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Review

Ligand based dendritic systems for tumor targeting

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Abstract

Medications that can selectively target tumors at the same time avoid access of the drug to nontarget areas, employ utilization of homing devices termed as ligands, that can bind to specific epitopes expressed on the surface of the necrotic mass of cells. Molecular signatures for transferrin, Epidermal Growth Factor, Sialic Lewis and folic acid are expressed on the surface of these cells. Dendrimers are nanosized, non-immunogenic, and hyper-branched vehicles that can be efficiently tailored for spatial distribution of bioactives, thereby reducing untoward cytotoxicity on normal cells. These nanoparticulate drug delivery vehicles provide a unique platform that has precisely placed functional groups so that multiple copies of ligands can be attached to it and facilitate targeting to the tumor surface or neo-vascularizing vessels proliferating around these cells. The article reviews the scope of ligand based dendritic system as a prospective for delivery of anti-cancer drugs, via active targeting with interception of minimal side effects.

Keywords: Tumors; Site-specific delivery; Receptors; Ligands; Nanoparticulate carriers; Dendrimers

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1. Introduction

In the context of drug delivery for tumors, development of "safe" and "effective" dosage form is the basic necessity. Chemotherapy aims at complete elimination of tumor mass, at the same time one needs to be aware of the toxicity associated with anti-cancer agents. Thus, a great emphasis needs to be laid on developing strategies that specifically and preferentially target tumors, at the same time promise reduced access to non-target areas. Kinetics of drug delivery for tumors must be spatial in origin. Thus, the carrier systems need to be so designed that they bind to target cells as a result of their intrinsic affinity. An attractive approach may be use of "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 (de Wolf and Brett, 2000; Molema, 2005). Cyclic peptide having Asn-Gly-Arg (NGR)-motif (CNGRC-peptide) is an aminopeptidase N (CD13) ligand (Curnis et al., 2000) that promotes homing of NGR-drug conjugates. The anti-tumor activity of CNGRC-TNF (Tumor Necrosis Factor) complex has been studied in RMA-T lymphoma and B16F1 melanoma and the complex showed greater anti-tumor activity then TNF alone (Curnis et al., 2002). Unusual genetic setup, molecular diversity and vasculature of tumors leads to expression of various surface markers and proteins, and these can be utilized as receptors of the ligands (Zurita et al., 2003). Various drug delivery systems have been reported in which angiogenesis related molecules have been accounted as ligands. Chemical conjugates of VEGF have been developed and reported to block tumor induced angiogenesis. VEGF (Vascular Endothelial Growth Factor) is an important factor that promotes angiogenesis (neo-vascularization) in tumors and its receptors (VEGF-R) are over-expressed on rapidly sprouting tumor vessels. Upon binding of VEGF to its receptors, the complex (VEGF/VEGF-R) is internalized, promotes intracellular delivery and helps in normalization of tumor vasculature (Olson et al., 1997). Also a number of carriers like liposomes, microemulsions, microparticles, dendrimers have being explored as transport vehicles for the delivery of bioactive at these necrotic sites. Dendrimers as nanoconstructs have a highly controlled architectural design, along with unique and myriad interfacial properties. The branched topology of these carriers imbibes them with properties that make them unique when compared with other vectors. This scores these amphiphilic molecules a potential vehicle for the delivery of anti-cancer therapeutic moieties (Lee et al., 2005). The present article discusses, as to how, different ligand conjugated dendrimers offer functional performance advantages at the molecular level for chauffeuring the drug to receptors on the tumor surface where the bioactive is internalized.

The rapidly expanding necrotic mass of tumor needs increased supply of nutrients. Increased rate of transcription, proliferation of tumor cells and altered morphology of blood vessels interpenetrating the tumor mass, leads to expression of various receptors on the surface of these cells (Siemann, 2006). The receptors have two essential components i.e. extra-cellular ligand binding domain and an intracellular signal-transmitting domain. These receptors have specific binding sites so that, only the ligand of given orientation can be recognized and thus a specific ligand-carrier binds to specific receptor where the system is either internalized or it liberates the therapeutic moiety at the extra-cellular site and the biological response is elicited (Kozasa, 2007) (Fig. 1). The ligands should have affinity to selectively bind to biologically relevant target, and is the basic necessity when the matter concerns more specifically for the delivery of an anti-cancer drug. Various literatures have reviewed about the morphology of cancerous cells in all its existing forms i.e. leukemic cells, sarcomas, solid tumors. Thus, it becomes too essential to elucidate the various receptors present on these heterogenic mass, and that they can be very useful to provide the access of drugs, oligonucleotides (ON), peptides etc. into the tumor vasculature for its complete eradication with no undue side effects.

