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Applications of Dendrimers in Cancer Therapy

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ABSTRACT

Dendrimer chemistry was first introduced in 1978 by Fritz Vogtle and coworkers. He synthesized the first "cascade molecules", today known as dendritic molecules. The dendrimer architecture permits control over properties such as shape, size, density, polarity, reactivity and solubility. Dendrimer density functions and starburst limits can be easily modeled mathematically. Dendrimers have stimulated wide interest in the field of chemistry and biology, especially in applications like drug delivery, gene therapy and chemotherapy. A treatment of Cancer mainly focused on the targeting the active drug molecule at the site without affecting the neighbour cells and dendrimers have this property which is useful in diagnosis and treatment purpose which is a new hope in this area of Cancer treatment.

Key words: Dendrimers, Cancer Therapy and PAMAM Dendrimers.

1. INTRODUCTION

The word "dendrimer" originated from two words, the Greek word dendron, meaning tree, and meros, meaning part. Dendrimer chemistry was first introduced in 1978 by Fritz Vogtle and coworkers. He synthesized the first "cascade molecules", today known as dendritic molecules. In 1985, Donald A. Tomalia, working in the field of polymer chemistry, synthesized the first family of dendrimers [1], these contributions to the field have paved the way for continuing research in this promising area. The term 'dendrimer' refers only to an architectural motif and not a particular compound. To date greater than 160 various polymers with dendritic structures are reported in the literatures. The surface groups of dendrimers are amenable to modification and can be tailored for specific applications. The dendrimer architecture therefore permits control over properties such as shape, size, density, polarity, reactivity and solubility. They are produced in an iterative sequence of reaction steps, in which each reaction results in a new so called generation. Dendrimer density functions and starburst limits can be easily modeled mathematically. These features are related to core multiplicity, the branching multiplicity of the monomer units, and the branch lengths, as well as the core and branch volumes [2]. Due to their multivalent and monodisperse character, dendrimers have stimulated wide interest in the field of chemistry and biology, especially in applications like drug delivery, gene therapy and chemotherapy.

2. DENDRIMERS IN CANCER DIAGNOSIS AND TREATMENT

2.1. Dendrimers Have Attractive Properties for Cancer Treatment

Cancer epitomizes the challenges faced during drug delivery: an anticancer drug must be able to seek out subtle changes that distinguish a transformed cell from the other 200 or so types of healthy cells found in the body and then provide a sufficiently high dose of a toxic agent to selectively kill the cell while not harming its healthy neighbors. Therefore, even though dendrimers can be endowed with many favorable properties for drug delivery, an ultimate challenge – ergo, a ''real-world'' test of these versatile nano-devices will be whether they can successfully meet the formidable tasks of diagnosing and treating of malignant disease [3,4].

3. DENDRIMER-SIZED PARTICLES PASSIVELY ACCUMULATE AT THE SITES OF TUMORS

To begin the discussion of properties that make dendrimers attractive vehicles for cancer treatment, we revisit the concept that encapsulation or covalent linkage of small molecule drug candidates to a dendrimer enhances the pharmacological properties of the drug. In cancer chemotherapy, these desirable size-based features are reinforced by the enhanced permeability and retention (EPR) effect that improves the delivery of macromolecules to tumors. The EPR effect is based on unique pathophysiological features of a solid tumor, such as extensive angiogenesis resulting in hyper-

