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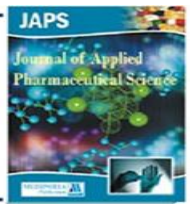

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Cancer Therapeutics- Opportunities, Challenges and Advances in Drug Delivery

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ABSTRACT

This review highlights the characteristics of the cancer that provide opportunities for drug delivery carriers to target cancer. These opportunities include EPR effect, high interstitial pressure of tumour, low pH of endosomes of tumor cells, overexpressed receptors, angiogenesis etc. Chemotherapy is one of the most important treatments currently available among the various approaches. The present status of chemotherapy is far from being satisfactory. Its efficacy is limited and patients have to suffer from serious side effects, some of which are life-threatening. The newer approaches to cancer treatment not only supplement the conventional chemotherapy but also aim to prevent damage to the normal tissues and overcome drug resistance. The innovative approaches of cancer treatment require new concepts of drug delivery in cancer. This concept requires the unique surface morphology which ultimately determines the fate of new drug delivery systems. The Innovative methods must also shoot out the associated problems of cancer like multidrug resistance by tumor cells. Progress in developing various controlled and targeted drug delivery systems has reviewed here with an emphasis on dendrimeric drug delivery system. Dendrimeric system appears to be promising in cancer chemotherapy especially via ligand/receptor mediated endocytosis as it posses numerous properties (especially surface property) to target cancer.

Keywords: Targeted drug delivery; Cancer therapy; Chemotherapy, Dendrimers.

INTRODUCTION

Cancer is a leading cause of death worldwide. It accounts for about 7 million deaths/year (12.5% of deaths worldwide). It has been estimated that there will be 16 million new cancer cases every year by 2020 (WHO, 2007). Cancer progresses from the uncontrolled growth of cells to the formation of a primary tumour mass, vascularisation and subsequent spread (metastasis) of cancer cells to other parts of the body where secondary tumours may form. The main types of cancer leading to overall cancer mortality are lung, stomach, liver, colon and breast cancer. Nearly all cancers are caused by abnormalities in the genetic material which may occurs due to the effects of carcinogens, such as tobacco, smoke, radiation, chemicals or infectious agents. These genetic abnormalities push the cell towards unrestricted growth by affecting two general classes of genes i.e. Proto-oncogenes and Tumor suppressor genes. Proto-oncogenes codes for proteins that stimulate cell devision, the mutated form called oncogenes (Gibbs et al., 1994). Tumor suppressor genes codes for proteins that inhibit cell division and these inhibitory messages are generally inactivated or lost during tumour development (Hinds et al., 1994). This causes loss of normal functions such as accurate DNA replication, control over the cell cycle, orientation and adhesion within tissues, and interaction with protective cells of the immune system. Tumors can grow up to 1–2mm^3 sizes without requirement of blood supply as diffusion is sufficient at this level to support the supply of nutrients and removal of wastes from tumor cells. Tumour develops in various

stages. At first, clusters of genetically identical cells are formed, each cell dividing more rapidly than its normal neighbor's cells. When the cell mass reaches a sufficient size (1–2mm^3 size), the cells release chemicals to recruit surrounding connective tissue and vascular cells to the tumour and induce angiogenesis from existing blood vessels (Bhat et al., 2008). In some cases, a tumor can acquire a genetic mutation that enables it to secrete molecules (e.g., protease) that can degrade the surrounding tissue as well as blood vessel wall structure from which tumor can enter and migrate to distant tissues. Therefore, angiogenesis process could be an important target to suppress tumor growth and metastasis. If metastatic tumor cells enter a lymphatic duct or node, they can travel through the lymphatic system to metastasize to distant locations. This is frequently observed in breast cancer. Several different treatment techniques are in use or under development today, which can generally be grouped in five categories: surgery, radiation, chemotherapy, targeted, and immunotherapy (Si-Shen et al., 2003). The choice of therapy depends upon the location, grade and stage of the tumor, as well as the general state of the patient. Although, it has been observed that cancer chemotherapy is one of the best approaches to eradicate cancer. The success of chemotherapy depends on the selection of optimum carrier system. These carries includes nanoparticles, nanotubes, nanorods, dendrimers, liposomes, solid lipid nanoparticles, microspheres etc. Their newer form like stealth version (stealth nanoparticles, stealth dendrimers, stealth liposomes etc.) not only target to cancer by decreasing RES uptake but also enhances loading capability of the system. The complex of stealth version with targeting moiety enhances the targeting efficiency of the system.

PROBLEMS IN CANCER CHEMOTHERAPY

Apart from the various approaches of treatment, cancer chemotherapy is one of the major therapeutic approaches to combat cancer. The aim of the ideal cancer chemotherapy is to deliver the correct amount of drug with desired controlled rate and for sufficiently long duration of time to the site of action (cancer cells), while prevent the normal cells to obtain the desired therapeutic response. In order to achieve this goal, drug delivery systems must hold sufficient amount of drug and root out the problems like drug resistance based on cellular or non-cellular mechanism, altered biodistribution, biotransformation as well as clearance of anticancer drugs from the body. The delivery systems should meet the requirements like prolonged circulation (which can obtain by PEGylation), sufficient tumour accumulation (by considering EPR effect), uptake by tumor cells (by active targeting) and controlled drug release (by optimizing delivery system) with a profile matching the pharmacodynamics of the drug.

Biodistribution of Drug

Intravenous administration of conventional anticancer drugs (Conventional chemotherapy) are distributed throughout the whole body via the bloodstream, and affects both malignant and rapidly dividing normal cells of the bone marrow, gut, lymphoid tissue, supermatogenic cells, fetus as well as hair follicles (Links et al., 1999). Such treatments have problems like severe side effects, high patient risks, repeated treatments, altered biodistribution of drug and the acquisition of multidrug resistance (MDR) by the cancer cells (Brigger et al., 2002).

Multidrug Resistance (MDR)

Acquisition of drug resistance by cancer cells is a factor influences the success of cancer chemotherapy and it could be aquired by non-cellular as well as cellular mechnism. The non-cellular resistance occurs due to poorly vascularized tumor regions which can effectively reduce drug access to the tumor and thus protect cancerous cells from cytotoxicity. The acidic environment in tumors can also confer a resistance mechanism against basic drugs. These compounds would be ionized, preventing their diffusion across cellular membrane. High interstitial pressure and low microvascular pressure may also retard or impede extravasation of molecules. Cellular mechanism involves over-expression of the plasma membrane P-glycoprotein (P-gp), which is capable of repelling drugs from the cell, causes decreased sensitivity and intracellular accumulation of drugs. This is more pronounced with drugs which enters the cell by passive diffusion through lipid bilayer. Upon entering into cell, these drugs bind to P-glycoprotein, which forms transmembrane channels and uses the energy of ATP hydrolysis to pump these compounds out of cells (Fig. 1). The co-administration of P-gp inhibitors with encapsulated anticancer drugs in nanoparticles have been proposed to prevent P-gp-mediated MDR (Krishna et al., 2000). Limitation also exist because of the lower potency of some drugs after being linked to targeting moieties when targeting portion is not cleaved correctly or at all.

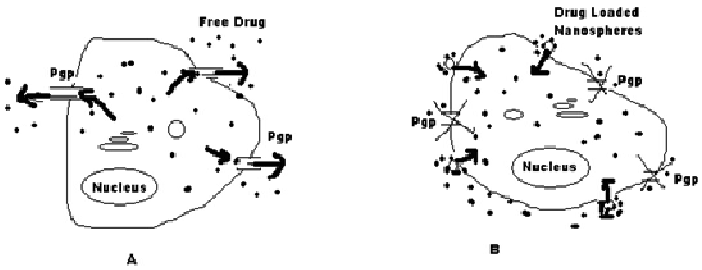

Fig. 1. (A) Free drug effluxed by Pgp, resulting in the absence of therapeutic efficacy. (B) The release drug from nanospheres adheres at the tumor cell membrane, resulting in microconcentration gradient at the cell membrane, which could saturate Pgp and reverse MDR.

