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Review Article

Tumour and dendrimers: a review on drug delivery aspects

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Abstract

Tumour is a morbid state, characterized by spontaneous outgrowth of an abnormal mass of cells. The evolution of tumours is random, disorganized, a condition of numerous mutations. The properties are biased and incompletely comprehended. It is a malignant or benign condition that encompasses its own rules of morphogenesis, an immortal state that elucidates different physiology. It is a pathological crisis that still haunts the minds of scientists, physicians and patients, a complete cure of which is still a dream to be realized. The unpredictable microenvironment of cancerous cells in all of its existing forms i.e. leukaemic cells, solid tumours and sarcomas is well documented. This phenomenon expressed by cancerous sites in the body poses various obstacles towards drug efficacy. Thus, it has become necessary to address briefly the issues relating to tumour physiology, its vasculature and angiogenesis. The information could provide insight towards the development of tumour-targeted drug delivery. The salient features regarding these have been discussed.

Tumour physiology and vasculature

Under normal conditions, cells reproduce, grow, divide, multiply and eventually undergo apoptosis. This maintains proper balance and functioning of the organs. However, tumour cells are subjected to uncontrolled proliferation of cells, evade apoptosis and hence they develop as an abnormal mass of cells that can be life-threatening if untreated at an early stage. Tumours bear highly irregular chaotic architectural vasculature. 'The genesis of blood vessels is an abnormal state' in tumours as there is abrupt formation of newer vessels and existing vessels tend to become disorganized. However, 'the genesis of blood vessels occurring elsewhere in the body is not necessarily abnormal'. Such eruptions occur as a result of genetic aberrations from pre-existing vessels or in a specialized microenvironment from endothelial cell progenitors of bone marrow (Jain et al 1987; Haroon et al 1999; Carmeliet & Jain 2000; Ribbati et al 2001; Peppas & Blanchette 2004; Gjini 2005). Upon undergoing change in the organizational pattern of a gene, the normal cell cycle is disrupted. The gene (proto-oncogene) is thus converted into an oncogene and loses its normal growth regulation. As a consequence of mutations, abnormal growth and proliferation of cells begin along with the expression of various surface markers and proteins (Table 1), which facilitate their growth and supplement the cells with necessary nutrients and oxygen at the expense of normal cells. The abnormal signal proteins may induce faster proliferation of cells via transcription. Thus, a gene in a normal cell on being converted into an oncogene proliferates under the stimuli of these agents and leads to development of tumours (Folkman 2002; Siemann 2006). There is a change in chromosomal structure and the genetic setup begins to develop in such a way so as to delay senescence. Pericytes are the connective tissues that grow around basement membrane. This growth of pericytes around tumours provides them with adequate protection against tumour targeting. They have been known to facilitate tumours by promoting cell-to-cell attachment and hence their stabilization (Banfi et al 2005; Molema 2005).

Blood from the capillaries may diffuse or drain out into a newer network consisting of vessels and lymph nodes called the lymphatic system, which circulates like blood in various tissues and organs. Blood and lymph communicate to each other at lymph nodes and the lymph, via the thoracic vein, finally enters the general circulation. Lymphatic metastasis is characteristic of tumours. Metastasis is the movement of tumour cells to other sites and is a highly hazardous state of the biosystem. Every single cell that moves to another place may

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Table 1 Surface markers and proteins over-expressed in tumours

Carcinoma	Surface markers and proteins
Breast	C-myc gene (Peng & Hsu 2001; Aref et al 2001) amplification
	MAPK-1 (Arspe 2005)
	Activator protein-1 (Asthana et al 2005)
	MLH-1 (Aulenta et al 2003)
Colorectal	MSH-II (Aulenta et al 2003)
	Ki-ras2 genes (Azzam & Domb 2004)
	CD-95 L (Backer et al 2005)
Hepatocellular	Activator protein-1 (Banfi et al 2005)
	α-fetoprotein (Barth et al 1994)
Lung	PIK3CA gene (Barth et al 2004)
	Laminin-10/11 (Behr 1997)
	Ki-ras2 genes (Azzam & Domb 2004)
	hG-CSF (Benns et al 2000)
Leukaemic	CD-95 L (Berger & Benjmain 2003)
	Abl kinase (Bhadra et al 2004)
	CD-44 (Bhadra et al 2003)
Ovarian	P185 HER2 (Bhadra et al 2003)
	(P^{16}/P^{15}) (Bhadra et al 2005)

develop into a new tumour, giving rise to various necrotic regions. Upon treatment even if a single cell or colony is left out, it can again lead to an entire tumour (Mareel 2004; Arspe 2005). Present cancer therapy should be based on the philosophy, 'even a single cancer cell should not remain untreated in the body and nothing less than complete elimination of tumour from an individual can be accepted'. This forms the objective behind chemotherapy. Tumours invade the stroma of an organ or tissue and metastasize to lymph vessels promoting formation of newer vessels (lymphangiogenesis). Lymph node sinuses can be the dominant regions for tumour metastasis. Cancer generally proceeds in the direction of lymph flow, but can also be in the reverse direction. Tumours may move from one site to another mostly via lymphatics giving rise to newer necrotic regions. As the cells grow at tumour sites they generate mechanical stress. The compromised vasculature and poor lymphatic drainage create interstitial stress, which hinders the blood supply and obstructs transport of bioactives (Alitalo & Carmeliet 2002; Kaul et al 2003). Irregular development of basement membrane, the presence of fenestrae (400–800 nm), widened inter-endothelial junctions, vasodilatation and formation of small blood vessels to nourish the tumour cells makes them more porous. Hence the permeability of tumour vessels is several times higher than normal vessels (Modi et al 2004; Sevenson & Tomalia 2005).

Rapid proliferation of cells develops an oxygen crisis, causing an alteration in the genome of expressing cells that exhibit resistance to hypoxia. Survival of tumour cells in anaerobic conditions makes them glycolytic. Further, tumour cells transcribe hypoxic inducible factor (HIF-1) that causes change in the programme of gene expression. HIF-1 induces pyruvate dehydrogenase kinase (PDK-1), a chemical that prevents apoptosis (Kim et al 2003; Mennon et al 2003). Normal cells lacking HIF-1 fail to generate PDK-1 and succumb to apoptosis. Hypoxia induces macrophage infiltration at tumour sites. Macrophages induce formation of new vessels (angiogenesis) via induction of various prognostic mitogens

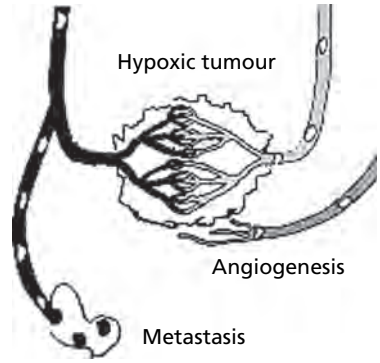

Figure 1 Avascular tumour growth.

(Crowther et al 2001) (Figure 1). Another important aspect of ischaemic tumour microenvironment is that the extracellular pH (~7.0 or less than 7.0) (Engin et al 1995) is slightly acidic compared with the normal tissues (pH~7.4). Although tumour cells may maintain intracellular pH, the acidic extracellular environment is an important aspect of mutagenesis. This forms the basis for systems having pH-based delivery. It induces expression of various signals that lead to oncogenic changes enabling cells to survive under unfavourable conditions. The cells generate various pumps so as to remove intracellular H^+ ions, preventing intracellular acidification and developing slightly alkaline conditions optimum for cellular growth. The growing tumour is a rapidly expanding capillary network that helps to defy the laws of mortality (Kraus & Wolf 1996; Wahl et al 2002).

Angiogenesis

The word angiogenesis elucidates development, growth and expansion of new vessels, which is necessary for proper functioning of the body. However, its malfunction may lead to severe pathological crisis such as cancer or degradation of the body. As the angiogenic switch is turned on (Figure 2), it facilitates growth of numerous vessels that supports tumour growth (Ribatti & Presta 2002; Bergers & Benjamin 2003).

Gjini (2005) reported a new concept of 'tumour vasculature normalization' that aimed to carefully monitor the dosage regimen of anti-angiogenic therapy, maintaining proper equilibrium between angiogenic stimulators and inhibitors. The concept aimed at the treatment of tumours via inhibiting production and release of various chemical factors that promoted rapid proliferation of newer vessels, at the same time facilitating the production and release of various chemical factors that promoted apoptosis. Various factors that play an important role in tumour angiogenesis are summarized below.