vascularization, limited lymphatic drainage, and increased permeability to lipids and macromolecules. These features, which help ensure adequate nutrient supply to meet the metabolic requirements of rapidly growing tumors [5-6], can be turned to the tumor's disadvantage by the use of nano-sized therapeutic agents. The EPR effect was discovered when selective accumulation of the SMANCS conjugate (styrene-maleic anhydride-neocarzinostatin) was observed at the site of tumors while similar accumulation was not seen with neocarzinostatin alone [7-8]. The EPR response was subsequently demonstrated for similarly-sized liposomes, thereby establishing that this effect was largely a function of particle size and did not solely depend on the chemical or biophysical properties of the macromolecule. Specifically, in one study optimal tumor delivery occurred for liposomes having a size distribution between 70 and 200 nm in diameter [9]. An independent study showed efficacy for liposomes loaded with daunorubicin in the same size range; specifically, those@142 nm in diameter exhibited an inhibitory effect against Yoshida sarcoma whereas smaller (@57–58 nm) and larger (@272 nm) liposomes had weaker or no effect [10]. Over time, cautionary notes were raised that tempered initial enthusiasm for exploiting the EPR effect for cancer treatment. For example, the porosity of the vasculature in tumors can be highly variable even with a single vessel that can be leaky to one size of particle in one region but not in another [11]. Experimentally addressing this issue was complicated by the size polydispersity of traditional nanoparticles used to exploit the EPR effect, which were typically either lipids or conventional polymers that rendered a significant proportion of intended drug inactive. Fortunately this issue – the ability to match exact and uniform sizes needed to target an individual tumor is highly tractable with dendrimers because selection of an exactly-sized entity is possible (Table-1) compared with the large size distributions that plague liposome and most polymeric materials [12].

The ability to construct monodisperse populations of dendrimers in the size range needed to exploit the EPR effect is an encouraging step towards the passive exploitation of tumor properties. Once the basic issue of size was resolved, however, secondary challenges (and opportunities) arose from observations that the chemical properties of the nano-sized particle can play significant roles in modulating the EPR effect. By way of a specific example, ''conventional'' polymeric materials showed efficacy at a smaller size range, occurring at 60 nm for both water soluble and hydrogel forms of poly (vinyl alcohol) (PVA) [13], whereas almost identically-sized 57 nm egg phosphatidylcholine (EPC)-liposomes were ineffective. As reported above, liposomes about twice this size showed maximal efficacy, so it was not unexpected that the EPC-liposomes were ineffective. Interestingly, however, hydrogenated egg phosphatidylcholine (HEPC)-liposomes in this size range (specifically, 58 nm) were active, illustrating that the exact chemical properties of the material is a critical design parameter. In this respect, the many options for dendrimer ''building blocks'', as well as the ability to further tune surface properties provide many opportunities to endow dendrimers with favorable ''Passive'' properties for tumor targeting.

Table -1: Generation by generation specifications for PAMAM Starburst dendrimers.

Generation	Physical or structural parameter			
	Molecular weight (Daltons)	Diameter (Å)	Surface groups ($-NH_2$)	Radius of gyration (Å)
G0	517	15	4	4.93
G1	1430	22	8	7.46
G2	3256	29	16	9.17
G3	6909	36	32	11.2
G4	14215	45	64	14.5
G5	28826	54	128	18.3
G6	58048	67	256	22.4
G7	116493	81	512	29.1
G8	233383	97	1024	36.4
G9	467162	114	2048	46.0
G10	934720	135	4096	55.2
G11	1869780	167	8192	68.3

4. MULTIFUNCTIONAL DENDRIMERS CAN SELECTIVELY TARGET BIOMARKERS FOUND ON CANCER CELLS

4.1. Methods for Targeting Specific Biomarkers of Cancer

As discussed above, dendrimers can achieve passive EPR-mediated targeting to a tumor simply by control of their size and physicochemical properties. Passive targeting, which localizes the nano-particle in the close vicinity of a cancer cell, can be immediately useful for diagnostic purposes or for the delivery of radioisotopes capable of killing any cell within a defined radius. In general, however, most delivery strategies require that the anticancer agent directly attached to, or be taken up by, the target cell. The ability to append more than one type of functionality to a dendrimer allows the inclusion of ligands intended to bind specifically to cancer cells in the design of a multi-functional drug-delivery nanodevices. Although a wide range of

targeting ligands have been considered, including natural biopolymers such as oligopeptides, oligosaccharides, and polysaccharides such as hyaluronic acid, or polyunsaturated fatty acids [14], discussion here is limited to folate, which is an exemplary small molecule tumor-targeting agent, as well as monoclonal antibodies directed against tumor associated antigens (TAAs).