Clearance by Reticuloendothelial System (RES)

Rapid blood clearance by the reticuloendothelial system (RES) is a problem in cancer therapeutics. It composed of monocytes and macrophages that are located in reticular connective tissue (for example, in the spleen). These cells are responsible for phagocytosing and removing cellular debris, pathogens and foreign substances from the bloodstream. The particle size, hydrophobicity, surface charge (Juliano, 1976) and composition of system influences the clearance profile of the delivery system. This problem can be overcome by PEGylation of

the delivery system. It was found that liposomes coated with synthetic polymer polyethyleneglycol (PEG) had significantly increased half-life in the blood (Blume et al., 1990). The pegylated liposomes are long circulating due to a highly hydrated and protected liposome surface, constituted by the hydrophilic polymers that inhibit protein adsorption and opsonization of the liposomes (Klibanov et al., 1990) (Fig. 2). This technique has also been proposed for other delivery systems in order to reduce their clearance by reticuloendothelial system (RES).

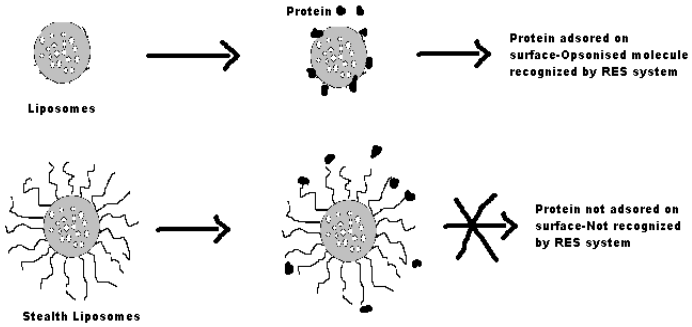

Fig. 2. Clearance of conventional and stealth liposomes by Reticuloendothelial System (RES).

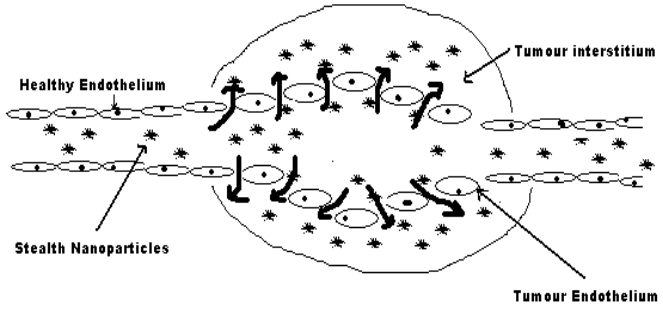

Fig. 3. Accumulation of steath nanoparticles in tumour interstitium due to EPR effect.

Hydrophobicity of Anticancer drugs

Another problem in the cancer chemotherapy is that most of the anticancer drugs are hydrophobic in nature. Because of the toxicity of most of the anticancer drugs to cancer as well as normal cells, it is a prerequisite condition to develop an i.v formulation rather than oral formulation. The i.v. formulation must hold the drug within the system to provide sustain relesae to minimise the exposure of the drug towards the normal cells. For the above objective of i.v. formulation, the drug must be soluble in aqueous media which is mostly rare in case of anticancer drugs. For example: paclitaxel is one of the most commercially successful anticancer agents with a worldwide sale of US$1.5 billion in 1999, there has been much effort in developing a better dosage form to avoid the usage of the adjuvant. Paclitaxel is highly hydrophobic with water solubility less than 0.5 mg/L. The adjuvants which are used for solubilization of the drug consisting of Cremophor EL (polyoxyethylated castor oil) and dehydrated alcohol (Si-Shen et al., 2003). It causes serious side effects, including hypersensitivity reactions, nephrotoxicity, neurotoxicity and cardiotoxicity, hyperlipidaemia, abnormal lipoprotein patterns, erythrocyte aggregation, and peripheral neuropathy (Weiss et al., 1990).

CANCER THERAPEUTICS

To avoid problems of cancer chemotherapy, nanotechnological targeted cancer chemotherapy has been proposed. Such nanotechnological targeted system includes nanocapsules, nanoparticles, nanorods, nanofibers, nanocrystals, nanotubes, stealth nanoparticles, liposomes, stealth liposomes, pH-sensitive liposomes, temperature sensitive liposomes etc. Such delivery implies for selective and effective localization of pharmacological active moiety at pre-identified (eg. over expressed receptors in cancer) target in therapeutic concentration while restricting its access to non target sites thus reducing toxicity, maximizing therapeutic index as well as improves the biodistribution of drug which is a major factor in success of cancer chemotherapy. Targeting to cancer cells by nanotechnological devices can be achieved by considering the characteristics of both i.e. the cancer which includes highly disordered leaky vasculature, high hydrostatic pressure, high requirements for nutrition, angiogenesis, RGD based strategy, EPR effect and the presence of over-expressed receptors. The formulation factor, which includes particle size, surface charge, hydrophilicity, hydrophobicity (determine RES uptake) and covalent attachment of ligands to carrier systems specific for over expressed receptors also plays an important role in targeting of nanodevices.

Enhanced Permeability and Retention Effect (EPR)

Conventional chemotherapy with anticancer drugs has no tumor selectivity and is randomly distributed in the body, resulting in a severe side effects associated with anticancer drugs with low therapeutic index. To improve tumor selectivity, researchers sought to develop conjugates bearing tumor-specific antibodies or peptides (Khandare et al., 2006). However it has been found that macromolecular drug delivery system with prolonged blood circulation time (by PEGylation) can accumulate by passive retention mechanism in tumors even in the absence of targeting ligands (Duncan et al., 2003). The microvasculature in tumor tissue is not very uniform. The tumor blood vessels are highly disorganized generally characterized by abnormalities such as a relatively high proportion of proliferating endothelial cells, increased tortuosity, relatively thin walled, leaky and have irregular diameter with less supporting pericytes or smooth muscle cells. Tumor-associated endothelial cells are abnormal in shape and grow on top of each other, and remain in a proagiogenic and inflammatory cytokine-rich environment (Thurston et al., 1999). The pore sizes in solid tumor vasculature vary from 100 to 780 nm (Yuan et al., 1995), which is much larger than the junctions in normal tissue where the gaps are usually less than 6 nm (Drummond et al., 1999). This leaky vasculature structures are necessary to provide oxygen and nutrients for fast-growing cancer renders the vessels permeable to macromolecules. Also the decreased lymphatic drainage allows macromolecule to not remove efficiently and retained within the tumor mass (Maeda et al., 1989).

This passive targeting effect is often referred to as the enhanced permeability and retention effect (EPR-effect) (Fig. 3) which was first identified by Maeda et al. (Matsumura et al., 1986, Maeda et al., 2001).