2. Receptors on the tumor surface

In order to replenish the needs of tumor cells, various receptors are over-expressed on their surface (Fig. 2). These receptors help tumor mass by providing them with the bioactives required for neo-vascularization. The nutrients and other molecules having affinity for their specific receptors move to the receptors, and once the molecules bind to them, the receptor–nutrient complex is internalized via receptor mediated endocytosis. Alternatively the delivery systems can be manipulated in such a manner that they mimic the nutrients, and hence gain access to these receptors

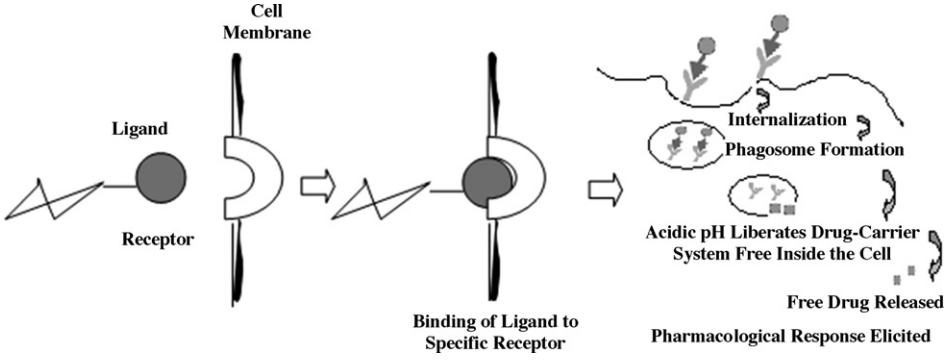

Fig. 1. Site-specific delivery of a carrier system can be achieved via, conjugation of a carrier to a ligand.

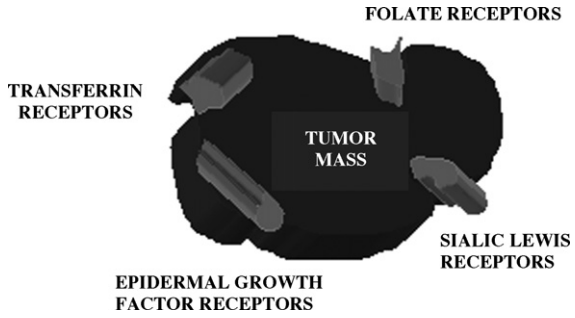

Fig. 2. Various receptors expressed on the tumor mass.

(Table 1). Various receptors on the tumor cells are summarized as follows.

2.1. Transferrin receptors (T_fR)

Transferrin is a β globulin (β_1-glycoprotein) and facilitates the transport of ferric ion (Fe^{3+}) through transferrin receptors on the plasma membrane. The intracellular delivery of Fe^{3+} is mediated via receptor mediated endocytosis and the transferring receptors move back on the surface to again bind to Fe^{3+} ions (Pun et al., 2004). These receptors have been shown to be over-expressed on rapidly growing and fast multiplying cells. The T_fR expression on tumors is about 10 folds higher in comparison to non-tumor cells. The average number of transferrin receptors is reported to be 10^5 per cell in RILQ Tlymphoma cells but less than 5×10^3 per cell in thymus and 5×10^4 per cell for spleen. The weight of the receptors per gram of tissue was found 550 μg for tumor cells and 330 μg for 4 thymus and spleen (Pun et al., 2004). In fact use of transferrin as a ligand has been explored as a suitable delivery system for site-specific delivery to tumors. Poly-L-lysine (PLL) covalently linked with transferrin for delivery of oligonucleotides (ONs) when exposed to human leukemic (HL-60) cells have been stated to promote apoptosis to a greater extent as compared to free ONs. Further it has been proved that the complex entered the cell by receptor mediated endocytosis (de Abrew, 1981). In another approach, PEGylated poly(cyanoacrylate) nanoparticles were conjugated to transferrin for delivery of paclitaxel (Ptx). PEGylation prevents aggregation of nanoparticles and transferrin effectively determines tumor site (Citro et al., 1992). PEGylation can be considered an important step as it prevents coalescence of nanoparticles, stabilizes them by providing a protective brush layer and by concealing the charge prevents nonspecific interaction (Pun et al., 2004).

2.2. Epidermal Growth Factor receptors (EGF-R)

EGF is an important factor that controls the disposition of neoplastic cells and potentiates transcription and proliferation of cells. EFG-receptors (EGF-R) are present across the cell membrane and can be sub-divided into three major parts: (i) extra-cellular part (rich in amino acid cystein), (ii) connecting segment (lipophillic in nature), and (iii) intracellular protein (tyrosine kinase) (Earp et al., 1995). Activation of tyrosine kinase promotes sequence of steps that promote cell growth. EGF-Rs expression on tumors is 100 folds greater than nontumor cells and hence, it provides a potential target for immunotherapeutic agent (Schwechheimer et al., 1995). Several monoclonal antibodies (mAbs) that target EGF-R have been known to inhibit proliferation of cell lines in culture, the most important being mAb-225 used to target lung tumor. However, immune stimuli was observed against the injected mAb in phase-I studies and thus subsequently a chimeric mAb-225 was prepared that illustrated no such reaction and has been used to target toxins such as Pseudomonas exotoxin-A efficiently (Fan and Mendelsohn, 1998). 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). PEG-stabilized liposomes were conjugated to trastuzumab using *N*-hydroxy succinimide (NHS)-PEG-Disteaoryl phosphatidyl ethanolamine (DSPE) and were loaded with water soluble boronated acridine and water soluble phenantridine. The formulation showed receptor specificity and was internalized in tumors to a greater extent in comparasion to unconjugated liposomes (Kullberg, 2003).