Vascular endothelial growth factor (VEGF)

This is the key factor involved in controlling disposition characteristics of cancer. Binding of VEGF to its tyrosine kinase receptors facilitates triggering of a cascade of events that leads to transcription, receptor mediated growth and proliferation of cells. Oncogenesis leads to production of VEGF, which is an important mitogen in cancer angiogenesis, and its

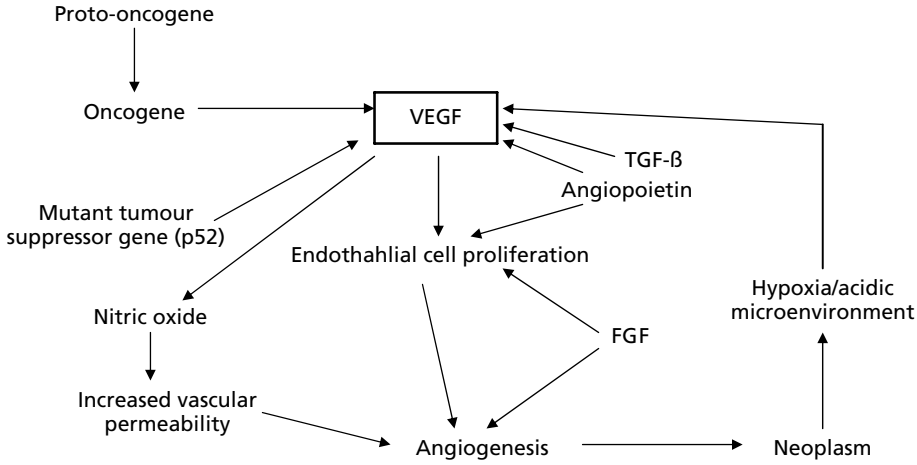

Figure 2 Angigoenesis: promoting tumours.

expression is up-regulated by hypoxia and an acidic environment. The anti-cancer drug Avastin binds to VEGF, inhibiting its interaction with endothelial cells, thereby preventing their proliferation and hence exerting an anti-angiogenic action. VEGF causes expression of matrix metalloproteinase (MMP) that causes breaks in the interstitial collagen of pre-existing vessels. MMP also promotes leakage of endothelial cell membrane (ECM) and subsequent release of mitogens (Jackson 2002; Saif 2006). The endothelial cells transcribe and newer vessels develop; these immature vessels are stabilized by deposition of pericytes and extracellular matrix (Jackson 2002). Activity of MMP has been evaluated to be up-regulated as a result of mutations. VEGF also mediates mitogen-activated protein kinase signal transduction cascade and facilitates production of nitric oxide (Weis et al 2004). Succession to these events helps in increasing permeability of blood vessels. Thus, VEGF or its receptors can prove to be potential targets for tumour therapy to eliminate existing tumours and prevent further growth of tumorous cells. Antibodies neutralizing VEGF resulted in suppression of tumorous outgrowths (Kim et al 1993). Depleting VEGF leads to apoptosis of tumour endothelial cells, decreasing vessel diameter and permeability leading to normalization of tumour vasculature (Carmeliet 2005; Gupta & Qin 2005). Chemical conjugates of VEGF have been utilized as carrier molecules. Upon binding of VEGF to its receptors VEGF-R, the complex VEGF/VEGF-R is internalized and selectively kills tumour endothelial cells (Olson et al 1997).

Angiopoietins

Angiopoietins induce proliferation of endothelial cells and growth of tumours. Angiopoietin 1 (Ang1) activates survival factor Akt and P13k, promotes macrophage infiltration, and secretion of VEGF, prevents apoptosis and enhances hypervascularization. Pericytes have been known to promote its release. The genetic setup begins to develop in such a way so as to delay cellular senescence and accelerate sprouting of newer vessels (Arspe 2005; Gupta & Qin 2005). Angiopoietin 2 (Ang2) helps in phosphorylation of endothelial cells. Ang2 is expressed at angiogenic sites and induces phosphorylation of tyrosine kinase receptor Tie-2. This promotes proliferation and migration of endothelial cells via secretion of VEGF. It expedites creation of new vessels and prevents death of existing vessels, thus providing a better environment for tumour growth and disposition, worsening conditions for the cancer patient. Ang2 exhibits these actions only in the presence of VEGF, otherwise it may lead to a relapse of the vessels. Inhibiting Ang1 and Ang2 would prevent formation of newer vessels and hence inhibit angiogenesis (Gale & Yancopoulos 1999; Tillmar & Welsh 2004; Arspe 2005; Nakayama et al 2005).

Oncogenes and tumour suppressor genes (TSG)

Proto-oncogene is a class of gene which in normal conditions helps in growth and division but on mutation it becomes activated (oncogene), causing cancer. Mutations can be due to a change in chromosomal pattern or dysfunctioning of the cell cycle. Thus, it is an altered gene leading to abnormal growth of cells and mutations in TSG (p53). Adenovirus mediates p53 (wild type) expression and postpones DNA replication thereby promoting apoptosis. While normal TSG prevents tumour growth and formation of newer vessels that inhibits angiogenesis, the mutant 'p52' prevents apoptosis and causes an increase in VEGF (Gupta & Qin 2005).

Transforming growth factor β (TGF-β)

TGF-β acts via two types of receptors: TGF type-I and TGF type-II. TGF-β stimulates production of angiogenic factors and exerts an inhibitory effect on anti-angiogenic moieties like angiostatin. However, MMP inhibitors have been found to reduce TGF-β-induced invasiveness (Gupta & Qin 2005).

Fibroblast growth factor (FGF)

FGF exerts its activity via tyrosine kinase receptors to promote the replication process. This promotes the formation of pericytes at tumour sites and stimulates VEGF mRNA production. It can provide an alternative pathway for tumour progression in the absence of VEGF and is highly expressed on the stroma of epithelial carcinomas. Mutations that cause tumour induce

genetic signals that help in expression of FGF. It provides synergistic effects for tumour growth and metastasis. Monoclonal antibodies (mAbs) that target Flk-1 receptors promote FGF-mediated tumour regression (Arspe 2005; Gupta & Qin 2005).

Approaches for tumour specific delivery

It is clear from the previous section that because of the associated irregularities concerning tumour, its growth within the body is widespread. Thus, providing justification on its proposed therapy becomes a very challenging task. However, there are a few strategies (Figure 3) that can be focused on to proceed in the elimination of the tumour. A large number of carriers have been reported using the fundamentals of one or more of these approaches, which have proved to be quite successful (Table 2).

Intracellular communication and tumour specific delivery

The intrinsic activity of the drug carrier system should be such that it causes intra-tumoral delivery of drug, thus giving a high concentration of drug at the target site and minimizing the possible side effects, reducing dosing frequency and increasing patient compliance. The active moiety or the drug coupled to the carrier can access the tumour tissues or cells via various mechanisms such as simple diffusion, facilitated diffusion, enhanced permeation and retention (EPR) effect, active transport or specific receptor-mediated endocytosis (RME) (Peppas & Blanchette 2004). The drug needs to traffic various membrane barriers to reach the tumour tissue. To deliver the drug within the tumour site it is required that the bioactive be either attached to various linkers that cleave in the acidic environment of the tumour or encapsulated within some vesicular carrier, or coupled via such bond, which

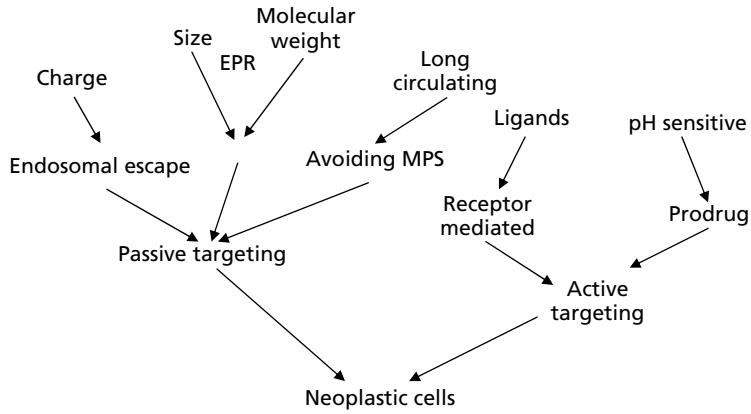

Figure 3 Targeting tumour: active and passive approaches.

Table 2 Various carrier systems used in tumour therapy

Carrier systems	Formulation	Remarks
Nanoparticles (Carmeliet & Jain 2000)	PEG modified gelatin nanoparticles loaded with DNA	Intracellular trafficking to tumours and nuclear delivery
Liposomes (Fischer et al 1999)	FA-PEG-DOPE modified liposomes containing DNA	Efficient for pH sensitive endosomal DNA delivery
HPMA polymer (Folkman 2002)	HPMA-(Gly-Phe-Leu-Gly) linker-TNP 470 complex	Selective accumulation in tumour vasculature and prevents TNP-470 from crossing blood–brain barrier
Liposomes (Friend & Pangburn 1987)	Folate conjugated liposomes	Efficient delivery of cytotoxic drug to tumours
Nanospheres (Fujita et al 1997)	Gelatin nanospheres complexed to DNA	Efficient vehicle for gene delivery
Nanoparticles (Gale & Yancopoulos 1999)	RGD-modified nanoparticles	Selective localization of drugs in tumours
Nanoparticles (Galle & Krammer 1998)	Dextran–drug conjugate incorporated in chitosan nanoparticles	Efficient vehicles to suppress tumour volume
Nanoparticles (Gaur et al 2000)	PLGA nanoparticle conjugated with anti-cancer drug	High-loading efficiency and comparable activity to official formulations
Microspheres (Gillies & Frechet 2004)	Alginate microspheres containing naked DNA	Gene delivery for tumours
Polylysine (Gillies & Frecht 2005)	Transferrin conjugated polylysine	Gene transfer agent

cleaves in the tumour microenvironment. The strategy may apply a pro-drug concept or an active targeting approach, so that the drug is converted into an inactive moiety or attached to a linker that liberates drug only on encountering tumour and not the normal cells. This exploits the pathophysiological difference between normal and malignant cells, such as acidic pH and expression of various tumour specific antigens (Patri et al 2002a; Gillies & Frechet 2004) (Figure 4). Micelles of PLLA-b-PEO polysulfadimethoxine are pH sensitive due to acidic sulfonamide groups and have been reported to be useful anti-cancer agents (Lee et al 2003).