Angiogenesis

Angiogenesis is a process of development of new blood vessels from pre-existing vessels. The new blood vessels supply nutrients and oxygen into the cancerous mass and remove metabolic waste products from tumors. The critical role of tumor angiogenesis in cancer progression has been first recognized by Judah Folkman in 1971 (Folkman, 1971). Blood vessels are required to supply oxygen and nutrients and to remove waste products from living tissue. The vascular networks of tissues provide the component cells with oxygen and nutrients. The vascular network is a stable system, and no significant regeneration occurs in the healthy human body, where new blood vessel formation is typically only seen during embryonic development or wound healing, and in response to ovulation. Tumors can grow up to 1–2mm^3 sizes without requirement of blood supply as diffusion is sufficient at this level to support the supply of nutrients and removal of wastes from the tumor cells. As solid tumors become larger than 2mm^3 it enters in a state of cellular hypoxia that initiates tumour angiogenesis. Tumor angiogenesis is critically important for the growth of solid tumors progression, since it supports tumor growth and metastasis (Folkman et al., 1971). Therefore, angiogenesis process could be an important target to suppress tumor growth and metastasis. Tumor angiogenesis is a complex process and involves the tight interplay of tumor cells, endothelial cells, phagocytes and their secreted factors, which may act as promoters or inhibitors of angiogenesis (Table 1).

Table 1. Positive and negative regulators of angiogenesis.

Angiogenesis Activators	Angiogenesis Inhibitors
Fibroblast growth factors	Thrombospondin-1
Placental growth factor	Angiostatin
Angiogenin	Metallo-proteinase inhibitors
Interleukin-8	Platelet factor 4
Hepatocyte growth factor	Genistein
Vascular endothelial growth factor	Interferon alpha
Transforming growth factors	Prolactin 16-kd fragment
Granulocyte colony-stimulating factor	Placental proliferin-related protein
Platelet-derived endothelial cell growth factor	Transforming growth factor

The angiogenic promoters send signals to the surrounding normal tissues in which specific gene activation and protein expression encourages growth and proliferation of new blood vessels (Ferrara, 2000). Several endogenous positive regulators have been identified such as vascular endothelial growth factor (VEGF), transforming growth factors, fibroblast growth factors, angiogenin, epidermal growth factor, as well as several smaller molecules (such as adenosine, PGE, etc.) secreted by tumor cells to mediate angiogenesis. IL-8, COX-2 and inducible nitric oxide synthase (iNOS) are among the inflammatory angiogenic molecules secreted by tumor cells, which influence the growth and development of tumor vasculature and metastasis (Yoshida et al., 1997). Among these molecules, VEGF and bFGF are more important for sustaining tumor growth (Carmeliet et al., 2000).

Angiogenesis occurs in several well-characterized stages. First, biological signals known as angiogenic growth factors activate receptors present on endothelial cells present in pre-existing venular blood vessels. Pericytes then retract from the abluminal surface of capillaries. Endothelial cells then release and activate proteases such as urokinase (uPA) and metalloproteases, which degrade the extra-cellular matrix in order to allow endothelial cells to escape from the original (parent) vessel walls. The endothelial cells proliferate into the surrounding matrix and form solid sprouts connecting neighboring vessels (Folkman et al., 1971). As sprouts extend toward the source of the angiogenic stimulus, endothelial cells migrate in tandem, using adhesion molecules, called integrines. These sprouts then form loops to become a full-fledged vessel lumen cells migrate to the site of angiogenesis (Gimbrone et al., 1972) (Fig. 4). The inhibition of angiogenesis by angiogenesis inhibitors in tumours is an attractive therapeutic strategy for eradicating cancer. Efforts in this direction have led to the clinical trials of many antiangiogenic drugs (Kerbel, 2000, Brannon et al., 2004).

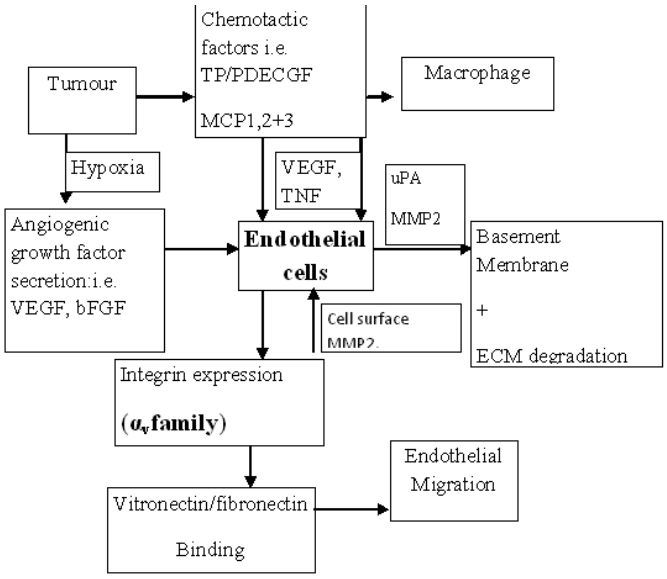

Fig. 4. Schematic diagram of tumour angiogenesis.

RGD based strategy

Intrigrins are a family of transmembrane heterodimeric glycoproteins receptors whose intracellular domines associate with cytoskeletal elements (e.g., vinculin and actin at focal adhesion complex). It consist of α and β subunits and are expressed on tumor-associated endothelial cells (Brannon-Peppas et al., 2004, Ruoslahti, 2002). The expression of the α_v family of integrins is restricted to vascular endothelium, vascular smooth muscle, melanoma, glioblastoma, monocytes and macrophages. Integrins

$\alpha_v\beta_3$ and $\alpha_v\beta_5$ are vitronectin receptors and are differentially expressed by a number of cell types. In particular endothelial expression of the $\alpha_v\beta_3$ dimer is a strong marker for angiogenesis in wound healing and in the development of tumour blood vessels. Integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$, which are either barely detectable or entirely absent from normal blood vessels but are abundantly expressed on tumoral vessels, represent potential pharmacological targets for antiangiogenic therapy (Park et al., 2008)

Targeting of small peptides towards integrins was investigated by phage display library selection of peptides targeting to tumor blood vessels (Temminga et al., 2005). The peptide sequence showing the most efficient binding to the $\alpha_v\beta_3$-integrin receptor was the Arg–Gly–Asp (RGD) tripeptide (Fig. 5). The Integrins bind to ECM via RGD motifs; these interactions signal cell attachments and can affect cell locomotion, differentiation.

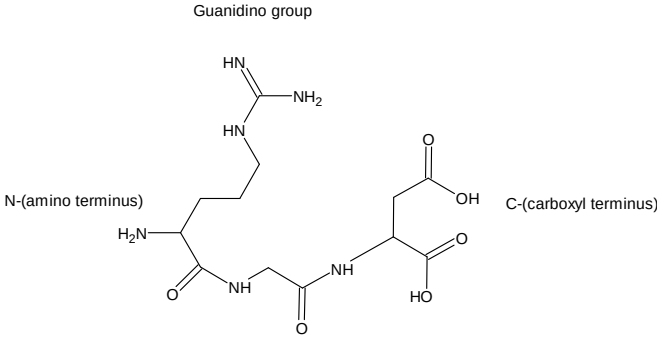

Fig. 5. RGD sequence with various terminuses.

Integrin-ECM interactions can utilize the same intracellular pathways used by growth factor receptors. For example Integrin mediated adhesion to fibronectin can triggers elements of the MAP kinase, Phosphtidylinositol 3-kinase, and protein kinase C pathways. In this manner, extracellular mechnical forces can be coupled to intracellular synthetic and transcriptional pathway. Schiffelers et al. (Schiffelers et al. 2003) repored doxorubicin encapsulated in PEG-liposomes with an integrin targeting RGD-variant coupled to the PEG-terminus, showed superior antitumor activity when compared to doxorubicin encapsulated in non-targeting pegylated liposomes. Several antibodies and peptides capable of functionally blocking the $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins have been demonstrated to inhibit neovascularization in tumor- bearing mice (Ruoslahti, 2002, Hammes et al. 1996).

