclaimed for two special attributes viz 1. The hybridoma they derived are immortal and could synthesize both diagnostic and therapeutic antibodies permanently and 2. The antibodies of hybridoma are chemically specific homogenous molecules derived from single clones (IgM and IgG).

Monoclonal antibodies have clinical utility as specific probes for blood group antigens, tumour cell surface antigens, tumour endothelial cell surface antigens and for detecting various viral, bacterial and parasitic antigens. They are used as diagnostic reagents to detect both major and minor blood group incompatibilities, cross reactivity of Ag-Ab, transfusion incompatibilities, infections, preleukemic cancers, high frequency antigens, Anti-Rh(D) reactions, alloimmunization of mothers, septic shock syndromes, blood products of transfusion etc.

Besides diagnostic functions of mABs, their value as therapeutic agents has been established in recent years by the research investigations that revealed their neutralizing functions over microbes, and their utility as anticancer agents and against several proinflammatory disease proteins. Though the diagnostic and clinical implications of mABs have been delineated by more than three decades of research, the impetus and reappraisal of their physio-chemical characteristics and their biological properties are deemed to go a long way since, mABs are high molecular weight proteins (-150 kDa) with highly complex secondary and tertiary structures, subject to post-translational modifications such as glycosylation. Various assays and analytical techniques to achieve the above mAbs characterization (Physico-Chemical/Biological) in order to design mAb drugs include i) capillary electrophoresis with laser induced fluorescence detection; ii) mass spectrometry techniques viz Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; and electron spray ionization mass spectrometry; iii) Nuclear magnetic resonance; iv) Liquid chromatography combined with mass spectrometry v) Surface Plasmon resonance vi) Circular Dichroism in near-and-far-UV spectra vii) Flow cytometry in combination with ELISAs. With all

these above high precision and state of the art technologies, the clinical strategies of mAb will become more reliable, highly precisioned, more qualitative and reproducible and of all more safe. With more quality controls encompassing in-process controls, product controls, material control, cell lines production, cell bank system, the derivation of such safe, tailor made and genome/personalized medicines is a reality of the future.

Gene therapy

It is known that genes which are the determiners of heredity, dictates the synthesis of a plethora of structural and functional proteins which bring about by their interaction and coordination, both the cellular and total body homeostasis. Knowledge about the inborn error metabolic diseases and other gene mutation oriented ones has derived an insight and understanding of the range of defects in our genetic map. More recently the genetic attribution to other such common human diseases as cancer, heart disease and diabetes has been well established. It has been elucidated that many of these abnormalities require environmental stimulation to initiate changes in the genetic components and without the genetic predisposition the disease might never arise. To date over 4000 diseases have been recorded from simple genetic disorders (monogenic).

Cystic fibrosis

The molecular and cellular basis of Cystic fibrosis involves defective Cl⁻ transport in epithelial cells including the cells lining the respiratory and gastro-intestinal tract. In 1984, it was demonstrated that Cl⁻ channels fail to function normally in epithelial cells from cystic fibrosis patients. In 1989, the gene for cystic fibrosis was isolated by molecular cloning. The gene sequence encodes a protein called Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) belonging to ABC transporter family. The mutation of the gene makes defective Cl⁻ transport. Gene therapy provides the potential of replacing normal CFTR genes into cultured cells of cystic fibrosis patients in order to restore Cl⁻ channel function. Viral vectors can transmit CFTR cDNA to the respiratory epithelia and the above gene therapy was initiated in 1993. (Colkins, F.S 1992). Further clinical trials using adenovirus vectors for gene therapy to cystic fibrosis have been conducted in the later years (Hoogerbrugge et al 1995; Morsy caskay 1999, Prince 1998, Boucher 1999).

Rheumatoid arthritis

In musculo-skeletal connective tissue inflammatory diseases especially Rheumatoid arthritis the destruction of articular cartilage results from the interaction of various auto immune cellular and humoral factors. Matrix Metalloproteinases (MMPs) and serum proteinases are construed for much of this destruction. Natural in vivo inhibitors like TIMPs and serpins bind to these proteinases tightly and eliminate them. However within a micro environment like the specific connective tissue foci, these inhibitors are overwhelmed by the proteinases resulting in their destruction. (Weiss 1989) or their inactivation (Boswell and Carell, 1987). Several transcription factors control the repression of the genes for the synthesis of MMPs. In addition to production of H₂O₂ (oxidants), other ROSs like oxidized LDL and NOs activate the NFκB in T cell lines and the translocation of NFκB to the nucleus and its further binding to specific genes encode several inflammatory proteins such as TNF α, IL - 1, IL - 6, IL - 8, and IL - 2 receptor β chain, inducible nitric oxide synthase (iNOS), MHC class I antigens, E selectins, Vascular cell adhesion molecule, serum amyloid A precursor and C - MYC. (Sahreck et al 1991; Liao et al