Drug or drug carrier systems, once internalized via phagocytosis, are encapsulated in endosomes when acidic pH (5.5–6.5) and endolytic enzymes (proteases, lipase, glycolases) set to release the drug, which is metabolized before eliciting the desired action. In this regard positively-charged carriers have been proved to undergo endosomal escape consequent to destabilizing endosomal membrane, preventing release of drug in the endosomes (Tkachenko et al 2004). Christiano (1998) reported the use of synthetic viruses and molecular conjugates as non-viral agents for endosomal escape. Delivery of protein/DNA polyplex along with adenovirus led to the adenovirus accompanying the polyplex entering the same endosome by receptor-mediated endocytosis, and subsequent lysis of the endosome by virus, facilitating endosomal release of DNA.

Enhanced permeation and retention (EPR) effect

Blood vessels in tumours bear leaky vasculature due to angiogenesis, presence of fenestrae (400–800 nm) and widened inter-endothelial junctions (Sevenson & Tomalia 2005). This provides a good opportunity for intracellular access of drugs. Although particles up to a size range of 800 nm will enter these vessels, they will eventually escape the capillaries. However, the macromolecules within this size range will enter these vessels, where they will be retained or lodged in capillaries due to their relatively 'high molecular weight'. Thus leaky tumour vasculature, as well as the poor lymphatic drainage, provides enhanced residence time for macromolecules in tumours. This phenomenon is termed the EPR effect. The small molecules may easily escape the capillaries due to insufficient pore cut-off size (400–800 nm); the macromolecules may prove to be an engineered design for being effectively localized at the site preventing their escape (Kaul et al 2003). These macromolecules may be taken inside as a consequence of extravasation of the vesicles, where enzymes work in co-ordination to release the drug (Modi et al 2004). *N*-(2-Hydroxy propyl) methacrylamide (HPMA) copolymer conjugated to doxorubicin has been shown to reduce cardiotoxicity by 4–5-times due to efficient localization mediated via the EPR effect (Duncan et al 2005). Thus, a drug conjugated to a macromolecule may lead to a far better distribution of the drug (Friend & Pangburn 1987) and EPR provides a passive transport mechanism for the entry of drug into malignant cells (Kaul et al 2003). Macromolecules such as albumins (Lowenthal et al 2005), globulins (Selby 1990) and synthetic polymer accumulate in the tumour tissues because these tissues have a vascular network characterized by both enhanced permeability of the neovasculature and a lack of the lymphatic recovery system (Matsumura & Maeda 1986). Also, increasing molecular weight of carriers may prevent their elimination by the kidneys (Michallet et al 2004). Thus, they circulate in the body for a prolonged duration, providing sustained delivery until they are eventually degraded or metabolized by the liver (Orive et al 2005) (Figure 5).

Avoiding mononuclear phagocytic system (MPS)

Uptake of drug by liver, spleen and macrophages is a great setback in delivery of drugs to tumour sites. Hydrophobic particles have a greater tendency to be eliminated; larger sized particles are more prone to reticulo-endothelial system (RES) uptake compared with smaller size particles such as nanometric particles. They may be opsonized by serum proteins and then cleared. On the other hand, carriers or active moieties possessing hydrophilic surfaces prevent their recognition by the MPS. Copolymer micellar carriers, having a particle size range from 20 to 100 nm, are small enough to avoid RES uptake (Kwon & Okano 1996). Biodistribution studies of hydrophilic nanoparticles prepared from poly(vinyl pyrollidone) have been shown to bypass uptake by liver and spleen (Gaur et al 2000). Coating with specific barriers that promote stearic hindrance for drug uptake by liver and spleen is yet another strategy to develop long circulating drug delivery units avoiding MPS clearance. Preparing water soluble derivatives

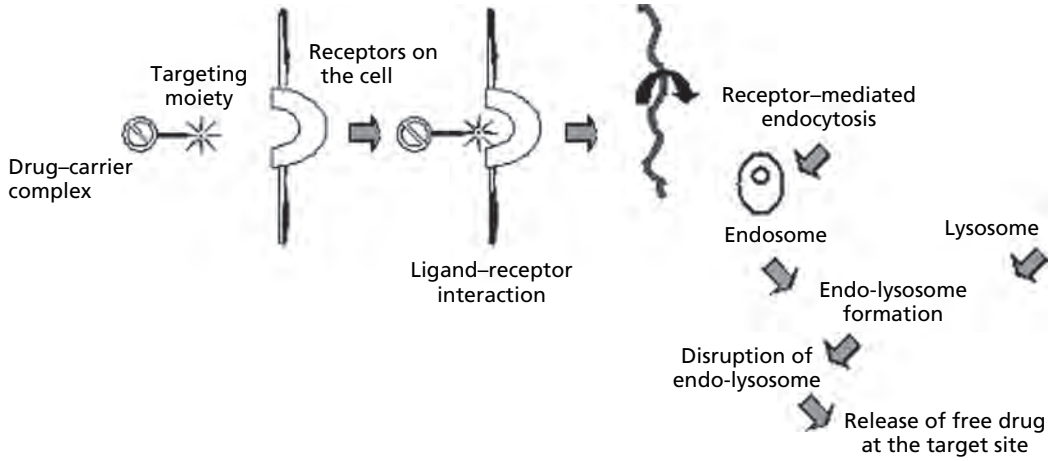

Figure 4 Site-specific delivery: ligand–receptor interaction.

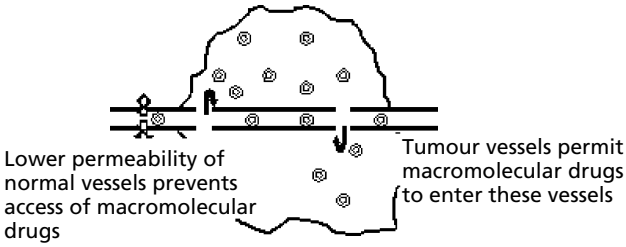

Figure 5 Enhanced permeation and retention.

and a reduction in particle size also provide suitable strategies to escape the RES (Peppas & Blanchette 2004); reported use of polyethylene glycol (PEG; macrogol) (fluoroalkylated PEG) as surfactant (Peng & Hsu 2001) has been shown to prevent RES uptake by increasing the hydrophilic character; negatively-charged carriers are more rapidly opsonized than positively-charged ones. Uptake of drugs by the RES follows Michaelis–Menten kinetics (Wood & Thakker 1982), therefore the critical point of saturation concentration specifies the limits for doses. Increasing the dose of moieties may prevent escape from the MPS due to saturation of uptake sites, but therapeutic indices, unwanted side effects and dose-related drug toxicity need to be considered (Davis 2000).

Gene therapy

This can be considered a new visionary for the treatment of immunogenic diseases. Tumours are caused as a consequence of mutations in the genome by, addition, deletion or change in chromosomal pattern. Suitable vectors such as anionic liposomes (Rosenberg et al 1990), poly-L-lysine (Ledley 1995), polyethylene oxide (PEO) (Benns et al 2000), retrovirus (Fischer et al 1999) etc. have been reported to access the nucleus and rectify the condition (Figure 6). Viral and non-viral vectors can be aimed at treating this condition. Viral vectors can be immunogenic; the random insertion in the host genome can possibly cause oncogene activation, tumour suppressor gene inactivation or nonspecific inflammation (Bordignon et al 1995), also the manufacturing of viral vectors is difficult and they have limited capacity to carry DNA. Thus, the safety concern may circumvent the use of non-viral vectors. However, non-viral vectors also possess the major drawback of having inefficient and comparatively low transfection ability (Abaan & Criss 2002; Azzam & Domb 2004; Khalil et al 2006). Recognizing the gene (Patri et al 2002b) and delivery of a specific gene into the target site can play a crucial role in life-threatening diseases (Wang et al 1996; Eliyahu et al 2005).