2.3. Lectin receptors (SL-R)

Anomalous composition of neoplastic cells because of atypical deposition of glucose and related monomers and abnormal glycosylation leads to expression of 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 and Robinson, 2002). Glycoconjugates used for targeting can be subdivided into: (i) one in which macromolecule is an active therapeutic moiety itself, and (ii) another in which the macromolecule aids in delivery of therapeutic moiety to the desired site (Davis, 1999). Protein–carbohydrate interaction in biological

Table 1
Various ligand coupled systems for tumor specific delivery

Carrier system	Receptors	Cell lines	References
Poly-L-lysine	Transferrin	HL-60	Citro et al. (1992)
Poly(cyanoacrylate) nanoparticles	Transferrin		Xu et al. (2005)
Trastuzumab	Epidermal Growth Factor	BT-474 and SKBR-3	Yakes et al. (2002)
Poly-L-lysine	Sialic Lewis	A-549	Stewart et al. (1996)
Dioleylphosphatidylethanolamine liposome	Folate		Reddy et al. (1999)

science is well known and it promotes cell–cell recognition and adhesion, but because lectins (carbohydrate binding proteins) have lower affinity to monomeric sugars, the linear molecules need higher glyco conjugation than globular and branched moieties (Lee and Lee, 1995). Stewart et al. conjugated phosphorothioated ONs to PLL containing fucose molecules, and observed increase in cellular uptake by 15 times. Further they also showed that the inhibition of Interstitial Cell Adhesion Molecule (ICAM-1) in A-549 lung carcinoma was dose dependent in case of fucosylated PLL–ON complex while no such inhibition was observed in non-fucosylated PLL–ON complex (Stewart et al., 1996).

2.4. Folate receptors (F-R)

Folic acid (FA) is a vitamin necessary for the synthesis of purines and pyrimidines, and is expressed on variety of tumors. Upon binding of the ligand the ligand–receptor complex is internalized via receptor mediated endocytosis (Kukowska-Latallo et al., 2005). However, direct conjugation of the drug to FA can result in decreased affinity for folate receptors or lead to alteration of therapeutic affinity of the drug (Cho et al., 1997). Due to increased demand of folic acid by tumor cells the cells begin to give rise to increased number of F-R so as to capture more of FA and hence can serve as prominent site for entry of methotrexate, an anti-cancer drug, which is an analogue of FA. Also by use of folic acid as a ligand one can maneuver the drug to gain access inside the tumor vasculature (Pobojewski, 2005). The receptors are expressed on basolateral surface (blood side) of tumor cells as compared to their expression on apical surface on normal side. This is an eminent property of these receptors, and the use of FA as targeting ligand bears high specificity when delivered via blood (Lu and Low, 2003). Ratnam et al. delivered folate conjugated liposomes for successful treatment of acute myelogenous leukemia and found that the system was capable of evading P-glycoprotein (Pgp) mediated efflux of drug (Ratnam et al., 2003). Reddy et al. developed folate conjugated transfection complex of DNA plasmid that is internalized by tumors. They prepared liposomal formulations supplemented with folate PEGDioleylphosphatidylethanolamine. *N*-Citraconyl-dioleylphosphatidylethanolamine was also added for pH-sensitive drug delivery. The targeting ligand improved transfection efficacy and targeting property as compared to formulation without any ligand (Reddy et al., 1999).

3. Nanoparticles mediated drug delivery to tumors

Nanoparticles having size range of 1–10 nm have capacity to diffuse with greater ease inside the tumor cells. This helps to overcome limitations relating to chemotherapy using free drug such as poor in-vivo in-vitro correlation and overcome other possible resistances offered by tumors (Sinek et al., 2004a). Poly(ε-caprolactone) nanoparticles (MW: 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). Micelles prepared from PEG/phosphatidyl ethanolamine (PEG-PE) having PEG of molecular weight ranging from 750 to 5000 Da upon i.v. administration provided selective accumulation in Lewis lung carcinoma and proved to be a potential delivery system for poorly soluble drugs (Lukyanov et al., 2002). Copolymer micelles having particle size range from 20 to 100 nm are small to bypass uptake by liver and spleen (Orive et al., 2005). Nasongkla et al. (2006) prepared multifunctional polymeric micelles having ability to target tumor cells. Doxorubicin and superparamagnetic iron oxide (SPIO) nanoparticles were loaded in the micelles. In-vitro MRI and cytotoxicity studies showed specific and selective cytotoxic response of these micelles. In-vivo studies of the pegylated liposomes having APRPG as a targeting moiety in mice bearing colon 26 NL-17 carcinoma, conjugated to distearoylphosphatidyl ethanolamine (DSPE) elucidated selective accumulation in the tumor mass (Maeda et al., 2004a). PEG-APRPG modified liposomes encapsulating Adriamycin enhanced suppression of the tumor mass to a greater extent than non-targeted liposomes (Maeda et al., 2004b).

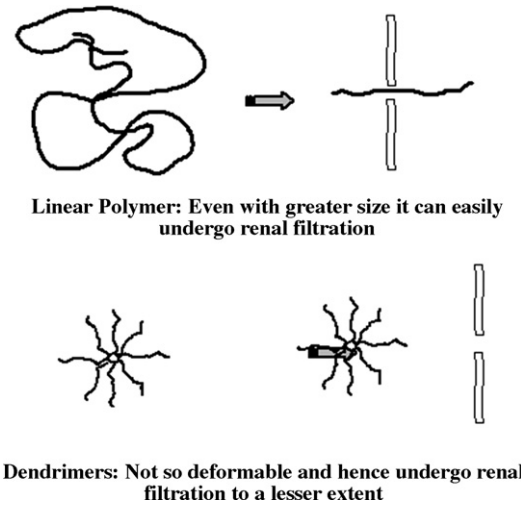

Fig. 3. Long circulating dendrimers can bypass renal elimination. Linear polymer: even with greater size it can easily undergo renal filtration. Dendrimers: not so deformable and hence undergo renal filtration to a lesser extent.