Ligand based strategy (Active tumor targeting)

Cancer cells often display increased cell surface expression of proteins that may be found at low levels on normal cells (tumor associated antigen), as well as protein that are found exclusively on cancer cell surface (tumor specific antigen). Active targeting for such expressions is an attractive approach which uses "ligands" that facilitate homing of therapeutic moieties at specific epitopes. The specific and selective binding of ligand to its receptor, determines the biodistribution of anti-cancer drugs and hence exert control over pharmacokinetic properties of the drug. (Wolf et al., 2000, Molema, 2005). Active targeting not only reduces the side effects by targeting to specific tumor receptors but also facilitates receptor mediated endocytosis across the plasma membrane than the normal endocytosis (Table. 2).

Table 2. Currently used targeting moieties with examples

Targeting ligands	Target	Example of tumour target
RGD,Arg–Gly–Asp	Cellular adhesion molecules, $\alpha_v\beta_3$-integrin	Vasculature endothelial cells in solid tumours
NGR, Asn–Gly–Arg	Aminopeptidase N (CD13)	Vasculature endothelial cells in solid tumours
Folate	Folate receptor	Cancer cells that overexpress the folate receptor
Transferrin	Transferrin receptor	Cancer cells that overexpress the Transferrin receptor
GM-CSF	GM-CSF receptor	Leukaemic blasts
Anti-tenascin	Extracellular-matrix protein overexpressed in tumours	Glial tumours, breast cancer
Anti-VEGFR	Vasculature endothelial growth factor receptor (FLK1)	Vasculature endothelial cells in solid tumours
Anti-CD22	CD22, a B-cell surface antigen	Non-Hodgkin's lymphoma and other B-cell lymphoproliferative diseases
Anti-CD25	CD25, α-subunit of the interleukin-2 receptor on activated T cells	Hairy-cell leukaemia, Hodgkin's and other CD25+ lymphoma
Galactosamine	Galactosamine receptors on hepatocytes	Hepatoma

Table 3. Current cancer drug delivery technologies.

Lipid Based Systems	Spesific Strategies
Lipid Based Emulsion	Antibody based therapy
Conventional Liposomes	Carbohydrate based therapy
Long circulating Liposomes	Delevery of proteins and peptides
pH-sensitive Liposomes	RGD based formulations
Immunoliposomes	Albumine based drug carrier
Solid Lipid Nanoparticles	Antiangiogenesis therapy
Polymeric Systems	Fatty acid as a targeting vector
Nanoporticles	Tumour actived prodrug therapy
Microsheres	Heat activated targeted drug delivery
Steath Nanoparticles	PEG technologies
Drug-polymer conjugate	Biodegradable polymeric devices
Polymer-DNA complexes	Angiolytic agents
Polymer-protein conjugate	**Biological Therapies**
Dendrimers- Dendrons, steath dendrimer,	Gene Therapy
Diblock & Triblock dendritic copolymers	Antisense Therapy
Micelles-Immuno micelles, thermo-responsive micelles,	RNA Interference
ph responsive micelles	Genetically Modified Bacteria

Transferrin receptor (TfR) is one of the tumor epitops that have shown to be over-expressed on rapidly growing and fast multiplying cells. The TfR expression on tumors is about 10 folds higher in comparison to non-tumor cells. Transferrin is a β globulin (β1- glycoprotein) molecule that facilitates the transport of ferric ion (Fe3+) through transferrin receptors on the plasma membrane. The intracellular delivery of Fe3+ is mediated via receptor mediated endocytosis and the transferring receptors move back on

the surface to again bind to Fe3+ ions. (Pun et al., 2004). PEGylated Poly(cyanoacrylate) nanoparticles when conjugated to transferrin for delivery of paclitaxel (Ptx) have shown grater accumulation to tumor site while PEGylation prevents aggregation of nanoparticles thus potentiat its targeting efficiency.(Tripathi et al., 2002). Folate targeting is an interesting approach for cancer therapy. Folic acid (FA) is a vitamin necessary for the synthesis of purines and pyrimidines, and its receptors are overexpressed on variety of epithelial tumors of various organs like colon, lung, prostate, ovaries, mammary glands and brain. Upon binding of the ligand the ligand-receptor complex is internalized via receptor mediated endocytosis (Kukowska- Latallo et al., 2005). It has been found that folate conjugated liposomes for acute myelogenous leukemia shows that the system was capable of evading P-glycoprotein (Pgp) mediated efflux of drug (Ratnam et al., 2003). This gives the information about the capability of folate to bypass cancer cell multidrug-efflux pump.

The other receptors includes EGF-Rs whose expression on tumors is 100 folds greater than nontumor cells and hence, it provides a potential target for immunotherapeutic agent (Schwechheimer et al., 1995) Human Growth Receptor (HER-2) is a member of EGF family and their number is augmented in several tumors (Artemov et al., 2003). Trastuzumab is a mAb against HER-2 that has been shown to arrest G-1 phase of cell cycle (Yakes et al., 2002). Luteinizing hormone- releasing hormone receptor (LHRH-R) is another kind which barely presents in surface of most normal cells but overexpress in ovarian and some other cancer cells (Grundkar et al., 2002). A composition of LHRH-PEG-camptothecin has been developed by Dharap et al (Dharap et al., 2003) which shows grater accumulation to tumors cells when compared to PEG-camptothecin system. Tumor also overexpress various surface binding lectin-like receptors, that have very high affinity for carbohydrate molecules (Dennis et al., 1999) These lectin-like receptors contains carbohydrates like Sialic Lewis-X SL(X). Glycotargeting exploits interaction of endogenous ligands with carbohydrate moieties (mannose, galactose, fructose, lactose) (Davis et al., 2002).

ADVANCES IN DRUG DILEVERY SYSTEMS

The use of current drug delivery technologies (Table. 3) in cancer therapy requires drug delivery to cancer tissues only, meaning that a drug delivery system should hold the anticancer drug in the blood and then allow a continuous drug release at the cancer site. The basic requirements of any drug delivery systems to deliver chemotherapeutic agent to the cancer are, first it should be long circulating in nature, second it must provides controlled and sustained release of drug and finally it must show sufficient tumour accumulation (Au et al., 2001). All these requirements can be fulfilled by proper selection of drug delivery system. For cancer therapeutics the systems can be categorized into two major classes i.e. lipid based systems and polymer based systems. All these systems have their own advantages and disadvantages. Lipid based systems are easy to prepare having increases bioavailability as compared to free drug and it can solubilize hydrophobic drugs. Targeting is possible by attaching ligand to them. Biocompatibility and prefential accumulation in tumour are other advantages but they are mostly unstable system and removed by RES. The polymeric systems show the same advantages as lipid based system but mostly are immunogenic (Egilmez et al., 2005). To removes shortcoming of delivery systems, surface modification is usually required.

Liposomes have been used as delivery vehicles for stabilizing drugs, overcoming barriers to cellular and tissue uptake, and for directing their contents toward specific sites in vivo. Due to increased surface hydrophobicity of conventional liposomes, they esily gets opsonized and thus uptake by phagocytic cells of mononuclear phagocyte system (MPS). This is particular useful for targeting to tumors of RES organs (like liver cancer). The formulation parameters which including lipid composition, vesicle size (Gabizon et al., 1990), lipid membrane fluidity (Banno et al., 1986), surface charge (Lee et al., 1992), cholesterol content (Gregoriadis et al., 1979), and steric stabilization (Emanuel et al., 1996), have been optimized to extend the therapeutic index of liposomal drugs over that of the corresponding conventional formulations. The stealth liposomes types of liposomes have been developed by attaching PEG molecule on surface which reduces opsonization process by steric hindrance or by increasing surface hydrophilicity. Stealth liposomes have the advantages of long circulating nature which potentiat the targeting efficiency. The biocompatible PEG molecules can act as a spacer for tumor specific ligand molecules so that they can represent to tumor receptors in an efficient way (Emanuel et al., 1996, Mori et al., 2005). Stealth liposomes of doxorubicin (doxil) have been used successfully in patients with metastatic breast cancer, head and neck cancer, ovarian cancer and unrecectable hepatocellular carcinoma. (Torchilin, 2005). The variation of liposomes for release of drug after uptake to tumor includes pH sensitive liposomes (destabilize upon encountering the low pH environment of endosomes), heat sensitive, enzyme sensitive and photosensitive liposomes (Anderson et al., 2005).