Targeting tumour angiogenesis

Tumour angiogenesis is a consequence of disturbance in the activity of angiogenic promoters and angiogenic inhibitors. The strategy is based on two approaches, inhibition of angiogenic factors and their receptors, or anti-vascular therapy (Siemann 2006). It aims to prevent formation of new vessels and damage the existing ones. Angiogenesis inhibitors seem to interrupt endothelial cell function. The strategy up-regulates the activity and amount of angiogenic inhibitors, preventing

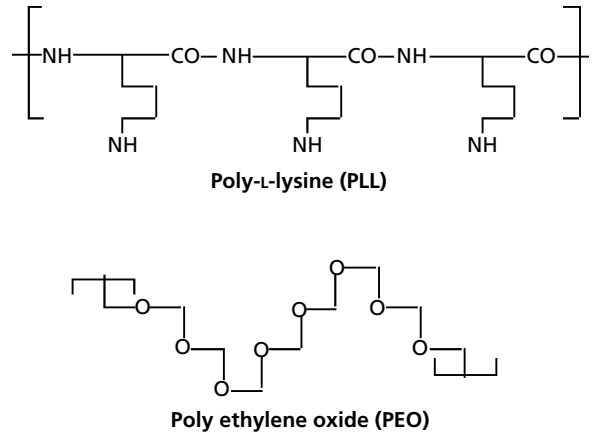

Figure 6 PLL and PEO (non-viral vectors).

endothelial cells from giving rise to new vessels. Endothelial cells can be better subjected to drug targeting than tumour cells as they are less prone to become resistant to drug therapy. Destroying endothelial cells employs only a few cells for death of a large tumour area. Gaining knowledge of antigenic markers on the surface of tumorous endothelial cells can enable choice of suitable ligands. As a result tumour vessels relapse and apoptosis of the endothelial cell is encountered (Peppas & Blanchette 2004; Arspe 2005). Combretastatins (CA4P), a class of anti-cancer drug, have been found to alter the shape of endothelial cells consequent to membrane blebbing causing necrosis of tumour vessels (Tozer et al 2001). Angiogenic inhibitor o-(chloracetyl-carbamoyl) fumagillol (TNP-470) conjugated to HPMA promoted regression of Lewis lung carcinoma by effectively inhibiting endothelial cell production and proliferation (Folkman 2002; Fainaro et al 2004).

This approach extends to delivery of agents that prevent synthesis and release of inhibitors for VEGF, FGF and MMP. Targeting VEGF inhibits supply of nutrients and oxygen to tumour cells and prevents further growth. Down-regulating tumour receptors hinders tumour growth and promotes pericyte destruction. This prevents significant side effects as compared with other strategies. However, it is a difficult concept due to variation in temporal and spatial expression of various growth factors (Ribbati et al 2001; Fainaro et al 2004).

Dendrimer-based controlled/targeted anti-tumour delivery and diagnosis

In just a couple of decades dendrimers have attained recognition among the few leading systems utilized as nanoscale units or carriers of active moieties such as gene, oligonucleotides (ODNs), DNA and bioactives for wide-ranging applications.

Dendrimers are highly branched polymeric nano-carriers synthesized in a reiterative fashion. They are the most recent uni-molecular, polymeric, non-immunogenic system that has attracted the scientific community worldwide for tumour specific delivery. These nanoparticulate carrier systems are globular

in shape, monodispersive in nature, and have unique organization and highly-controlled architecture. The synthesis can be optimized to control their size, properties, composition and reactivity. However, a number of repetitive synthesis steps poses the greatest challenge in large-scale production of dendrimers (Holister et al 2003; Majoros et al 2004).

Dendrimers consist of an initiator core, interior generations composed of repeating units attached to the initiator core, and terminal functionality attached to the outermost generation (Jain & Khopade 2001). Based on the cores, repeating units and surface functionalities, a number of dendrimers have been reported: poly propylene imine (PPI) dendrimer; poly amidoamine (PAMAM) dendrimer; poly-L-lysine (PLL) dendrimer; melamine dendrimer; and citric acid dendrimer etc.

Characterization of dendrimers, in-depth analysis and imperfections can be assessed by: infrared spectrometry (IR), nuclear magnetic resonance (NMR), matrix-associated laser desorption ionization-time of flight (MALDI-TOF), electron spray ionization (ESI), vapour phase osmometry (VPO), laser light scattering (LLS) and sodium dodecyl sulfate-poly acrylamide gel electrophoresis (SDS-PAGE) etc. (Erichmann et al 2000; Jain & Khopade 2001; Shi et al 2005). Dendrimers could prove to be efficient archers in the battle against cancer. The terminal groups can be fabricated in such a way so as to provide specific charge and affinity to bind the drug and release it at the desired pH or on encountering a specific enzyme or microenvironment. The exo-groups can be embedded to provide attachment to a large number of drug moieties, ligands, and mAbs for specific delivery. Also, dendrimers can be tailored in such a way that few branches uphold the drug and remaining ones can be engineered with targeting moiety or ligands (Dian 2002; Kannan et al 2004; Khandare et al 2005) (Figure 7). The presence of multiple groups provides them with the inherent capability of targeting the drugs to tumour sites, to deliver therapeutic payload of drug as well as a marker for diagnostic imaging of the tumour regression (Kolhe et al 2006). Shukla et al (2006) synthesized AlexaFluor (AF)-tagged G5 PAMAM dendrimers conjugated to anti-HER2 monoclonal antibody (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 (Shukla et al 2006). Similarly, Thomas et al (2004) tailored G5 PAMAM dendrimers with 60 bca and J591 antibodies that bound to CD14 and prostate specific membrane antigen (PSMA), respectively, and labelled with fluorescein isothiocyanate (G5-FI-60B, G5-FI-PA). Flow cytometric and confocal studies on HL60 (from human myeloblastic leukaemia) and LNCaP (from prostate cancer) cell lines that express CD-14 and PSMA antigen showed the receptor specificity as the conjugates bound to specific antigen expressing cells and the control G5-FI lacked specific affinity for any of the cell lines (Thomas et al 2004). With the rise in subsequent generations, an exponential rise in density of functional groups provides increased sites of attachment (poly-valency) (Horan et al 1999; Liang et al 2000). As the size increases with the generation there is also an increase in encapsulation efficiency. Significantly PAMAM dendrimers have a size range from 2.3 nm in G-2 to 5.3 nm in G-5 (Zeng & Zimmerman 1997). In this regard dendrimers can prove to be as important carriers for the delivery of anti-cancer drugs. Various polymers and other hyperbranched structures have randomly distributed functional groups, polydispersive nature, no characteristic shape, are coiled in on themselves and lack uniform molecular weight distribution. Various carrier systems such as micro-emulsions tend to be unstable, whereas dendrimers are highly stable carriers and can be stored for longer periods. Also, the exceptionally high drug loading capacity of dendrimers adds to their properties and ultimately provides a greater accumulation of drug at tumour site (Asthana et al 2005). Certain large sized liposomes and microspheres are unable to enter tumorous vasculature and the presence of liposomes at the tumour site does not ensure the delivery of the drug, and the threat of bypassing the site cannot be ruled out (Mastrobattista et al 1999).

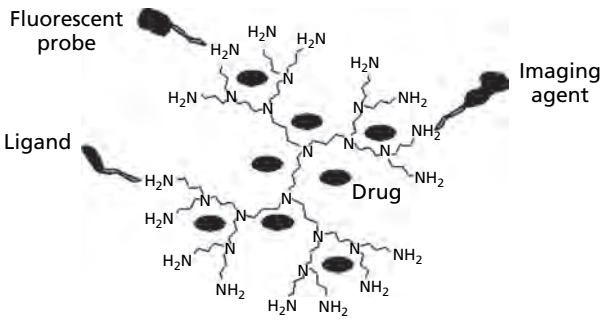

Figure 7 Dendrimers have precisely placed functional groups that can be simultaneously conjugated with fluorescent probe, imaging agent and ligand.

One advantage of macromolecular drug delivery vehicles is derived from the nature of the tumour tissue vasculature (Maeda et al 1992; Ambade et al 2005). The nanometric size of dendrimers is beneficial for their entry into hyper-permeable vasculature, while their high molecular weight and lymphatic dysfunctioning causes their localization as well as preventing escape via the EPR effect (Klajnert & Bryszewska 2000; Gillies & Frechet 2005). The unique core shell design of dendrimers helps in the incorporation of both hydrophilic and hydrophobic moieties and the presence of hydrophilic terminal surface groups prevents their opsonization or their recognition by macrophages and elimination by the RES (Sevenson & Tomalia 2005). Their surfaces can be elegantly modified so that the drugs can be physically entrapped, encapsulated, or conjugated by covalent bonds, hydrogen bonds, or ionic interactions (Dian 2002; Kannan et al 2004) (Figure 8).