4. Dendrimers

In last half a decade dendrimers have emerged as one of the most promising nano-particulate carrier system that has greatly attracted the scientific community. Dendrimers are uni-molecular polymeric systems synthesized in a re-iterative manner. At the same time their synthesis can be so optimized as to control their size, shape, molecular mass, composition and reactivity. Dendrimers have hyper-branched structure with precisely placed functional groups that bear important role in controlling the properties of therapeutic moieties that are encapsulated or complexed with it. Most eminent properties of dendrimer are its monodispersive nature, globular shape, highly controlled architecture, which also makes them efficient carrier system for drugs. In contrast, various polymers and other hyper-branched structures have randomly distributed functional

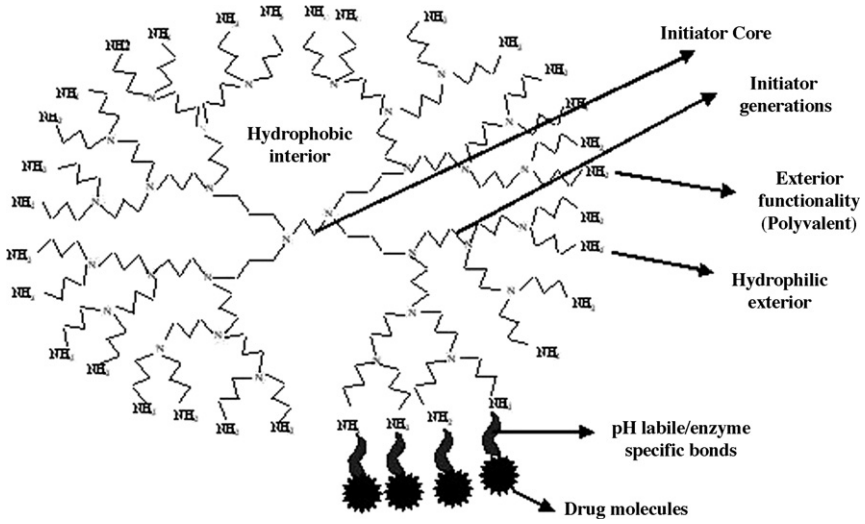

Fig. 4. Dendrimer: Unique architecture facilitates (a) encapsulation, (b) conjugation of drugs, (c) prevent their opsonization by MPS, and (d) provides numerous sites of attachment.

groups, polydispersive nature, no characteristic shape, coiled in them and lack uniform molecular weight distribution (Jain and Khopade, 2001; Asthana et al., 2005). Carrier systems like micro-emulsion tend to be unstable, whereas dendrimers are highly stable carriers and can be stored for longer periods (Asthana et al., 2005). However, the multi-component liposomal formulations are non-covalently associated systems are challenging to formulate and stabilize when compared to dendrimers with covalently associated drugs (Drummond et al., 1999).

Dendrimers consists of three characteristic scaffolds (i) multifunctional initiator core, (ii) inner generations, which consist of repeating branched units; and (iii) exterior surface groups, attached to the outermost generation (Tomalia et al., 2001). Sinek et al. have reported that nanoconstructs having size range from 1 to 10 nm are capable of diffusing directly into tumor cells (Sinek et al., 2004b). Significantly PAMAM dendrimers have size range of 2.3 nm in generation-2 (G-2) to 5.3 nm in G-5 (Svenson and Tomalia, 2005). In this regard dendrimers can prove to be an important carrier for the delivery of anti-cancer drugs.

The terminal functionalities of the dendrimers, which are involved in complexation of therapeutic moieties, are termed as exo-receptors and the groups present in the interior responsible for drug entrapment are termed endo-receptors. Dendrimers may contain same functional group at the terminal junctions and this can be an important feature for substrate binding to these functionalities. Multivalency provided by the dendrimer can play a dominant role for increased affinity of substrates to its complementary receptors, and is purely by co-operation or on statistical basis. This multiple binding mimics nature (e.g. protein–protein & protein–membrane binding) resulting in significantly enhanced activity. Thus, attaching multiple copies of ligand to dendritic surface promotes increased access to the target area where otherwise movement of the carrier system/ligand by simple diffusion is problem (Zeng and Zimmerman, 1997).

Nanoparticulate architecture of dendrimers (about ∼10 nm for 10.0G) favors its entry in the highly permeable tumor vasculature and its high molecular weight causes its localization and prevents its escape. The process termed as Enhanced Permeation and Retention (EPR) effect (Gillies and Frechet, 2005). How-

Table 2
Ligand coupled dendrimers for tumor specific delivery

Type of dendrimers	Ligand	Receptors	Fluorescent moiety	Cell lines	Reference
G5 PAMAM	Anti-HER2 mAb	HER2	AlexaFluor	MCA-207	Shukla et al. (2006)
G5 PAMAM	60 bca/J591 Ab	CD14/PSMA	Flourescein isothiocyanate	HL60/LNCaP	Thomas et al. (2004)
G6 PAMAM	17α-ethinyl-estradiol	Estrogen	Flourophore TMR	MCF-7	Harrington et al. (2006)
Ester terminated	Folic acid	Folate			Kono et al. (1999)
G5 PAMAM (neutral/hydroxyl/acetyl/carboxylate)	Folic acid	Folate		KB	Patri et al. (2005)
G5 PAMAM	Folic acid	Folate	Flourescein isothiocyanate	KB	Thomas et al. (2005)
G5 PAMAM	J591 anti-PSMA mAb		Flourescein isothiocyanate	LNCaP	Patri et al. (2002)
G5/G7 PAMAM	Folic acid	Folate	Flourescein	KB	Choi et al. (2004)
Glc-Nac coated PAMAM		Lectin		B16F10	Vannucci et al. (2003)