1993, Lander et al 1993; Baeurle and Henkel 1994). In addition metabolic substrates such as free sugars, proteoglycans, glucosaminoglycans, (GAGs), Lactic acid etc at the metabolites level may also enhance inflammation in arthritogenesis. (Subramaniyan and Ramalingam, 2007) Inappropriate activation of NF-κB is believed to have lead to pathological diseases, besides rheumatoid arthritis, such as autoimmune diseases, asthma, septic shock and lung fibrosis. Inappropriate inactivation of NFκB has also been linked to apoptosis and delayed cell growth (Brown and Brown, 2002). In light of the above information, it is made clear that connective tissue damage in case of RA is not a singular event but a cascade involving several mediators, genes, proteins, reactive oxygen species and antibodies etc. Hence a valid therapeutic approach to chronic inflammatory joint diseases involves a search for the key natural/synthetic proteinase inhibitors and/or their inactivation/inhibition of nuclear import of transcription factor like NFκB, inhibition of the pro inflammatory genes and also the employment of clinically useful anti-rheumatic compounds with anti oxidant properties. (Williams et al 1992, Kopp and Ghosh 1994, Schreck et al 1992, Traenckher et al 1994; Brown et al 1995; Didonato et al 1995; Clen et al 1996)

Lysosomal storage disease: (Gaucher's)

Lysosomal storage diseases have been implicated to genetic defects and the failure and/or deficiency of the enzyme synthesis results in failure of catalyzation of the specific substrate hydrolysis. The above cellular malfunction leads to pathological consequences in affected organs. The best incidence is the Gaucher's disease which is the most common of the lysosomal storage disease afflicting predominantly the Jewish population. Among the three types of this disease, Type II and III manifest severe pathological features like splenomegaly and hepatomegaly, bone lesions, extensive neurological damage and ultimately early death. The lack of enzyme glucocerebrosidase in these patients prevents the hydrolysis of glucocerebrosidase to glucose and ceramides. (Fig). Though enzyme replacement therapy has been advocated by Christian de Duve in as early as 1960s and the clinical trials have been successfully demonstrated in 1970s, the prohibitive cost of such treatment conceived that gene therapy may be more promising, in which normal glucocerebrosidase gene could be introduced into bone marrow cells from which the macrophages are derived. The seeding of such macrophages in organs like liver and spleen could correct the inborn error and consequently the disease.

Antisense therapy

Antisense drug development is yet another recently evolved biotechnological strategy which blocks the real 'sense m-RNA' by the 'antisense m-RNA' synthesized through gene insertion inside the cellular genome. In this, protein translation is inhibited which is required off in the disease alleviation. Such antisense drugs are in clinical trials to treat diabetes, hepatitis c, cancers, psoriasis, Crohn's disease and rheumatoid arthritis. The drug designing in this line is met with limited success due to certain limitations in vivo. (Ricki Lewis, 2005) McGraw Hill, Human Genetics: Concepts and applications.

Cancers and molecular medicine

Over the last few decades, substantial evidences implicated that both oxygen and organic free radical intermediates in the biomolecular interactions contribute to the multistage carcinogenesis. (Cerutti 1985; Kensler and Trush 1985; Trush and Kensler 1991 a, b, Guyton and Kensler 1993, Ti and Trush 1995). Several exogenous modulators of cancer initiation, promotion and progression include

UV, ionizing radiation, redox cycling chemicals, redox metals, mineral dusts etc. Conversely endogenous modulators include mitochondrial mechanism, NAD(P)H oxidases, lipoxygenase, Xanthine oxidase, transition metals, Cytochrome 450, endogenous hormones etc. Both sources generate reactive oxygen species (ROS). Elevated ROS has been noticed in patients with genetic disorders such as Down's syndrome, Bloom's disease etc. Besides the above factors, microbial mechanism of malignancy has also been well established. It has been found recently that chronic inflammation of visceral organs and other tissues due to bacterial and parasitic infections, causes the infiltration of the affected tissues by freely moving phagocytic cells primarily polymorphonuclear leukocyte (PMNs), monocytes and macrophages. When these phagocytes release hydrolytic enzymes and ROS, the normal defense mechanisms which limit the phagocytes are abrogated and the sustained production of ROS chronically develops neoplastic growth. (Kozumbo et al 1985; Weitzman and Gordon 1990; Cerulti and Trump 1991; Ohshima and Bartsch 1994; Rosin et al 1994 a, b, Wiseman and halciweld 1996). The association of urinary bladder cancer by the infection of *Schistosoma haematobium*; colonic cancer by *S. falciparium*, Cholangio carcinoma by *Opisthorchis sp* and *Clonorchis sp* infections and gastric cancer by *Helicobacter pylori* has been well documented (IARC 1994; Kantor et al 1988; The EUROGAST Study group 1993). Recently the *H. Pylori* has been demonstrated to be endowed with the capacity to excrete antibiotics through active efflux mechanism and develop antibiotic resistance. Such resistance on the part of microbes could aggravate neoplastic growth of tissues. (Falsafi et al, 2009) In all the above mentioned instances of carcinogenesis, the predisposing causative factors are invariably associated with excess ROS production.