Dendrimers cross the highly permeable tumour membrane (the cell wall of those cells that are tumorous in nature) by endocytosis, paracellular transport and extravasation. Binding the dendrimers with other high molecular weight moieties might increase their circulation time as these units bypass the glomerular filtration limit, thereby decreasing renal elimination. With an increase in dendrimer generation there is an increase in molecular weight of dendrimers, thus its hydrodynamic volume increases causing slower elimination and longer circulation time (Chen et al 2000; Piehler et al 2000; Lin et al 2004). Use of dendrimers in tumours can be as

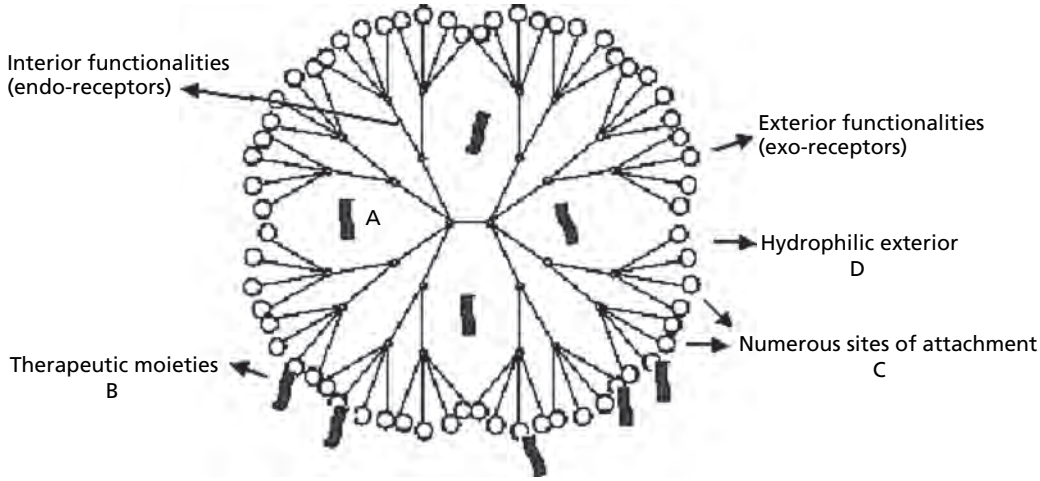

Figure 8 Dendrimers; (A) physical entrapment of drugs, (B) conjugation of drugs, (C) poly-valency and (D) resist RES uptake.

potential non-viral vectors for gene therapy, taking advantage of controlled immunogenicity and transfection capability of dendrimers (Mislick et al 2000). Cationic dendrimers can be used to deliver DNA into cells effectively. The encapsulated DNA is also prevented from being recognized by enzyme systems that degrade it. The interaction of amine groups of PAMAM dendrimers with phosphate groups of DNA elicits their transcription property. Dendrimers aid in crossing the biological barriers, which helps to increase transfection efficiency (Cloninger 2002; Aulenta et al 2003).

It is also important to note that dendrimers bear the capability of solubilizing some insoluble anti-cancer drugs such as paclitaxel (Ooya et al 2003), 5-fluorouracil and methotrexate. The increased solubility leads to increased loading, and by tailoring the surface of dendrimers targeting can be achieved (Chauhan et al 2003; Gupta et al 2006a) (e.g. methotrexate, doxorubicin HCl). This helps to prevent side effects and increase the therapeutic effect of the drug (Kojima et al 2000; Gupta et al 2006b). Melamine-based dendrimers modified on their periphery were loaded with methotrexate and 6-mercaptopurine. When administered to C3H mice intraperitoneally, the drug–dendrimer formulation was found to exert reduced hepatotoxic action as compared with free drug. Alanine transaminase (ALT) level was used to probe liver damage. Forty-eight hours after dosing it was found that the ALT levels of drug-loaded dendrimers were 27% (methotrexate) and 36% (6-mercaptopurine) lower than those of animals treated with the drug alone (Neerman et al 2004).

Cancer therapy necessitates targeting to a specific oncogenic cellular mass called a tumour. Different strategies have been reported earlier to target tumours such as pH-based drug release, ligand attached to dendrimer periphery etc. The use of dendritic polymers as drug delivery vectors and as pharmaceutical actives has received increasing interest (McCarthy et al 2005). Potentially numerous dendrimers can be designed to overcome pathological as well as physiological barriers. Dendrimers can be aimed for selective and effective localization of pharmacologically active therapeutics at pre-identified sites and restricting access to non-target sites. Dendrimers have been reported for their ability to transfect cells and can be designed as carriers for anti-cancer agents to tumour sites. Dendrimers have also been known to sustain drug release (e.g. methotrexate, doxorubicin HCl) and therefore increase the therapeutic effect of the drug (Kojima et al 2000; Quintana et al 2002; Kukowska Latallo et al 2005).

Folate conjugated dendrimers

Conjugating drug-loaded dendrimer to a specific targeting ligand can be an effective method to deliver drug at the desired site. Ligands possess some functional moieties that are recognized by cell surface receptors that bear affinity to bind them. The ligand is then taken internally to the site of action to release the therapeutic moiety attached to it. Thus, these ligands can serve as potent stimulators or inhibitors of a pharmacological, pathological response. Folic acid (FA) is a vital nutrient for the growth of cancer cells and can be used as a ligand due to high binding affinity and non-immunogenicity. Cell surface receptors of folic acid are over-expressed on many types of cancer cells. Thus folate-conjugated dendrimers can be effective anti-cancer agents having high affinity for these cancer cells (Quintana et al 2002). Methotrexate, a structural analogue of folic acid, can be incorporated into dendrimers to be effectively delivered to the cancer tissues with reduced toxicity. To gain access to the tumour surface, via the circulatory system, the drug needs to be hydrophilic, and to penetrate the cell membrane the lipid solubility must be high. The simultaneous occurrence of both properties in the drug is difficult. Folate-conjugated dendrimers enter the cell by RME, bypassing cellular barriers, allowing hydrophilic drugs to enter cancer cells of tumour xenografts (Leamon & Low 1994; Lee et al 1996; Caliceti et al 2003). Folate conjugates have been found to be quite stable even after endocytosis in tumours and remain functional for quite some time. Binding of folic acid to folate receptors (FR) is pH sensitive and once they reach acidic endocytic vesicles release the conjugated moiety, and the folate receptors move back to the tumour cell surface. Conjugation of dendrimers to folic acid has been widely explored. Biodistribution studies show encapsulating methotrexate

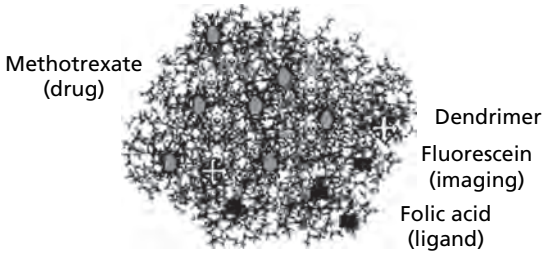

Figure 9 Dendrimer branching structure drugs and other moieties attached.

into folate-conjugated dendrimers has better tumour targeting potential with minimum side effects as compared with free drug, regressing tumours to a greater degree; similar results were obtained with doxorubicin (Neerman 2006; Reddy & Low 1998) (Figure 9).

Glyco-dendrimers

The unusual course of glycosylation on the cancer cell surface over-expresses specific antigens that can serve as potential targets for immune recognition through lectin-like receptors expressed on the surface of immune cells, that include natural killer (NK) cells, $CD8^+$ and $CD4^+$ lymphocytes (Bezouska et al 1994; Pospisil et al 1995). The glyco-dendrimers find use as antigens, raising mAbs against tumour tissues selectively. The dendrimer formulation can be processed in three ways: fully carbohydrate-coated dendrimers; dendrimers with carbohydrate moieties at periphery; and carbohydrate-centred dendrimers (Roy 1996). Surface modifications of PPI, PAMAM, and PLL dendrimers to glyco-dendrimers have shown their tumour-targeting potential (Lindhorst & Kieburg 1995; Glabus et al l996). These carriers closely resemble the natural carbohydrate ligands. The carbohydrate-coated system adds to selectively localizing the drug at the desired site. The macromolecular system provides longer circulation and precise delivery and seems to surpass various ligand-binding systems. Weak interaction chemistry between carbohydrate and receptor proteins needs a large amount of carbohydrate molecules to attain significant binding and elicit the desired effect. Glyco-dendrimers provide enhanced interaction between endogenous proteins and carbohydrates. The glyco-dendrimers can elicit twofold actions: they can themselves act as therapeutic agent; and act as vehicles for an active moiety. With inherent modifications and fine-tuning they can be used in gene therapy (Davis & Robinson 2002; Wang et al 2002). They can be modified to interact with asialoglycoprotein receptors present on hepatocarcinoma cell surface. T-cell antigen GalNAc has been attached to glyco-dendrimers and has been proved to be directed to tumours. RME provides intracellular trafficking of these dendrimers and they have been found to be highly effective against colorectal carcinomas, melanomas and breast tumours (Vannucci et al 1994; Roy & Baek 2002).