4.2. Targeting By Folate, A Small Molecule Ligand

Folate is an attractive small molecule for use as a tumor targeting ligand because the membrane-bound folate receptor (FR) is over expressed on a wide range of human cancers, including those originating in ovary, lung, breast, endometrium, kidney and brain [15]. As a small molecule, it is presumed to be non-immunogenic, it has good solubility, binds to its receptor with high affinity when conjugated to a wide array of conjugates, including protein toxins, radioactive imaging agents, MRI contrast agents, liposomes, gene transfer vectors, antisense oligonucleotides, ribozymes, antibodies and even activated T-cells [16-17]. Upon binding to the folate receptor, folate-conjugated drug conjugates are shuttled into the cell via an endocytic mechanism, resulting in major enhancements in cancer cell specificity and selectivity over their non-targeted formulation counterparts. Recently, folate has been enlisted in innovative dendrimer-based targeting schemes [18].

4.3. Targeting By Monoclonal Antibodies

Of the many strategies devised to selectively direct drugs to cancer cells, perhaps the most elegant (and demanding!) is the use of monoclonal antibodies that recognize and selectively bind to tumor associated antigens (TAAs) [19-22]. TAA-targeting monoclonal antibodies have been exploited as delivery agents for conjugated ''payloads'' such as small molecule drugs and prodrugs, radioisotopes, and cytokines [23,24]. The field of ''immunotherapy'' envisioned almost a hundred years ago, and given renewed impetus a quarter century ago by the development of monoclonal antibody technologies, has nonetheless progressed erratically over the past two decades as many pitfalls have been encountered. Current prospects remain mixed but hopeful; optimistically, progress marked by commercial interest with companies providing their immunotherapeutic drug candidates with flashy trademarked names, such as ''Armed AntibodiesTM'' [25]. Similarly, the rosy opinion that this field is ''on the verge of clinical fruition'' has been published recently [26]. Perhaps, more realistically, one recent synopsis holds out ''hope'' for a major clinical impact for this strategy within the next 10 years. Although a detailed discussion of the many pitfalls encountered in immunotherapy efforts is beyond the scope of this chapter, one key issue – readily addressed by dendrimers – is the requirement that an extremely potent cytotoxic drug be used in targeted antibody therapy. This point is illustrated by the fact that the greatest progress in this field has occurred for immunotoxins, which are antibody–toxin chimeric.

Molecules that kill cancer cells via binding to a surface antigen, internalization and delivery of the toxin moiety to the cell cytosol. In the cytosol, protein toxins, such as those from diphtheria or pseudomonas, catalytically inhibit a critical cell function and cause cell death [27]. The high potency of immunotoxins for killing cancer cells is dramatically illustrated by ricin, where the catalytic activity of this ribosome-inactivating enzyme allows a single immunotoxin conjugate to kill a cell upon successful uptake and trafficking to the site of action [28,29]. A drawback of immunotoxins is their significant immunogenicity, which limits repeated use; from a broader perspective, their repeated use is made necessary by difficulties in providing a sufficiently high drug load to eradicate all cancer cells despite the high potency of conjugated toxin. An alternative approach of radio immunotherapy, where high energy radio nuclides are conjugated to TAA-targeting antibodies, also shows promise but suffers from indiscriminate toxicity (the surrounding healthy tissues, as well as off-target tissues, become irradiated in addition to the target cancer cells) [30]. A third possible approach for immunotherapy, the conjugation of commonly-used small molecule drugs to TAAs, is hindered by the relatively low potency of most low molecular weight therapeutics. To illustrate this point,@10 000 TAAs occur on a typical cancer cell [31], making this number the upper limit for the number of targeting antibodies that can bind to the cell. The widely used anticancer drug cisplatin, to give one example, requires internalization of at least 50 X this level of drug molecules for therapeutic efficacy.