Apart from lipid technology, polymeric technology has been used for improving bioavailability of anticancer agent. Polymeric technology can be classified as polymer drug conjugate and drug loaded polymeric vesicles. Drug polymer conjugate needs covalent attachment of polymers to drug molecules, which ultimately reduces the toxicity, enhances serum half-life, and shows intra tumor accumulation as compared to free drug (Vicent et al., 2005). The associated disadvantages includes RES uptake, inability to target and it cannot be used with biologicals.

Nanoparticles may be defined as being submicronic (<1 µm) colloidal systems generally, but not necessarily, made of polymers (biodegradable or not). According to the process used for the preparation of the nanoparticles, nanospheres or nanocapsules can be obtained. Surface morphology and formulation compositions determine its biodistribution. Nanoparticles can accumulate to cancer passively by EPR effect as lymphatic drainage system is compromised in tumor cells. Targeting can also achived via surface engineering of nanoparticals. The folate grafted

to PEGylated cyanoacrylate nanoparticles had found a 10-fold higher apparent affinity for the folate-binding protein (FBP) than free folate did (Stella et al., 2000). Poly (ε-caprolactone) nanoparticles (Mol Wt-15,000) loaded with tamoxifen when administered to mice (MCF-7 breast cancer cell lines) achieved higher concentration in tumors as compared to free drug solution. The studies also showed that the nanoparticles had greater retention time within the tumor mass (Shenoy et al., 2005).Certain types of nanoparticles were also found to be able to overcome MDR resistance, which is due to the presence of the P-glycoprotein efflux system localized at the cancerous cell membrane. These points we had already discussed earlier. The large scale production of polymeric nanoparticles is problematic and cytotoxicity of the polymers after internalization into cells is a crucial aspect (Emanuel et al., 1996).

The Solid lipid Nanoparticals (SLN) combine the advantages of other innovative carrier systems (e.g. physical stability, protection of incorporated labile drugs from degradation, controlled release, excellent tolerability) while at the same time minimising the associated cancer problems. SLN formulations for various application routes (parenteral, oral, dermal, ocular, pulmonar, rectal) have been developed and thoroughly characterised in vitro and in vivo (Pinto et al., 1999, Dingler et al., 1999, Demirel et al., 2001). The main features of SLN with regard to parenteral application are the excellent physical stability, protection of incorporated labile drugs from degradation, controlled drug release (fast or sustained) depending on the incorporation model, good tolerability and sitespecific targeting. Potential disadvantages such as insufficient loading capacity, drug expulsion after polymorphic transition during storage and releatively high water content of the dispersions (70–99.9%) have been observed (Wissing et al., 2002).

Polymeric micelles are thermodynamic aggregates of amphiphilic block copolymer molecules in selective solvents above the critical micelle concentration (cmc). Due to their small size micelles can avoid uptake by the RES resulting in prolonged circulation of the system which ultimately enhance targeting potential. The advance version includes immunomicelles, thermoresponsive micelles, ultra sound sensitive formulation and pH responsive assemblies for targeting (Egilmez, 2005). Once the micellar vehicles are introduced into the body, they are virtually infinitely diluted below cmc and become thermodynamically unstable. The leads to disruption of micellar structures so gives burst release of entrapped drugs. On the other hand dendrimers with amphiphilic moieties are known to exhibit micelle-like behavior and have ‘‘container’’ properties in solution (Kojima et al., 2000). Dendritic micelles are unimolecular micelles in which the hydrophilic and hydrophobic segments are connected covalently therefore shows grater stability as compared to micelles.

Dendrimers in Anticancer tharapy

Dendrimers are hyperbranched, globular, monodisperse, nanometric polymeric carriers, having definite molecular weight, shape, size, and host-guest entrapment properties (Fig. 6). Dendrimers have made its position as a versatile gene and drug carriers by means of numerous potential advantages over the other carrier systems, which include structure uniformity, monodispersity and high purity (Tomalia et al., 1985), less toxicity

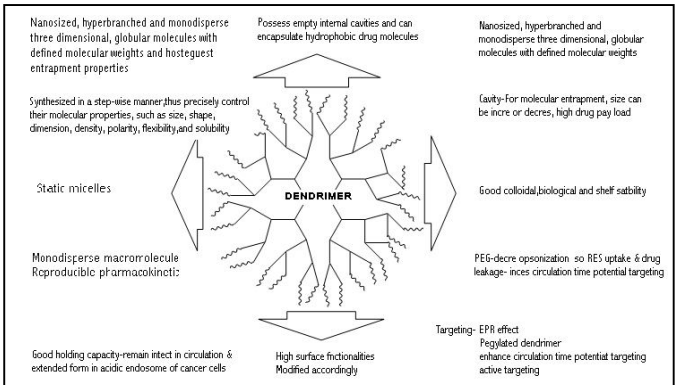

Fig. 6. Properties and application of dendrimers.

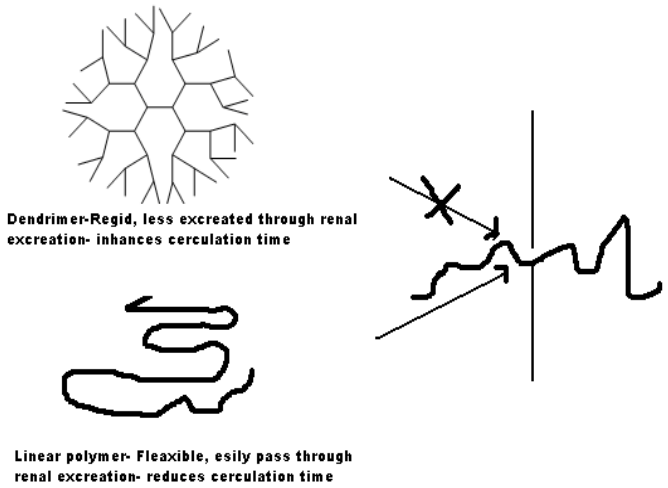

Fig. 7. Renal filtration of dendrimers and linear polymer.

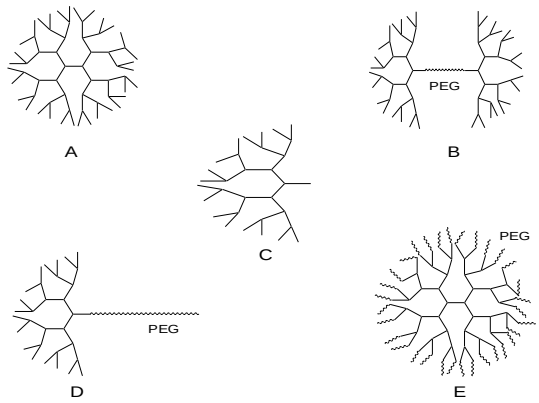

Fig. 8. Variation in dendrimeric technologies. (A)Dendrimers, (B) Triblock dendritic copolymers, (C) Dendrone, (D) Diblock dendritic copolymer, (E) Steath dendrimers.

at low concentration and low generation (Fischer et al., 1999), efficient membrane transport (Stewart et al., 1996), high drug pay load (Kojima et al., 2000), low immunogenicity (Reddy et al., 1999), etc. Dendrimers (MW>40 KDa) were found to remain in blood for longer period as compared to polymers with lower molecular weight. Linear polymers are more flexible so easily undergo renal filteration while dendrimers are regid molecules in which fuctional units are covelantely attached with each other hence undergo renal filteration to a lesses extent (Fig. 7) Applications of dendrimers in the pharmaceutical as well as biomedical field includes the encapsulation and solubilization of hydrophobic drugs (Hawker et al., 1993), gene and drug delivery (Wang et al., 2010), protection of the bioactives from its environment with increased stability , as therapeutic agents (Dutta et al., 2007), as magnetic resonance imaging contrast agents, (Kobayashi et al., 2003) as vaccine (Tam, 1988), as artificial proteins and enzymes (Dandliker et al., 1997), and in immunoassays (Singh et al., 1994).