The multi step carcinogenesis is due to many different genes and by the more specific oncogenes. Though these genes may play critical roles in cancer development, ras genes and their mutations account for 15% of all types of human cancers, 25% of lung cancers, 50% colon cancers and 90% pancreatic cancers. The mutated oncogenic ras proteins drive the cell proliferation through signal transduction and MAP kinase pathway. Several research groups developed ras targeted inhibitor drugs. The potential of such molecular drugs and others may represent the major milestones in cancer therapy. (Bos 1989, Gibbs et al 1994). However the discovery of anti ras onco protein therapy may constitute only the tip of an iceberg in the ocean of cancer oncology. As several genes, and the so called immediate early genes encode transcriptional factors consequent to growth factor stimulation and alter the expression of a battery of other down-stream genes which establish new programmes of gene expression in the development of cancer, it is pertinent to search for new molecular medicines to combat the disease strategy of cancer.

Antioxidant defense system

Various antioxidant defense systems are in vogue inside the body system (in vivo) which have an effective scavenging function. Vitamin – E and C are widely accepted in the biological system as therapeutic antioxidants (Patra et al 2001). Vitamin C (Ascorbic acid) not only acts as antioxidant but revives other antioxidants like α – tocopherol viz, Vitamin E. The latter is an effective scavenger of lipid peroxyl radicals. It intercalates into cellular and/or subcellular membranes to function as a chain-breaking antioxidant. Its therapeutic remediating effect of inflammation has been well documented in rat/mouse model studies (Bennet 1986; Yoshikawa et al 1992; Murthy et al 1994). The role of vitamin C in conferring immunity to parasitic infections has been elucidated in sheep host *Ovis ovis*. The therapeutic features of vitamin – E include i) selective inhibition of H_2O_2 formation, ii) spares inhibition of NADPH oxidase, myelo peroxidase and exocytosis of elastase, iii)

readily scavenges O_2^- , iv) prevents the inactivation of α_1 – proteinase inhibitor, and v) effectively inhibits 5 – LO in vitro. In several inflammatory diseases, the decrease in disease severity is associated with a decrease in arachidonic acid metabolites and a reduction in circulating TNF α levels. The importance of arachidonic acid synthesis inhibitor drugs in cancer alleviation through anti heat shock protein (hsp) mechanism has recently been delineated. Hence the pharmacological/therapeutic control of inflammatory devastating diseases needs a fresh impetus of research in the direction of characterization of mediators and their receptors, identification and characterization of ligand binding sites on the receptors and development of monoclonal antibodies to neutralize the mediators etc. This new impetus will give hope to finding a “final common pathway” for many of human diseases in which inflammation is the starting point. The overt tissue damages in all oxidative stress induced diseases, implicates upon the abnormalities in various components of the immune system. Various self limiting immunological processes are down regulated and abnormal immune responses/cascades are up regulated to cause the above overt tissue damage (Strober and Ehrhardt 1993; Sartor 1994; Simmonds and Ranpton 1993). These include a) hyperactivity of abnormal T cells; b) abnormal epithelial cell accessory function; c) cytokines production; d) tip off balance between cytokine/anticytokines; e) changes in adhesion molecules; f) abnormal endothelial and mesenchymal functions; g) excessive production of ROM, ROS, NOs etc. Several human diseases are coordinated by the interplay of genetic, immunological and environmental factors. As current drug therapies involving anti inflammatory and immuno suppressive effects could not alleviate or overcome the serious damages caused by the generation of ROS, ROMs, pro inflammatory mediators and the genes, a new look and research emphasis is warranted to derive more insights to immunotherapy.