PEGylated dendrimers

PEG has a polyether skeleton, which is non-toxic and bypasses non-specific recognition from macrophages, protein adsorption and the MPS system, as a result of increased stearic hindrance, shielding positive charge and increased hydrophillicity (Gref et al 1995). PEGylation of drugs also aids the sustained and controlled delivery properties, helping delivery of potent drugs of low half-life. PEG-coated dendrimers can also be used to deliver proteins, as in the case of leukaemia, increasing their plasma half-life, stability, reducing the frequency of dosing, potentially reducing hypersensitivity reactions, and increasing tumour selective uptake (Yang et al 2004). Also, these provide scope as biocompatible dendrimers, reducing cytotoxicity and immunogenicity, a common drawback in PAMAM and PPI dendrimers. These systems alter pharmacokinetics of the drug increasing blood circulation half-life, sustaining delivery and reducing burst release. Increase in molecular weight provides EPR effect once the system enters the tumour via extravasation (Davis 2000; Modi et al 2004). The dendrimers have enhanced loading capability and greater disposition of drug at the tumour site. Kojima et al (2000) reported the effect of varying chain length of PEG on solubility. MPEG 550 G-3, MPEG 2000 G-3, MPEG 550 G-4 and MPEG 2000 G-4 PAMAM dendrimers encapsulating methotrexate and doxorubicin were evaluated. An increase in encapsulation efficiency with increase in chain length and dendrimer generation was predicted (Figure 10). Similarly, Yang et al (2004) coupled PEG with molecular weights 750, 2000 and 5000 to Starburst^{TM} G3.0 PAMAM dendrimers to solubilize the hydrophobic compound, pyrene. They demonstrated that micelles tended to dissolve more pyrene as compared with free pyrene in water.

Peptide dendrimers

These consist of amino acids acting as a core and the terminal functionalities can be either of amino-acid group or a peptide moiety, and possess highly controlled architecture, size and composition. Peptides have shown affinity to tumour cells, inhibiting endothelial cells and triggering their apoptosis. Doxorubicin HCl conjugated to peptides showed increased tumour

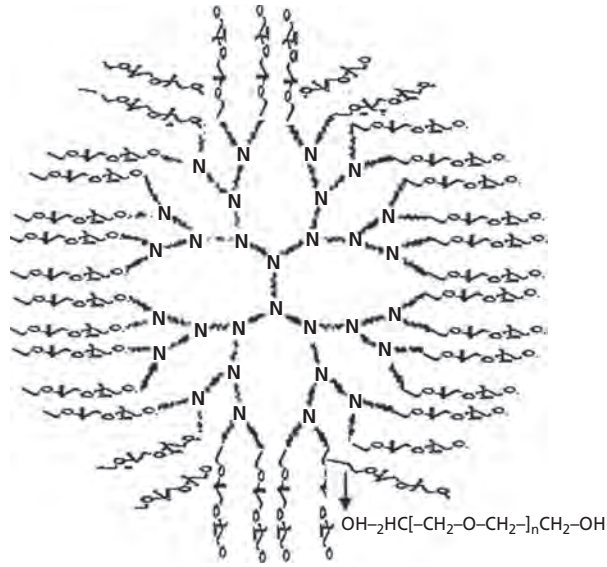

Figure 10 PEGylated dendrimer.

regression. Peptide dendrimers are a novel development in polymer science; these can mimic as proteinomimetics and find use in biomedical application. L-Lysine dendrimers can be used as molecular inhibitors for various angiogenic factors and can work as multiple antigen peptides to stimulate immune response generating antibodies against tumours (Bhadra et al 2005; Gillies & Frechet 2005). Peptide dendrimers can alter cell–cell interactions and cellular adhesion and they can be fashioned at their branching points (multivalency) as gene delivery carriers to enhance transfection capability. The peptide dendrimers have affinity for integrin receptors, which is a protein controlling cell–cell interaction in cancer cells. The dendrimer can prove to be efficient in non-covalent binding of DNA with site-specific cleavage. Non-covalent delivery can prevent metabolism mediated via enzyme nuclease, when compared with DNA delivered by other means, and helps crossing biological membranes more efficiently. Thus it can be a promising approach in DNA nanotech providing greater specificity and selectivity. Melamine dendrimers are potentially a newer class of peptide polymers that can be meticulously fabricated and explored for anti-tumour properties (Saddler & Tam 2002; Bhadra et al 2004; Fairman & Akerfeldt 2005) (Figure 11).

pH-sensitive dendrimers

The dendrimers are large enough to enter normal vessels but are of optimal size to enter the more porous tumour. The dendrimers can be formulated to make them pH sensitive. The change in hydrodynamic radii and conformation with pH can be an important concept to sustain and control drug release. Various dendrimers are sensitive to pH. At normal physiological pH (i.e. pH 7.4) the terminal amine groups in PPI dendrimers are not protonated and the branches converge to the central core. However, once engrossing the tumour site and entering the tumour vessels the dendrimers are subjected to lower pH; the groups at the exterior are protonated and repel each other, thus undergoing a change in 3-D conformation, moving branches outward and depositing the drug at the tumour site. This prevents the release of high potency drug at non-specific sites, preventing toxic effects and unwanted allergies (Patri et al 2002a; Gillies & Frechet 2004; Sevenson & Tomalia 2005).

pH-sensitive dendrimers can be fabricated by tailoring functional groups at the surface of dendrimers. This can be done by incorporating specific groups such as amine (NH_2) group, as in the case of PAMAM and PPI dendrimers, at the surface. The group protonates and becomes charged at lower pH causing structural changes in the nanostructure and releasing the drug (Zeng & Zimmerman 1997). Sideratou et al (2000) studied the pH-dependent release of pyrene from quaternized PPI dendrimers. Dendrimers having terminal –COOH moieties are less cytotoxic and hence cause side effects to a lesser degree. Acidic moieties such as the carboxylic acid (–COOH) group in citric acid dendrimers aggregate at lower pH and are charged only at basic pH (Namazi & Adeli 2005). The pH of physiological body fluids is 7.4, which can act as a buffer and maintain this pH. However, the intracellular pH at the tumour site is acidic, thus the dendrimers can be so designed to release the drug specifically on encountering the desired pH (Vasir et al 2005). Ester-terminated dendrimers are non-toxic, biocompatible and have shown tumour-selective affinity. Peptides such as L-lysine are also pH sensitive and charged at lower pH. Thus peptide-based dendrimers have potential for pH-sensitive delivery. Tailoring the surface with acetic-anhydride prevents non-specific interaction and can be attached to ligand to direct dendrimers to neoplastic cells of prostate. Also a linkage or spacer can be attached between dendrimer and drug that is pH sensitive. Thus hydrazone linkage and aconityl linkage can be used that are acid labile. The amide and ester bonds can be developed that undergo hydrolysis at lower pH (vanHest et al 1995).

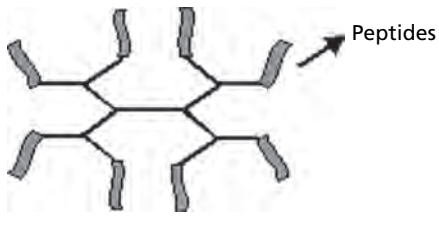

Figure 11 Peptide dendrimers.

Boron neutron capture therapy (BNCT)

Dendrimers are one of the most attractive polymers that have been used as boron carriers due to their well-defined structure and large number of reactive terminal groups, and can bind up to 1000 boron atoms per molecule of dendrimer (Wu et al 2006). Although the mAbs can be used as carriers of boronated compounds, lower loading capacity has been a major limitation. As a matter of fact, a large number of ^{10}B atoms (10^9) must be delivered per tumour cell (Alam et al 1989) and hence polyhedral cage-like structures of borane anions are needed to be linked to mAbs. However, the attempt reduces the solubility of the formulation, which ultimately leads to its precipitation or could result in decreased/diminished immunogenicity of the compound (Sneath 1976). However, mAb can be conjugated to a boronated dendrimer. This would promote site-specific delivery and prevent random distribution (Wu et al 2006). Backer et al (2005) reacted 5.0 G PAMAM dendrimers with decaborate molecules to produce a macromolecule with 1050–1100 boron atoms per dendrimer. This was conjugated to thiol groups of VEGF (a 121-amino-acid isoform of human VEGF with NH_2-terminal Hu-tag (a 1–15-amino-acid fragment of human RNase I)) at a 4:1 molar ratio using the hetero-bifunctional cross-linker sulfo-LC-SPDP. The dendrimeric formulation was tagged with a near-IR Cy5 dye to allow fluorescent imaging of the bioconjugate (VEGF-BD/Cy5) in-vitro and in-vivo. Internalization of VEGF-BD/Cy5 by PAE cells expressing 2.5×10^5 VEGFR-2 per cell was inhibited by excess VEGF, indicating a VEGFR-2-mediated mechanism of uptake. Fluorescent imaging of 4T1 mouse breast carcinoma revealed selective accumulation of VEGF-BD/Cy5 particularly at the periphery of tumour sites where angiogenesis was most active. Accumulation of VEGF-BD/Cy5 in 4T1 breast carcinoma was diminished in mice pretreated with a toxin–VEGF fusion protein that selectively killed VEGFR-2-overexpressing endothelial cells. Further the authors also predicted that VEGF-BD/Cy5 and its more advanced boron containing bioconjugates would be able

to kill endothelial cells in the tumour vasculature and promote occlusion and necrosis of the tumour mass.