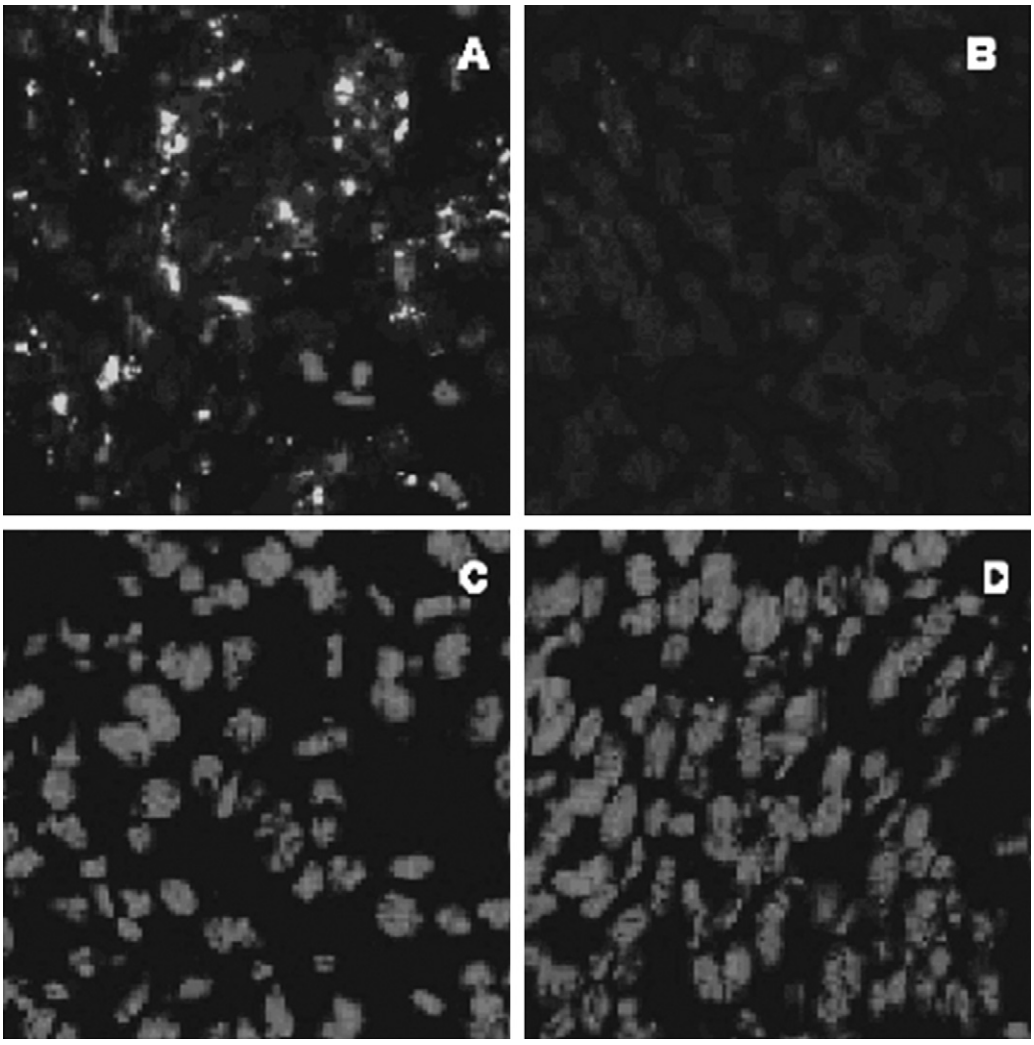

Fig. 5. Images of tumor sections. (A and B) HER2+ (A) and HER2− (B) tumors from an animal injected with G5-HN-AF. (C and D) HER2+ tumors from SCID mice injected with PBS (C) or G5-AF (D) (Shukla et al., 2006).

ever, for longer circulation, half-life is an important requirement. Dendrimers (MW > 40 kDa) were found to remain in blood for longer period of time when compared to those of polymers with lower molecular weight. Also, the more branched dendrimer's (3.0G) when conjugated to 8 chains of 5 kDa polyethylene oxide showed a significant higher residence time when compared to 2.0G dendrimer conjugated to 4 chains of 10 kDa polyethylene oxide (Gillies et al., 2005) (Fig. 3). The host-guest chemistry promotes the encapsulation of hydrophobic drugs at the same time also facilitates the attachment of hydrophilic moieties (Gupta et al., 2006; Tripathi et al., 2002). The hydrophilic exterior of these robust nanostructures prevents their recognition by mononuclear phagocytic system (MPS) and hence prevents their subsequent removal by opsonization. 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 micro-environment, the amine groups protonate, and they repel to undergo a conformational change, facilitating the release of drug (Svenson and Tomalia, 2005; Bhadra et al., 2003) (Fig. 4).