Critical cell cycle phases in cancers

In cancer progression, the very vital mechanism is the replication of DNA and the continuation of cell divisions with abrogation of normal control mechanisms. In the cell cycle phases of G_1 , G_2 , S, M the G_2 check point prevents the initiation of mitosis prior to the S phase. The control mechanisms prevent cells in G_2 from reentering S and the next cell cycle. Thus G_2 and S are the critical points in the cell cycle phases. Recent investigations have delineated the molecular basis of both the control mechanisms of the cell cycle in

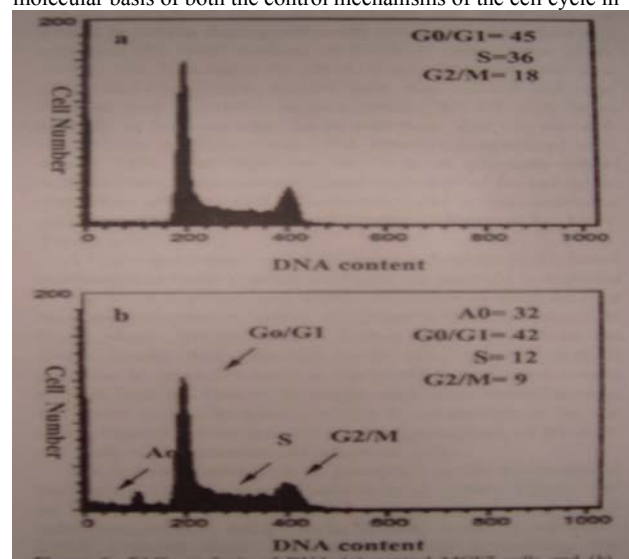


Figure 2. cytotoxic effect of Achatinin_H (lectin) on MCF7.

normal cells and their failure in cancer cells. These studies have revealed that the cell cycle of all eukaryotes is controlled by a conserved set of protein kinases which are responsible for triggering the major cell cycle transitions. The efficacy and therapeutic significance of anti-tumour compounds is assessed by their predominant antimetabolic property. Flow cytometry is one of the most powerful and specific methods for the integrated study of molecular and morphological events occurring during cell death and cell proliferation (Darz et al 1997). Indra et al (2007) have demonstrated that Achatinin H arrests the G₂ – M phase in MCF-7 cells, the major check point of the cell cycle through FACS analysis and suggested its therapeutic utility as a new type of anticancer agent towards such fast growing malignant cells. A search for such biomolecules and clinical trials of their cytotoxic reactions would go a long way in biomedicine development to cancer disease. Indra et al (2007) have also demonstrated evidence for apoptosis in MCF₇ breast cancer cells treated with achatinin. The extent of apoptosis is quantified by the formation of the typical DNA ladder (Fig 2 & 3).

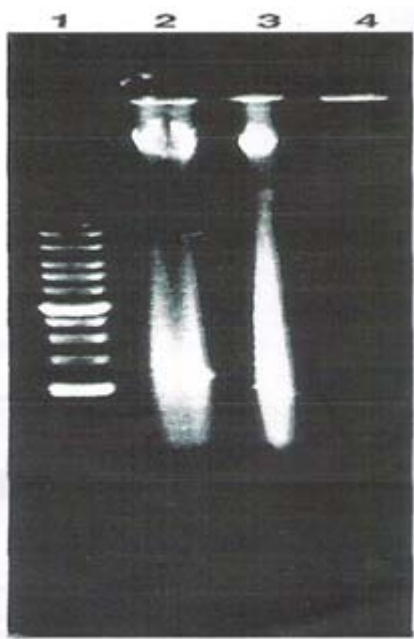


Figure 3. Agarose gel electrophoresis of DNA isolated from MCF7 treated with achatinin_H for 24 hr (lane 2), MCF7 treated with achatinin_H (lane 3), control (lane 4), DNA ladder (100 bp).

Green tea and black tea extract (PBS) nerium laetex (1:100) PBS, calotropis latex (1:100) PBS on the cell proliferation and cell survivability (MTT assay and trypan blue binding assay) of SP2/O myeloma cells (personal observation) revealed that the polyphenols and monophenols in both the hot and cold extracts arrested their growth in vitro. The latex extract of calotropis brought 100% death of cells while that of nerium brought 50% death of cells. In the MTT assay, the herbal extract treated cells (SP2O) showed significant loss of MTT reduction due to cell death as compared to control. (Figure 3) (Personal observation). Though similar reports on the antimetabolic/anticarcinogenic effect of some herbal and animal proteins are available, research investigations are warranted still to derive more insights regarding their mechanisms of cancer cell damage whether these natural agents bring about the cytopathy at the surface receptor level, or at the first/second messenger level or at the enzyme levels in cytoplasmic cascade, or at the nuclear import level or at mitochondria level by suppression of anti apoptotic BCL gene expression and/or eventually at the transcriptional level remains to be elucidated further.