The variation in dendrimeric technologies includes conventional dendrimers, steath dendrimers, dendrimeric block coplymers (Fig. 8). Sinek and coworkers have reported that nanoconstructs having size range from 1-10 nm are capable of diffusing directly into tumor cells (77). Significantly, PAMAM dendrimers have size range of 2.3 nm in generation-2 (G-2) to 5.3 nm in G-5 (78). In this regard dendrimers can prove to be an important carrier for the delivery of anti-cancer drugs. The unique property of pH triggered drug release by PAMAM and PPI dendrimers has been widely exploited for tumor specific delivery. At the physiological pH (~7.4) the tertiary amine groups of these dendrimers remain deprotonated and the branches converge to central core. This prevents the release of drug in the environment but once the dendrimers enter the tumor vasculature, which has somewhat more acidic microenvironment, the amine groups protonate, and they repel to undergo a conformational change, facilitating the release of drug (Boas et al., 2004) (Figure 9).

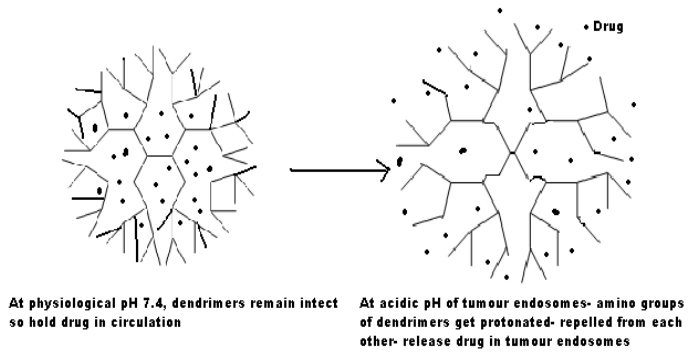

Fig. 9. Release of drug from amino dendrimers (PAMAM and PPI) depends on pH condition.

Presence of numerous tailorable surfaces on the dendrimer makes it possible to attach various ligands and thus delivery to their specific receptors on the tumor cell surface. Bhadra et al (2003) reported a PEGylated-PAMAM dendritic architecture (G-4) for delivery of the anticancer agent 5-fluorouracil (5-FU). The architecture display low hemolytic toxicity and high loading capacity. Kojima et al (Kojima et al., 2000) synthesized PAMAM dendrimers having PEG grafts and studied their ability to encapsulate two anticancer drugs: adriamycin (ADR) and mithotrixate (MTX). It was demonstrated that encapsulation increases with generation as well as molecular weight of PEG attached. It was also inferred that the increased encapsulation of MTX by dendrimer as compared with ADR was due to acid-base interaction between MTX (acidic in nature) and amino groups of dendrimer. Quintana et al (Quintana et al., 2002) designed dendrimer-based therapeutic conjugates with methotrexate (MTX) for tumor cell targeting. The author conjugates MTX to 5GPAMAM-FITC-FA (folic acid, FA) complex via amide and ester linkages. The conjugates internalized in the KB cell line of human epidermoid carcinoma. Plain MTX was found four fold less effective in killing tumor cells than drug conjugates through ester linkages. Frechet and co-workers synthesized ester terminated dendrimers encapsulating methotrexate (Mtx) and FA. The system showed selective affinity to tumor cells expressing F-R (Kono et al., 1999). Shukla and co-workers (2006) synthesized G5 PAMAM dendrimers conjugated to anti-HER2 monoclonal antibody by tagging the formulation with alexaFluor (AF) (G5-AF-HER2). In-vitro studies were performed on MCA-207 control and MCA-207 HER2 cells. Flow cytometric studies revealed the uptake of conjugate by HER2 expressing cells while no such affinity was found for MCA-207 control cells that did not express HER2. Patri and coworkers synthesized G5 PAMAM-FA-MTX complex for the studies. They modified the surfaces so that neutral (hydroxyl or acetyl) ornegatively charged (carboxylate) groups were obtained as terminal functionalities. Binding characteristic of all the modified dendrimers incorporating MTX with and without FA were performed on FR (+) KB cells. The results shows decrease in non-specific interaction of the dendrimers as compared to amine terminated ones and a greater access of the complex in FR (+) cells, which is further supported by lack of cytotoxicity in FR(-) cells (Patri et al., 2005). Thomas and coworkers formulated trifunctional dendritic device G5 PAMAM-FI-FA-MTX conjugate. Results demonstrate cellular internalization of the system and the G5 PAMAM-FI-FAMTX conjugate inhibited growth of FR (+) KB cells whereas non-targeted G5 PAMAM-MTX conjugate failed to promote tumor suppression (Thomas et al., 2005).

CONCLUSION

The issues relating to safety, efficacy, accumulation and disposal, toxicity are important aspects of drug delivery. Potential of dendrimers as vehicle for site-specific delivery of anti-cancer drugs seems to be promising approach but their high costs, complex synthesis procedure and cytotoxicity issues are a matter of concern when compared to other delivery systems.

Conflict of Interest

There is no conflict of interest regarding the manuscript.

REFERENCES

Anderson TL, Jensen SS, Jorgensen K. Advanced strategies in liposomal cancer therapy: problems and prospects of active and tumor specific drug release. Prog Lipid Res. 2005;44:68-97.

Artemov D, Mori N, Ravi R, Bhujwalla ZM. Magnetic Resonance Molecular Imaging of the HER-2/neu Receptor. Cancer Res. 2003;63:2723–2727.

Au JLS, Jang SH, Zheng J, Chen CH, Song S, Hu L,Wientjes MG. Determinants of drug delivery and transport to solid tumors. Journal of Controlled Release. 2001;74:31–46.

Banno Y, Ohki K, Morita T, Yoshioka S, Nozawa Y. Involvement of the membrane fluidity of lactosylceramide targeted liposomes in their intrahepatic uptake, Biochem Int 1986;12:865–871.

Bhadra D, Bhadra S, Jain S, Jain NK. A PEGylated dendritic nanoparticulate carrier of fluorouracil. Int J Pharm 2003;257:111-124.

Bhat TA and Singh RP. Tumor angiogenesis – A potential target in cancer chemoprevention. Food Chem. Toxicol. 2008;46:1334-1345.

Blume G, Cevc G. Liposomes for the sustained drug release in vivo. Biochim Biophys Acta. 1990;1029:91–97.

Boas U, Heegaard PM. Dendrimers in drug research. Chem Soc Rev. 2004;33(1):43-63.

Boehm T, Folkman J, Browder T, O'Really MS. Antiangiogenic therapy of experimental cancer does not induce acquired drug resistance. Nature. 1997;390:404–407.

Brannon-Peppas L, Blanchette JO. Nanoparticle and targeted systems for cancer therapy. Adv Drug Deliv Rev. 2004;56:1649–1659.

Brigger I, Dubernet C, Couvreur P. Nanoparticles in cancer therapy and diagnosis. Adv Drug Deliv Rev. 2002;54:631–651.

Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. Nat. Med. 2000;407:249–257.