5. Receptor specific dendritic nanoconstructs

The exo-groups at the surface of dendrimers can be so designed that few of the branches are conjugated to the drug and remaining ones are tailored with targeting moieties or ligands. The attachment of ligands to dendrimer confirms its destination to the target site and subsequently prevents the delivery of drug to non-target areas. Thus, presence of multiple functional groups (polyvalency) aids to target the drug at its desired location (Khandare et al., 2005; Dian, 2002). In case of tumors, the use of ligands conjugated dendrimers can be a highly promising approach for delivery of anti-cancer drugs (Table 2). Shukla et al. (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

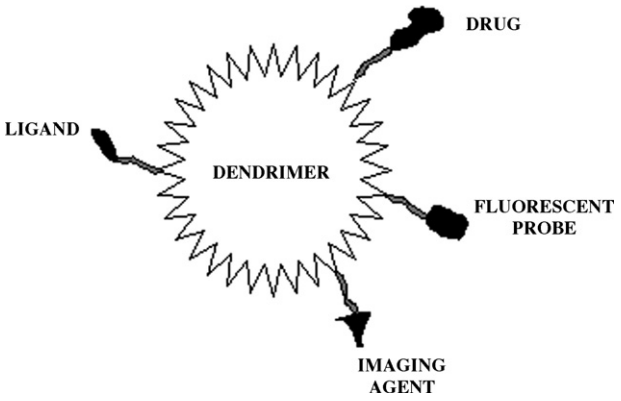

Fig. 6. Dendrimers as vehicles that can be simultaneously tailored with drug, fluorescent probe, imaging agent and ligand.

of conjugate by HER2 expressing cells while no such affinity was found for MCA-207 control cells that did not express HER2. The affinity of the conjugates for SKBR-3 cells (expressing HER2) was proved by confocal microscopy. Tumors were developed in SCID mice by subcutaneous infection of the control and HER2 expressing MCA-207 cells. In-vivo studies on HER2 expressing MCA-207 cells by confocal microscopy also depicted increased specificity of the conjugate for HER2 expressing cells. Fig. 5 clearly shows fluorescence of internalized conjugate in HER2(+) cells (A) and not in HER2(−) cells (B). Also no fluorescence is seen in HER2(+) cells when PBS (C) and control G5-AF (D) is given. In a similar fashion Thomas et al. (2004) tailored G5 PAMAM dendrimers with 60 bca and J591 antibodies that bind to CD14 and prostrate specific membrane antigen (PSMA), respectively and labeled with flourescein isothiocyanate (G5-FI-60B, G5-FI-PA). HL60 (from human myeloblastic leukemia) and LNCaP (from prostrate cancer) cell lines that express CD-14 and PSMA antigen were taken. Flow cytometric and confocal studies showed the receptor specificity as the conjugates bound to specific antigen expressing cells and the control G5-FI lacked specific affinity to any of cell lines.

Presence of multiple branching sites in dendrimer provides enhanced interaction to the receptor and hence provides spatial accessibility for targeting. Targeted delivery offers increased therapeutic index, reduction in the required dose as well as toxicity (Brigger et al., 2001; Luo and Prestwich, 2002). Dendrimer can thus provide a unique platform that can couple targeting moiety, drug, imaging agent and fluorescent probe simultaneously without affecting the integrity of individual components (Thomas et al., 2004) (Fig. 6). The ability to attach any or all of these molecules in a well-defined and controllable manner onto a robust dendritic surface clearly differentiates dendrimers from other carriers such as micelles, liposomes, emulsion droplets, and engineered particles (Svenson and Tomalia, 2005). 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 or on the angiogenic microcapillaries growing around these cells. Ligands possess certain structural analogues that are recognized by specific cell surface receptors. As the complex reaches the target site, it is internalized and subsequently releases the therapeutic moiety. The ligands thus play a prominent role in inhibiting or stimulating a patho-physiological response. Thus, conjugating these ligands to dendrimers will provide enhanced intracellular trafficking of these macromolecules in the necrotic tumor cells. Harrington et al. formulated estrogen dendrimer (G6 PAMAM) conjugate (EDC). Using estrogen that functions as a ligand, the research group used a derivative of 17α-ethinylestradiol for attachment of the former to the dendrimer. Cell uptake studies were performed on estrogen receptor (ER) positive MCF-7 cell lines and ER negative MDA-MB-231 cell lines, and EDC was tagged with a fluorophore TMR. Cellular fluorescence depicted the localization of EDCTMR complex in ER(+) cells (A), the complex when given along with estradiol (E2) showed decreased fluorescence. EDC has affinity for ER(+) cells and thus results in competitive binding between E2 and EDC–TMR complex. Hence the figure B shows decreased fluorescence, suggesting the uptake of EDC–TMR complex is due to ER binding (B). The TMR labeled empty dendrimer showed minimal uptake compared to E2 supplemented cells (C). ER(−) cells also showed minimal uptake of EDC–TMR conjugate in the cells (D) and no difference was observed with that of E2 supplemented cells (E). The results indicate that on attaching the estrogen ligand to the dendrimer the moiety substantially retained its affinity for the receptor ER for which it was originally meant (Harrington et al., 2006). Frechet and co-workers synthesized ester terminated dendrimers encapsulating methotrexate (Mtx) and FA. Conjugation was achieved between γ-carboxyl group of FA and hydrazide groups of dendrimer. The dendrimeric formulation had high solubility at physiological pH, although some amount of FA was precipitated at lower pH. The system showed selective affinity to tumor cells expressing F-R and the possible mechanism of uptake inside tumors can be receptor mediated endocytosis (Kono et al., 1999).