Similarly, 4.0G PAMAM dendrimer was reacted with the isocyanato polyhedral borane $Na(CH_3)_3NB_{10}H_8NCO$. EGF was derivatized with m-maleimidobenzoyl-*N*-hydroxysulfosuccinimide ester (sMBS). The reaction of boronated dendrimer with maleimide groups produced stable bioconjugates, which contained ~ 960 atoms of boron per molecule of EGF. The BSD–EGF initially was bound to the cell surface membrane and then was endocytosed, which resulted in accumulation of boron in lysosomes and showed high potential as a boron delivery agent for BNCT against tumour mass (Capala et al 1996).

Gene therapy

Genetic therapies required for the treatment of tumours and metastasic sites still remain a challenge despite the development of various viral and synthetic vector systems. However, dendrimers can prove to be a useful tool in this area and help in the elimination of necrotic mass. Boyd et al (2006) formulated 3.0 G and 4.0 G, cationic PLL based ^{3}H-dendrimers (BHALys $[Lys]_4$ $[^3H\text{-}Lys]_8$ $[NH_2]_{16}$ and BHALys $[Lys]_8$ $[^3H\text{-}Lys]_{16}$ $[NH_2]_{32}$) with terminal amine groups. They studied the effect of dendrimer size (molecular weight or generation), surface charge, and surface functionality on the pharmacokinetics and biodistribution of PLL-based dendrimers after intravenous administration. They proved the utility of dendritic systems as drugs or drug delivery systems. Dufes et al (2005) incorporated tumour necrosis factor-α (TNF-α) in PPI dendrimers, which was found to be effective in the treatment of A431 epidermoid carcinoma, C33a cervix carcinoma, and LS174T colorectal adenocarcinoma. Dendrimers by themselves have been shown to exhibit plasmid-independent antitumour activity, ranging from pronounced growth retardation to complete tumour regression. Dendrimers incorporating transcriptionally targeted TNF-α were found to be more efficacious than treatment alone of either dendrimer or TNF-α.

Dendrimers also elicit an important property, termed 'proton-sponge effect'. Cationic dendrimers have high buffering capacity due to the presence of protonable amine groups. Thus dendrimers can act as a weak base and exhibit resistance to acidification by endosomes. The decrease in pH may result in rapid osmotic swelling and increased osmotic pressure leading to endosomal rupture that facilitates translocation of DNA into the nucleus without any degradation (Behr 1997; Godbey et al 1999). Lai et al (2005) demonstrated that intracellular trafficking of G4 PAMAM dendrimers conjugated to FITC in various cell organelles was mediated by the proton-sponge effect. The combination of synthetic transfection agent and targeted anti-tumour gene thus can be a highly promising approach for the systemic treatment of experimental solid tumours.

Magnetic resonance imaging (MRI)

MRI is an important tool for visualization of tumours and helpful in improving breast cancer diagnosis. These agents can be useful to detect small tumours, providing much needed pathological information because of the high resolution with very high sensitivity (Aref et al 2001). Ability to simultaneously attach a targeting ligand, contrast agents and carry a high payload of anti-cancer drugs in the same dendritic molecule provides a platform for multifunctional nano-scale drug delivery devices that can serve as minimally-invasive diagnostic agents (Hudde et al 1999). PAMAM dendrimers (3.0, 4.0, 5.0 and 6.0 G) were conjugated with a bifunctional diethylenetriaminepentaacetic acid derivative to produce four novel macromolecular magnetic resonance imaging (MRI) contrast agents. Size-dependent changes in the pharmacokinetic properties were observed in biodistribution studies. ^{153}Gd-labelled 6.0 G PAMAM-conjugates remained in the blood significantly longer than all of the other preparations. High quality and detailed 3D-micro MR images and angiography of mice were obtained using PAMAM-$(1B4M\text{-}Gd)_{192}$ as a macro-molecular MRI contrast agent. Numerous fine vessels were visualized on subtracted 3D-MR angiograms with G6D-$(1B4M\text{-}Gd)_{192}$. The results showed that dendrimer could be effective to perform 3D-micro-MR angiography of mice so as to estimate the microvasculature of cancerous tissue for anti-angiogenesis therapy (Kobayashi et al 2001). Sato et al (2001) conjugated PAMAM dendrimers (3.0, 4.0, 5.0 and 6.0 G) to chelated gadolinium (Gd). The results showed that as the molecular size increased, the excretion of the ^{153}Gd-dendrimer conjugates was retarded. In conclusion, Gd-dendrimers could be retained in the fine vessels with high quality and detail, and could be adequate for visualizing small tumour vasculature. Kobayashi & Brechbiel (2004) formulated PPI dendrimer-gadolinium-based MRI contrast agents. Contrast agents were found to have a spherical shape and the molecular size altered the route of excretion. Contrast agents of less than 60 kDa molecular weight were excreted through the kidney, resulting in these agents being potentially suitable as functional renal contrast agents. Larger hydrophilic agents were found to be useful for lymphatic imaging. Finally, contrast agents conjugated with either mAbs or with avidin were able to function as tumour-specific contrast agents. They might also be employed as therapeutic drugs for either gadolinium neutron capture therapy or in conjunction with radio-immunotherapy (Kobayashi & Brechbiel 2004).

Photodynamic therapy (PDT)

The concept of PDT involves administration of a drug that has high affinity for tumour cells, followed by exposing the tumour cells to the moderate intensity light from an adequate source, such as a laser. This causes destruction of the tumour cells, which is thought to be mediated by oxygen (Kaestner 2003). Nishiyama et al (2003) studied the role of dendrimers as novel carriers of photosensitizer molecules for photodynamic therapy (PDT). 3.0G aryl ether dendrimer porphyrins (DP) were complexed with 32 quaternary ammonium groups (32(+)DPZn) and 32 carboxylic groups (32(−)DPZn). Protoporphyrin IX (PIX), which is a hydrophobic and relatively low molecular weight photosensitizer, was used as a control in that study. Confocal studies indicated that PIX induced severe photodamage to disrupt membranes and cell organelles (plasma membrane, mitochondrion and lysosome). At the same time the cells treated with dendrimer porphyrins showed least photodamage, as shown by the characteristic fluorescent pattern of such organelles even after photoirradiation. However, notably 32(+)DPZn achieved remarkably higher (1)O(2)-induced cytotoxicity against Lewis lung

carcinoma cells than PIX. Further, both dendrimer porphyrins had far lower toxicity as compared with PIX, demonstrating their highly selective photosensitizing effect in combination with a reduced systemic toxicity. Zhang et al (2003) developed porphyrin core based dendrimers for PDT. They proved that this dendritic system was a suitable module against the tumour cells. The dendrimers were encapsulated within poly(ethylene glycol)-b-poly (aspartic acid) micelles to reduce the toxicity and were found to be quite stable under normal physiological states of pH 6.2–7.4.

Recent scientific outlook

Dendrimers are one of the most recent carrier systems that have influenced the scientific community. The decade has seen a remarkable development in the aspect of drug delivery, where the cascade molecule dendrimer has substantially revolutionized the era of novel drug delivery. Since the innovation of PAMAM dendrimers by Tomalia et al (2001), this drug delivery application has opened novel avenues toward cancer chemotherapy (Table 3).

In this context Kukowska Latallo et al (2005) extensively studied acetylated PAMAM dendrimers conjugated to folic acid and methotrexate on its surface. After administration to mice, the animals gained weight and tumour growth was delayed for 30 days as seen against the control group.

Surface tailored dendrimers have proved to be an efficient vehicle for controlled drug delivery. Kojima et al (2000) used M-PEG to PEGylate PAMAM dendrimers and the loaded drug, adriamycin, showed sustained release properties. Ooya et al (2003) used polygycerol dendrimers ((polyethylene glycol) methacrylate) loaded with paclitaxel, which also displayed sustained-release characteristics. Tripathi et al (2002) explored the oral delivery aspect of dendrimers by modifying the surface of dendrimers with palmitoyl chloride, encapsulating 5-fluorouracil. The system sustained the release and improved oral bioavailability compared with free drug. Similarly, Shukla et al (2003) used dendrimers for BNCT of tumours. A lower generation G-3 PAMAM dendrimer was conjugated to boronated PEG with folic acid attached to the distal end. PEG of varying chain length was used. Biodistribution studies were performed on mice bearing folate receptor (+) murine 24 JK-FBP sarcoma and showed tumour selective uptake.