Dandliker PJ, Diederich F, Zingg A, Gisselbrecht JP, Gross M, Louati A, Sanford E. Dendrimers with porphyrin cores: Synthetic models for globular heme proteins. Helv Chim Acta. 1997;80:1773–1801.

Davis BG, Robinson MA. Drug delivery systems based on sugar macromolecule conjugates. Curr. Opin. Drug Disc. & Dev. 2002;5(2):279-288.

Demirel M, Yazan Y, Muller RH, Kilic F, Bozan B. Formulation and in vitro– in vivo evaluation of piribedil solid lipid particles. J. Microencapsul. 2001;18(3):359–371.

Dennis JW, Granovsky M, Warren CE. Glycoprotein glycosylation and cancer progression. Biochim. Biophys. Acta. 1999;1473:21-34.

Dharap SS, Qiu B, Williams GC, Sinko P, Stein S, Minko T. Molecular targeting of drug delivery system to ovarian cancer by BH3 and LHRH peptides. J Control Release. 2003;91:61-73.

Dingler A, Blum RP, Niehus H, Gohla S, Muller RH. Solid lipid nanoparticles (SLNk/Lipopearlsk)—a pharmaceutical and cosmetic carrier for the application of vitamin E in dermal products. J. Microencapsul. 1999;16(6):751– 767.

Drummond DC, Meyer O, Hong K, Kirpotin DB, Papahadjopoulos D. Optimizing liposomes for delivery of chemotherapeutic agents to solid tumors. Pharmacol Rev. 1999;51:691–743.

Duncan R. The dawning era of polymer therapeutics. Natl Rev Drug Discov 2003;2:347–360.

Dutta T, Jain NK. Targeting potential and anti–HIV activity of lamivudine loaded mannosylated poly(propylene imine) dendrimer. Biochim Biophys Acta. 2007;1770:681–686.

Egilmez NK. Advances in drug delivery for cancer therapy. Drug Delivery Report. 2005;28-31.

Emanuel N, Kedar E, Bolotin EM, Smorodinsky NI, Barenholz Y. Preparation and characterization of doxorubicin loaded sterically stabilized immunoliposomes. Pharm Res. 1996;13:352–359.

Ferrara N. VEGF: an update on biological and therapeutic aspects. Curr. Opin. Biotechnol. 2000;11:617–624.

Fischer D, Bieber T, Li Y, Elsasser HP, Kissel T. A novel non-viral vector for DNA delivery based on low molecular weight, branched polyethylenimine: Effect of molecular weight on transfection and cytotoxicity. Pharm Res. 1999;16:1273–1279.

Folkman J, Merler E, Abernathy C, Williams G. Isolation of a tumor factor responsible for angiogenesis. J. Exp. Med. 1971;133:275–288.

Folkman J. Tumor angiogenesis: therapeutic implications. N. Engl. J. Med. 1971;285: 1182–1186.

Gabizon A, Price DC, Huberty J, Bresalier RS, Papahadjopoulos D. Effect of liposome composition and other factors on the targeting of liposomes to experimental tumors: biodistribution and imaging studies. Cancer Res. 1990;50:6371–6378.

Gibbs JF and Oliff A. Pharmaceutical research in molecular oncology. Cell. 1994;79:193-198.

Gimbrone Jr MA, Leapman S, Cotran R, Folkman J. Tumor dormancy in vivo by prevention of neovascularization. J. Exp. Med. 1972;136:261–276.

Gregoriadis G, Davis C. Stability of liposomes in vivo and in vitro is promoted by their cholesterol content and the presence of blood cells. Biochem Biophys Res Commun. 1979;89:1287–1293.

Grundkar C, Gunthert AR, Miller RP, Emons G. Expression of gonadotropin releasing hormone II (GnRH-II) receptor in human endometrial and ovarian cancer cells and effect of GnRH-II on tumor cell proliferation. J Clin Endocrinol Metab. 2002;87:1427-1430.

Hammes HP, Brownlee M, Jonczyk A, Sutter A, Preissner KT. Subcutaneous injection of a cyclic peptide antagonist of vitronectin receptor-type integrins inhibits retinal neovascularization. Natl Med. 1996;2:529–533.

Hawker J, Wooley KL, Fréchet JMJ. Unimolecular micelles and globular amphiphiles: Dendritic macromolecules as novel recyclable solubilization agents. J Chem Soc Perkin Trans 1. 1993;1:1287–1297.

Hinds PW and Weinberg RA. Tumor suppressor genes. Current opinion in Genetics and Development.1994;4:135.

Juliano RL. The role of drug delivery systems in cancer chemotherapy. Prog Clin Biol Res. 1976;9:21–32.

Kerbel RS. Tumor angiogenesis: past, present and the near future. Carcinogenesis. 2000;21:505–515.

Khandare J, Minko T. Polymer–drug conjugates: progress in polymeric drugs. Prog Polym Sci. 2006;31:359–397.

Klibanov AL, Maruyama K, Torchilin VP, Huang L. Amphiphathic polyethyleneglycols effectively prolong the circulation time of liposomes. FEBS Lett. 1990;268:235–237.

Kobayashi H, Kawamoto S, Jo SK, Bryant HL, Brechbiel MW, Star RA. Macromolecular MRI contrast agents with small dendrimers: Pharmacokinetic differences between sizes and cores. Bioconj Chem. 2003;14:388–394.

Kojima C, Kono K, Maruyama K, Takagishi T. Synthesis of polyamidoamine dendrimers having poly(ethylene glycol) grafts and their ability to encapsulate anticancer drugs. Bioconj Chem. 2000;11:910–917.

Kojima C, Kono K, Maruyama K, Takagishi T. Synthesis of polyamidoamine dendrimers having poly(ethylene glycol) grafts and their ability to encapsulate anticancer drugs. Bioconjug Chem. 2000;11:910-917.

Kono K, Liu M, Frechet JMJ. Design of dendritic macromolecules containing folate or methotraxate residues. Bioconj. Chem. 1999;10:1115-1121.

Krishna R, Mayer LD. Multidrug resistance (MDR) in cancer: Mechanisms, reversal using modulators of MDR and the role of MDR modulators in influencing the pharmacokinetics of anticancer drugs. Eur J Pharm Sci. 2000;11:265–283.

Kukowska-Latallo JF, Candido KA, Cao Z, Nigavekar SS, Majoros IJ, Thomas TP, Balogh LP, Khan MK, Baker Jr JR. Nanoparticle Targeting of Anticancer Drug Improves Therapeutic Response in Animal Model of Human Epithelial Cancer. Cancer Res. 2005;65(12):5317-5324.

Lee KD, Hong K, Papahadjopoulosb D. Recognition of liposomes by cells: in vitro binding and endocytosis mediated by specific lipid headgroups and surface charge density. Biochim Biophys Acta. 1992;1103:185–197.

Links M, Brown R. Clinical relevance of the molecular mechanisms of resistance to anti-cancer drugs. Expert Rev Mol Med. 1999;1999:1–21.

Maeda H, Matsumura Y. Tumoritropic and lymphotropic

principles of macromolecular drugs. Crit Rev Ther Drug Carrier Syst 1989;6:193–210.

Maeda H. The enhanced permeability and retention (EPR) effect in tumor vasculature: the key role of tumor-selective macromolecular drug targeting. Adv Enzyme Regul. 2001; 41:189–207.

Matsumura Y, Maeda H. A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs. Cancer Res. 1986;46:6387–6392.

Molema G. Design of vascular endothelium-specific drug-targeting strategies for the treatment of cancer. Acta Biochim Pol. 2005;52(2):301–310.