Better results can be visualized for these hormone resistant tumors, via, site-specific delivery of drugs, radionucleides and toxins without destructing the normal tissues in the vicinity. Patri et al. synthesized G5 PAMAM–FA–MTX complex for the studies. They fashioned the surfaces so that neutral (hydroxyl or acetyl) or negatively charged (carboxylate) groups were obtained as terminal functionalities, because amine groups are involved in non-specific interactions with the cells. Binding characteristic of all the modified dendrimers incorporating MTX with and without FA were performed on FR(+) KB cells. The results elucidated 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). Similar results were achieved by Thomas and coworkers by using Fluorescene Isothiocyanate (FITC) as a detecting agent. They formulated trifunctional dendritic device G5 PAMAM–FI–FA–MTX conjugate (Fig. 7). Spectrofluorimetric analysis demonstrated 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.

In this concern such targeted delivery can help to prevent non-specific drug/carrier cytotoxicities and also evade resistance that may develop due to free drug (Thomas et al., 2005).

Although monoclonal antibodies possess affinity for target epitopes and they maintain their binding characteristics when loaded with drug, their major drawback is low loading capacity and hence they are frequently used as ligands of carriers of high loading capacity (Molema, 2005). Patri et al. tagged G5 PAMAM dendrimers with fluorescence for fluorescence and conjugated J591 anti-PSMA monoclonal antibody. The conjugates were found to target PSMA(+) LNCaP cells as depicted by the fluorescence and showed no selective affinity to PSMA(−) PC3 cells and very less fluorescence in the PSMA(−) cells due to the entry of the complex by simple diffusion (Patri et al., 2002).

Electrostatic interaction between the cationic amine terminal functionalities of PAMAM dendrimers with anionic phosphate groups in DNA enables them to be used as synthetic non-viral agents and showed enhanced transfection efficiency compared to naked DNA, particularly in cell lines derived from monkey and human neoplasm (Haensler and Szoka, 1993). Choi et al. engineered bi-functional (G5/G7) PAMAM dendrimer for DNA delivery to tumors. Instead of coupling all the moieties to a single dendrimers, the scientists developed the formulation using synthetic oligonucleotides, in such a fashion that two dendrimers self-assemble (Fig. 8). One dendrimer was coupled with FA and another tagged with fluorescein marker. The nanoconstructs assembled by DNA were found to have a profound affinity for FR(+) KB cells, and the system was internalized by receptor mediated endocytosis (Choi et al., 2004).

Dendrimers functionalized with glyco-coat (glycodendrimers) can play a dominant role in specifically recognizing lectin receptors on the tumor surface. Roy has suggested

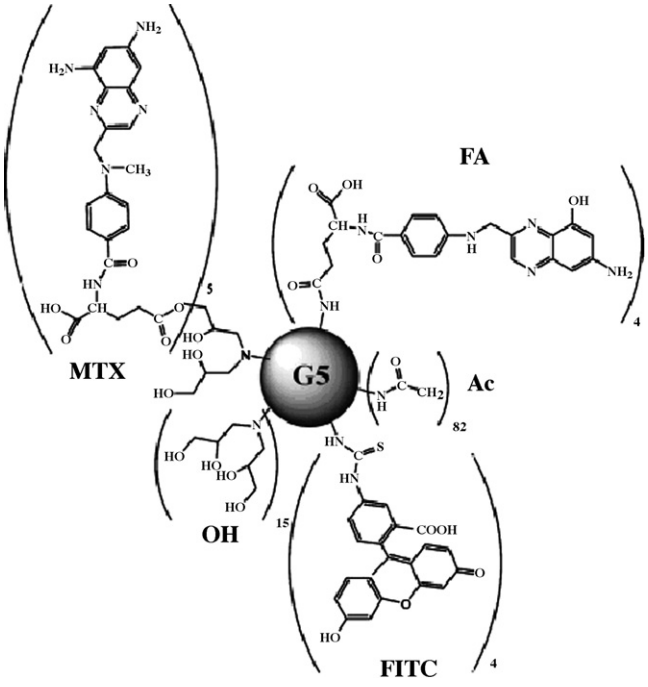

Fig. 7. Schematic representation of generation 5 (G5) dendrimer surface-functionalized With FITC, FA, and MTX (Thomas et al., 2005).

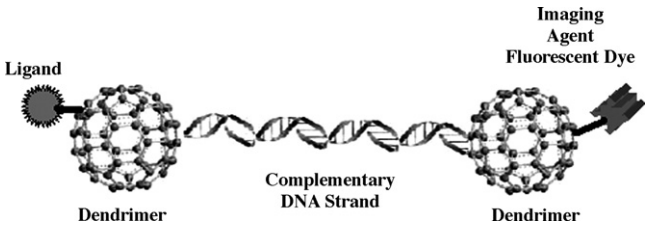

Fig. 8. Dendrimers attached to another by means of DNA strands. On one end imaging agent is attached and fluorescent dye on the other (Choi et al., 2004).