Dendrimer have been used successfully to provide temporal and spatial control over drug delivery. In this context Kannan et al (2004) studied unmodified PAMAM and hydroxyl terminated PAMAM complexed with ibuprofen in A549 lung carcinoma and found them to enter tumour cells faster than hyperbranched polyols. Roy & Baek (2002) provided evidence that glycodendrimers bearing immunodominant T antigen Gal NAc helped in receptor crosslinking and entry in tumours by receptor-mediated endocytosis. Padilla De Jesús et al (2002) synthesized PEO-dendrimer hybrid with doxorubicin covalently attached to it, and demonstrated pH-triggered release. Emanuele et al (2004) conjugated propranolol to lauroyl-PAMAM dendrimers, showing it to be delivered as a prodrug bypassing P-glycoprotein efflux via endocytosis-mediated epithelial transport.

Table 3 Various dendritic systems reported for tumour management

Dendrimer	Formulation	Cell lines
PAMAM	Anti-PSMA antibody conjugated to dendrimers (Kojima et al 2000).	LNCaP cells
	Modified to –OH terminal functionality and complexed with ibuprofen (Liang et al 2000)	A549 cells
	PAMAM – mPEG encapsulating methotraxate/doxorubicin (Olson et al 1997)	
	PAMAM-FITC-folic acid complex conjugated to methotraxate via amide/ester linkage (Peng et al 1993)	KB cells
	Acetylated PAMAM dendrimer conjugated to folic acid and methotrexate (Peppas & Blanchette 2004)	KB cells
	Surface modification of PAMAM dendrimers with palmitoyl chloride and loading 5-fluorouracil (Wu et al 2004)	
	G3 PAMAM dendrimer conjugate to Boronated PEG and folic acid (Wu et al 2006)	24 JK-FBP
	Conjugation of propranolol to lauroyl G3 PAMAM dendrimers (Yang et al 2004a)	CACo-2 cells
	Pegylation of PAMAM dendrimers encapsulated with 5-fluorouracil (Yang et al 2004b)	
	[^{111}In]Oligonucleotide DNA conjugated to G4 PAMAM dendrimer (Zeng & Zimmerman 1997)	
Glycodendrimer	Glycodendrimer bearing T antigen disaccharide ß-gal-(1-3)-α-GalNAc (Selby 1990)	
	G3.5 PAMAM dendrimer conjugated to glucosamine/glucosamine-6-sulphate (Zhang et al 2003)	
	Conjugation of PAMAM dendrimers with GlcNAc8 (Vannucci et al 2003)	B16F10
Polyglycerol	(Polyethylene glycol) methacrylate encapsulating paclitaxel (Namazi & Adeli 2005)	
Polyester	PEO-dendrimer hybrid with doxorubicin covalently attached by hydrazone linkage (Padilla de Jesús et al 2002)	

Quintana et al (2002) have explored PAMAM dendrimer as multifunctional nanoconstructs for delivery of anti-cancer agents, methotrexate and paclitaxel. While developing the dendritic units, the group also studied the various functional moieties such as carboxyl, hydroxyl and acetamide terminated dendrimers and their effect on the free availability of folic acid ligand. The molecular modelling studies provided the data that clearly indicated drastic difference of the mean diameter and the distance of the folic acid from the dendritic core. The results showed by flowcytometric studies on KB cells confirmed the greater access of acetamide capped dendrimers inclusion through folate mediation as compared with hydroxyl and carboxyl groups. All the folic acid moieties in the acetamide derivative appeared to extend away from the surface of the dendrimer. This further emphasized the effects of conformational changes leading to back folding of the folic acid ligands due to the repulsive forces from charged amines among the freely available amine terminals, and finally resulting in insignificance of the ligand attachment. Bhadra et al (2003) encapsulated 5-fluorouracil in PEGylated PAMAM dendrimers. The results showed reduced haemolytic toxicity compared with non-PEGylated systems. Also, the PEGylated system displayed high loading efficiency and sustained release of the drug. Shaunak et al (2004) formulated water-soluble conjugates of glucosamine/glucosamine-6-sulfate with G3.5 PAMAM dendrimer. The conjugates were shown to inhibit FGF mediated endothelial cell proliferation and angiogenesis in Matrigel and placental angiogenesis assays. Shaunak et al (2004) predicted that the dendritic system could also be fashioned for immuno-modulatory and anti-angiogenic activity. Vannucci et al (2003) tailored octavalent PAMAM dendrimers with N-acetyl-glucosamine residues (PAMAM-GlcNAc8). C57BL/6 mice were inoculated with B16F10 melanoma cells and PAMAM-GlcNAc8 was administered. A dose-dependent increase in tumour growth was observed and $CD4^{+}$ number increased simultaneously. The multivalent glycoconjugates were able to generate anti-tumour response via innate and acquired immunity.

Dendrimer coupled to various markers (mAbs, antigens, ODNs etc.) have been used in gene therapy. Patri et al (2002b) showed that dendrimers were capable of being directed to antigens and receptors on the tumour's surface. They conjugated antibody to PAMAM dendrimers and found it to target to prostate cells. Sato et al (2001b) conjugated antisense oligonucleotide-DNA (^{111}[In]oligo) with G4 PAMAM dendrimer. The complex enhanced tumour delivery by 24.4% of injected dose per gram of tissue (ID g^{-1}) at 24 h compared with ^{111}[In]oligo without carrier (0.8% ID g^{-1}). Thus the carrier proved to be an efficient agent for tumour specific delivery and has potential as a tracer for imaging and gene therapy.

The preferred routes for the delivery of dendritic delivery systems have been the intravenous (Barth et al 1994, 2004; Malik et al 1999; Bhadra et al 2003; Padilla De Jesús et al 2002; Yang et al 2004) or the intraperitoneal (Barth et al 1994) routes. Recent studies suggested their utilization as transdermal (Modi et al 2004), oral (Wagner et al 1991; Wang et al 2002), and lastly as an ophthalmic (Vandamme & Brobeck 2005) delivery vehicles which can be exploited for delivery of anti-cancer drugs (Table 4).

Table 4 Various routes for administration of dendrimers

Dendritic system	Routes of delivery
PAMAM-4.0 G /4.5G (Neerman 2006)	Transdermal
PAMAM Wu et al 2004; Yang et al 2004a)	Oral
PAMAM-PEG (Yang et al 2004b)	Intravenous
Polyester (Pudilla de Jesús et al 2002)	Intravenous
PAMAM-3.5 G (Pt) (Malik et al 1994)	Intravenous
Boronated dendrimers (Barth et al 1994)	Interperitoneal, intravenous
PAMAM (Barth et al 2004; Yang et al 2004)	Intravenous
PAMAM (Vandamme et al 2005)	Ophthalmic

Conclusion

The treatment of cancer in the majority of medical applications involves either a higher dose of anti-cancer agent or via normalization of the vasculature to enhance access to these sites. Both approaches lead to untoward side effects on the normal organization of the body due to narrow therapeutic index as well as interception of adverse effects at these doses. Thus, it was envisaged that this article would review preferred approaches by various scientific groups world-wide, via exploitation of abnormal vasculature at the tumour sites, making them sensitive targets for therapy with these highly toxic bioactives. Also it would be essential to evaluate various factors before development of any novel carrier that participates in the unique story of tumour vasculature development, its porosity, blood perfusion and permeability.

It is observed that if nano-carriers are hydrophilic in nature they prevent RES uptake, prolonging their systemic circulation time. Also as blood perfusion is enhanced in tumours, hydrophilic carriers provide greater chances of access and retention in these sites. On the other hand cationic carriers bearing net positive charge interact with cell membrane to a greater extent. They are more prone to be surrounded by albumins and proteins, which are anionic and are recognized by macrophages and hence cleared to a greater extent than neutral and negatively-charged systems.

Macromolecular carriers reported previously showed some deficiencies relating to delivery. Dendrimers provide the opportunity to explore the utilization of size and molecular weight-based delivery systems. Dendrimers are hydrophilic moieties that can be fabricated for tumour targeting and upon entry in tumours undergo conformational changes to deliver the therapeutic payload. PAMAM, PPI, PLL and melamine dendrimers have been explored and are still being investigated, as the results have been positive relating to tumour specific delivery. PAMAM dendrimers are the ones that have been most widely explored as carriers for tumour drug delivery. PPI and PLL dendrimers have also been tried for gene therapy of tumours and have proved to be very successful.

In conclusion, the potential of dendritic carriers for the treatment of tumours of various origins and anatomical sites needs vigorous research attempts to arrive at unambiguous generalization. The issues relating to safety and toxicity, as well as efficacy, need to be addressed simultaneously.

Future prospects

The future of dendritic drug delivery lies in fine tuning of the carrier to incorporate the variety of anti-cancer agents, proteins and ligands to ensure the vectorization of the dendrimer-complex/conjugate to a tumour mass. The major emphasis will be on biomimetism, with enhanced plasma stability and capability to target the neoplastic mass of tissues. A detailed study of these nanostructures would help in the evolution of newer concepts that could be intimately involved in sustaining the life process.

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