Mori A, Klibanov AL, Torchilin VP, Huang L. Influence of the steric barrier activity of amphipathic poly (ethyleneglycol) and ganglioside GM on the circulation time of liposomes and on the target binding of immuno liposomes in vivo. FEBS Lett. 1991;284:263–266.

Park JH, Lee S, Kim JH, Park K, Kim K, Kwon IC. Polymeric nanomedicine for cancer therapy. Progress in Polymer Science. 2008;33(1):113-137.

Patri AK, Kukowska-Latallo JF, Baker Jr JR. Targeted drug delivery with dendrimers: Comparison of the release kinetics of covalently conjugated drug and non-covalent drug inclusion complex. Adv. Drug Delivery Rev. 2005; 57:2203– 2214.

Pinto JF, Muller RH. Pellets as carriers of solid lipid nanoparticles (SLNk) for oral administration of drugs. Pharmazie. 1999;(54):506– 509.

Pun SH, Tack F, Bellocq NC, Cheng J, Grubbs BH, Jensen GS, Davis ME, Brewster M, Janicot M, Janssens B, Floren W, Bakker A. Targeted Delivery of RNA Cleaving DNA-Enzyme (DNAzyme) to Tumor Tissue by Transferrin- Modified, Cyclodextrin-Based Particles. Cancer Boil. & therapy. 2004;7(3):31-41.

Quintana A, Raczka E, Piehler L, Lee I, Myc A, Majoros I, Patri A.K, Thomas T, Mule J, Baker JR Jr.. Design and function of a dendrimer based therapeutic nanodevice targeted to tumor cells through the folate receptor. Pharm Res 2002; 19:1310- 1316.

Ratnam M, Hao H, Zheng X, Wang H, Qi H, Lee R, Pan X. Receptor induction and targeted drug delivery: a new antileukemia strategy. Expert Opin. Biol. Ther. 2003;3:563-574.

Reddy JA, Dean D, Kennedy MD, Low PS. Optimization of Folate-Conjugated Liposomal Vectors for Folate Receptor-Mediated Gene Therapy. J Pharm Sciences. 1999;88(11):1112-1118.

Ruoslahti E. Specialization of tumour vasculature. Natl Rev Cancer. 2002;2:83–90.

Schiffelers RM, Koning GA, ten Hagen TL, Fens MH, Schraa AJ, Janssen AP, Janssen AP, Kok RJ, Molema G, Storm G. Anti- tumor efficacy of tumor vasculature-targeted liposomal doxorubicin. J Control Release. 2003;91:115–122.

Schwechheimer K, Huang S, Cavenee WK. EGFR gene amplification rearrangement in human glioblastoma. Int. J. Cancer. 1995;62:145–148.

Shenoy DB, Chawla JS, Amiji M. Biodegradable Polymeric Nanoparticles for Tumor-Selective Tamoxifen Delivery, In Vitro and In Vivo Studies. Mater. Res. Soc. Symp. Proc. Mat. Res. Soc. 2005;845:1-5.

Shukla R, Thomas TP, Peters JL, Desai AM, Kukowska-Latallo J, Patri AK, Kotlyar A, Baker Jr JR. HER2 Specific Tumor Targeting with Dendrimer Conjugated Anti-HER2 mAb. Bioconj. Chem. 2006;17(5):1109-1115.

Singh P, Moll F, Lin SH, Ferzli C, Yu KS, Koski RK, Saul RG, Cronin P. Starburst dendrimers: enhanced performance and fl exibility for immunoassays. Clin Chem. 1994;40:1845–1849.

Si-Shen Fenga, Shu Chienc. Chemotherapeutic engineering: Application and further development of chemical engineering principles for chemotherapy of cancer and other diseases. Chem Engineering Science. 2003;58:4087– 4114.

Stella B, Arpicco S, Peracchia MT, Desmae¨le D, Hoebeke J, Renoir M, d'Angelo J, Cattel L, Couvreur P. Design of folic acid-conjugated nanoparticles for drug targeting. J Pharm Sci. 2000;89:1452–1464.

Stewart AJ, Pichon C, Meunier L, Midoux P, Monsigny M, Roche AC. Enhanced biological activity of antisense oligonucleotides complexed with glycosylated poly-L-lysine. Mol. Pharmacol.1996;50:1487-1494.

Tam JP. Synthetic peptide vaccine design, synthesis and properties of a high density multiple antigenic peptide system. Proc Natal Acad Sci. 1988;85:5409-5413.

Temminga K, Schiffelers RM, Molemad G, Kok RJ. RGD-based strategies for selective delivery of therapeutics and imaging agents to the tumour vasculature. Drug Resistance Updates. 2005;8:381–402.

Thomas TP, Majoros IJ, Kotlyar A, Kukowska-Latallo JF, Bielinska A, Myc A., Baker Jr JR. Targeting and Inhibition of Cell Growth by an Engineered Dendritic Nanodevice. J. Med. Chem. 2005;48:3729-3735.

Thurston G, Suri C, Smith K, Mcclain J, Sato TN, Yancopoulos GD, Mcdonald DM. Leakage-resistant blood vessels in mice transgenically overexpressing angiopoietin-1. Science. 1999;286:2511–2514.

Tomalia DA, Baker H, Dewald JR, Hall M, Kallos G, Martin S, Roeck J, Ryder J, Smith P. A new class of polymers: starburst dendritic molecules. Polym J. 1985;17:117–132.

Torchilin VP. Recent advances with liposomes as pharmaceutical carriers. Nat. Rev Drug Discov. 2005;4:145-160.

Tripathi PK, Khopade AJ, Nagaich S, Shrivastava S, Jain S, Jain NK. Dendrimer grafts for delivery of 5-fluorouracil. Pharmazie. 2002;57:261-264.

Vicent MJ, Greco F, Nicholson RI, Paul A, Griffiths PC, Duncan R. Polymer therapeutics designed for a combination therapy of hormone-dependent cancer. Angew Chem Int Ed Engl. 2005;44:4061–4066.

Wang X, He Y, Wu J, Gao C, Xu Y. Synthesis and evaluation of phenylalanine modified hyperbranched poly(amidoanime)s as promising gene carriers. Biomacromolecules. 2010;1(1):245-251.

Weiss RB, Donehower RC, Wiernik PH, Ohnuma T, Gralla RJ, Trump DL, Baker R, VanEcho DA, VonHoff DD, Leyland-Jones B. Journal of Clinical Oncology. 1990;8:1263–1268.

Wissing SA, Muller RH. Solid lipid nanoparticles as carrier for sunscreens: in vitro release and in vivo skin penetration. J Control Release. 2002;81:225–233.

Wolf de FA, Brett GM. Ligand-Binding Proteins: Their Potential for Application in Systems for Controlled Delivery and Uptake of Ligands. Pharmacol Rev. 2000;52(2):207–236.

World Health Organization: Cancer. /http://www.who.int/cancer/enS. Accessed February, 2007.

Yakes FM, Chinratanalab W, Ritter CA, King W, Seelig S, Arteaga CL. Herceptin-induced inhibition of phosphatidylinositol-3 kinase and Akt is required for antibody-mediated effects on p27, cyclin D1, and antitumor action. Cancer Res. 2002;62:4132-4141.

Yoshida S, Ono M, Shono T, Izumi H, Ishibashi T, Suzuki H, Kuwano M. Involvement of interleukin-8, vascular endothelial growth factor, and basic fibroblast growth factor in tumor necrosis factor a-dependent angiogenesis. Mol. Cell Biol. 1997;17:4015–4023.

Yuan F, Dellian M, Fukumura D, Leunig M, Berk DA, Torchilin VP, Jain RK. Vascular Permeability in a Human Tumor Xenograft: Molecular Size Dependence and Cutoff Size. Cancer Res. 1995;55:3752–3756.