that these artificial multiantennary glycans can bind to the lectin receptors, which possess more than one binding site, in two different manners. Firstly, the single binding site of lectin receptors may interact with all the terminal saccharide moieties of the dendrimer molecule; or secondly, the terminal branches on the dendrimer surface can be so far apart that they bind to two different protein molecules. Thus, a condition in which a glycocluster containing limited number of ligands bind to a single receptor's active site is termed as "classical" glycocluster effect and when the glycocluster bind to two different receptor's active site is "crosslinked" glycocluster effect. Thus, the glycodendrimers can prove a potent module for delivery of anti-cancer agents (Roy, 2003). Vannucci et al. coated PAMAM dendrimers with *N*-acetyl-glucosamine (Glc-NAc) and the studies were conducted on B16F10 melanoma cells. The results depicted carbohydrate mediated immune recognition and immunomodulation in tumor conditions. Intra-peritoneal administration of the formulation resulted in dose dependent increase in survival rate. Also there was an increase in CD 4+ count and IL-2 production. Further, the researchers predicted a greater binding interaction of the hyper-branched dendritic nano-construct, which is believed to be ligand for lectin receptors (Vannucci et al., 2003). Shaunak et al. formulated water soluble conjugates of glucosamine/alucosamine-6-sulphate with dendrimers. The conjugates inhibited fibroblast growth factor (FGF) mediated EC's proliferation and neo-vascularization in Matrigel and placental angiogenic assays. The researchers also stated that these highly engineered macromolecules can provide a suitable platform for immuno-modulatory and anti-angiogenic activities (Shaunak et al., 2004).

Although the ligand–dendrimer mediated delivery of anti-cancer drugs is a highly fascinating approach, it is necessary to study the conformation and orientation of ligands in relation to its receptors and is mandatory to state that a conjugated ligand may sometimes have decreased affinity to its receptors. Baker and co-workers explored the ability of PAMAM dendrimers as drug delivery vehicle for anti-cancer agents, methotrexate and paclitaxel. The research group also formulated carboxyl, hydroxyl and ester terminated dendrimers and evaluated their effect on the affinity of folic acid ligand towards F-R. Molecular modeling studies clearly showed a drastic difference of mean diameter (amine $\sim 29.5 \pm 0.2$ Å, carboxyl $\sim 34.3 \pm 0.2$ Å, hydroxyl $\sim 21.8 \pm 0.1$ Å and ester $\sim 19.8 \pm 0.1$ Å) and hence the distance of FA from the dendritic core. Flowcytometric studies on KB cells suggest folate mediated acetamide

capped dendrimers to have more accessibility to the receptors as compared to carboxyl and hydroxyl groups. This further strengthens the importance of conformation and orientation of the molecule. Thus, repulsive forces of charged amines of PAMAM dendrimers cause back folding of FA and hence resulting in less prominent binding (Quintana et al., 2002).

6. Conclusion

Despite the desperate efforts of various scientific groups, there are many challenges for cancer therapy. Treatment of cancer is accompanied not only by untoward toxicity but also compromised tumor vasculature that poses hindered transport of the bioactives and thus affecting drug's efficacy. Hence the present article was envisaged to review the preferred approaches by various scientific groups for site-specific delivery, via exploitation of the receptors that develop on the surface of tumors, so that these highly toxic moieties can gain access in the tumor micro-environment. It is also necessary to elucidate molecular signatures on tumors, which are biologically relevant targets for targeted delivery.

Irregular development of basement membrane, presence of fenestrae, widened endothelial junctions and formation of small blood vessels to nourish the cells make them more porous. This has created a vision that the nanoparticles can be dictated as a suitable vehicle for delivery of anti-cancer agents, considering that their pharmacokinetic and bio-disposition properties are regulated by their size, zeta-potential and lipophillicity. In this context the nano-size of dendrimer and presence of hydrophilic exterior both prevent its recognition by mononuclear phagocytic system, provides greater entry in tumor and the high molecular weight enhances its residence time in tumors, the phenomenon termed as Enhanced Permeation and Retention (EPR) effect. When discussing about EPR, it is necessary to state that the single vessel can be leaky to particles of one size, but may not permit other particles. This is a complicated situation in case of drug delivery vehicles polydispersive in nature and can thus render a significant amount of drug inactive. Hence the monodispersive nature of dendrimers is highly yielding when the topic concerns drug delivery to tumor mass (Sampathkumar and Yarema, 2007).

Molecular recognition of the receptors by dendrimer–ligand system, internalization of the macromolecule and subsequent release of drug may work in subsequence and serve a potential modality against this mutated and heterogenic network. The system has proved its potentiality as carrier, and highly appealing results have been obtained exploiting dendrimers as vehicles for delivery of anti-cancer drugs. These nanomolecules can be modulated such that they are snapped together with imaging; targeting and therapeutic agents so that they are able to fingerprint a tumor mass, are capable of ferrying a large dose of drug, delivering it to a specific site and potentially reducing the unpalatable side effects.

Although the potentials of dendrimers are many, these nanoconstructs still face a number of limitations that impose a restriction on them from being widely adopted. Their high costs, complex synthesis procedure and cytotoxicity issues are a matter of concern when compared to other delivery systems.

To summarize the potential of dendrimers as vehicle for site-specific delivery of anti-cancer drugs to tumors of varying origin and anatomical sites seems to be a highly promising approach. But it still needs vigorous studies by various research groups to arrive at unambiguous generalization. The issues relating to efficacy, safety, accumulation and disposal, toxicity as well as regulatory approval are to be addressed simultaneously. In any case nanotechnological tools (nanoparticles and dendrimers) appear to be promising in cancer chemotherapy especially via ligand/receptor mediated endocytosis.

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