Recent developments in stem cell therapy

Stem cells by virtue of their potentials and functional attributes viz., self proliferation, self maintenance, differentiation, self renewal, lineage, their use has largely been recognized in clinical therapeutics. Autologous stem cells transplantation has become a front line therapy for both haematological and non-haematological solid tumours. In autoimmune diseases which result from self reactive T-lymphocytes and auto antibodies, high dose chemotherapy/myelo ablative chemo-radiotherapy supported by autologous/allogeneous stem cell therapy has brought effective control of the autoimmune diseases. Animal model studies on muscular dystrophy revealed that implantation of precursor muscle stem cells into dystrophic muscle resulted in rectification through missing gene product expression by the implanted cells. Similarly stem cells have recently been employed for coronary heart diseases. The ex vivo maintenance and expansion of stem cells make stem cell therapy a novel platform and strategy in their own right.

Stem cells and cancer therapy

Stem cell therapy for cancer is based on the fact that cancer patients will possess immune cells primed to attack the tumour and these cells may be employed for experimental therapy when their cancer cells fails to respond to conventional treatments. Recently Cassian (2008) at the Fred Hutchison Cancer Research Centre in Seattle (USA) extracted CD₄ cells from a cancer patient, replicated them ex vivo by cloning technique. These cells when injected to the patient become offensive against cancer cells and the patient recovered fully with no signs. Nevertheless, for diseases like cancer, the etiology could not be exactly understood since the genetic profiles of these complications are still poorly understood. The chronicity of (the development) the diseases, multifactorial/multigenic nature of them, and the interplay of superimposed environmental agents make cancer problems into a Gordian knot. Nevertheless, the stepwise sequence of the disease manifestations in case of cancer such as

- i) Genic alterations in the proto oncogenes (point mutations, gene amplification, over expression, gene rearrangements)
- ii) Allelic losses
- iii) Inactivation of tumour suppressor genes
- iv) DNA hypomethylation on the part of the nuclear genomic structures; and
- v) Promotion of angiogenesis and metastasis through such key proteins as VEGF, fibrinogen, plasminogen, MMPs, CEAs etc and
- vi) The establishment of secondary cancers in different tissues through such molecules as integrins, cadherins and Ig sub-families will enable both the more accurate diagnosis of exact position/stage of cancer as well as the various therapeutic strategies. The various gene therapeutic strategies viz gene replacement (P₅₃) oncogenic inactivation by antisense approaches; genetic prodrug activation therapy (GPAT), transduction of genes encoding the synthesis of anticancer proteins and proteomic and immunomic approaches in a succinctly coherent and integrated way would ensure survival of cancer patients. Such an approach which is holistic, will remind us the words of Paul Ehrlich -

“I trust that we no longer find ourselves lost on a boundless sea, but we have already caught a distinct glimpse of the land where we hope, nay which we expect will yield rich treasures for Biology and Therapeutics”.

Molecularly Targeted Therapeutics: (MTTS)

Oncologic literature and evidences revealed that cancers result due to the accumulation of multiple biological and genetic events over a period of years. Molecularly targeted therapeutics (MTTs) act as specific cancer cell signals to arrest the growth of cancer cells. The targeted therapies are directed against specific cellular mechanisms involved in cancer including aberrations in signal transduction, cell cycle regulation, evasion of apoptosis and tumour angiogenesis. Recently genetic or metabolic pathway based taxonomy of disease was delineated through genomics and proteomics. Mt DNA analysis, Y-chromosome analysis have yielded much treasures of information regarding population cum geographic (phylogeographic) based origin of genomic mutations and the consequent causal relationship to human diseases like cancer. (Gopal 2009; Thangaraj 2007). The analysis of human genetic variations is the main driving force to understand the genetic diseases. Though targeted therapy has become a successful immunological concept to human diseases even the monoclonally derived antibody drugs to cancer such as herceptin, erbitux, avastin, campath, ritux, bexxar, zevalin, velcade etc which are in clinical use to a diverse group of cancer patients may be comparable to “one-size fits all - readymade” approach. In reality one type of drug may not be ideal for all patients. Hence it is emphasized that evaluation of anti cancer drugs and development need to be based on the biology of the tumour as opposed to nodal status, age, hormone status etc. Such an approach would prove to be clinically ideal and patient-oriented, abrogating the non-specific cell reactions/events. (Whittaker 2005).

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