A numerical analysis of the cisplatin example presented above indicates that each tumor-targeting antibody would have to be modified with a large number of small molecules to be effective as an anticancer drug (in this case, roughly 50 cisplatin molecules upon superficial analysis). Modification of an antibody with multiple radioisotopes, toxins, or even small molecules to increase the efficacy of cell killing, however, diminishes or eliminates the inherent specific antigen-binding affinity of an antibody. Therefore, to maximize drug loading while

minimizing the deleterious effects on the biological integrity of the host antibody, an attractive approach is to use a linker molecule, such as a dendrimer, that can be highly conjugated (or internally loaded) with drug while modifying only a single site on the surface of the antibody [32]. Methodology to covalently attach antibodies to dendrimers that preserve the activity of the antigen–antibody binding site [33,34] e.g., by chemical modification of their carbohydrates and subsequent linkage to PAMAM [35], has opened the door for the inclusion of dendrimers in immunotherapy [36,37], thereby enhancing the future prospects of this chronically ''almost-there'' strategy.

5. DENDRIMERS IN CANCER DIAGNOSIS AND IMAGING

5.1. Labeled Dendrimers are Important Research Tools for Biodistribution Studies

The synthetic ability to attach both a tumor-targeting antibody and a potent payload of anticancer drugs to the same dendritic molecule provides a platform for multifunctional

Nano-scale drug delivery devices, Before this technology can be applied in the clinic, however, its safety and efficacy must be demonstrated; towards this end, fluorescently-modified dendritic conjugates have been used extensively to characterize cell targeting, surface binding, uptake and internalization, and even sub-cellular localization [38]. The radio labeled counterparts appropriate for animal studies have allowed detailed examination of the Biodistribution of dendrimers. Several radio-isotopes have been conjugated to dendrimers, including 3H [39], 14C [40], 88Y [41], 111In [42], and 125I [43-46]. These studies have established that the chemical and physical properties of dendrimers can be tuned to favor distribution to or away from specific organs and, ultimately, to achieve favorable Biodistribution to tumors. The methods used in these experiments, however, typically requiring post-administration dissection of the host animal to allow the analysis of organ sequestration and tissue distribution of the radioisotope, are clearly not applicable to clinical practice. Instead, they have served as an important stepping stone along the path towards non- or minimally-invasive diagnostic procedures, which are proceeding mainly by the development of MRI contrast agents [47-55].

6. STEPS TOWARDS THE CLINICAL REALIZATION OF DENDRIMER-BASED CANCER THERAPIES

6.1. The Stage is now set for Dendrimer-based Cancer Therapy

The use of dendrimers for cancer treatment is still in its infancy with few, if any, applications successfully translated to the clinic. Consequently, their use as diagnostic agents constitutes both an important goal in and of itself, and also a valuable ''baby step'' towards the ultimate goal of curing cancer. As discussed, the process of actual killing cancer cells entails the complicated process of drug uptake followed by release of the drug into the cytoplasm or nucleus and is clearly a more demanding process than cell surface labeling, or even localization to the vicinity of the tumor, sufficient for diagnostic purposes. Nonetheless, in some cases, the transition from imaging to therapy will be closely linked, as evidenced by efforts now underway to combine antibody-targeted MR imaging nanoparticles with the delivery of anti angiogenic genes intended to inhibit the vascularization to the V2 carcinoma model in rabbits [56]. Another promising strategy – boron neutron capture therapy – has undergone impressive development over the past decade and is presented next as a successful demonstration of the promise of dendrimer-based cancer therapies.

7. BORON NEUTRON CAPTURE THERAPY

Cisplatin-based therapies illustrate the need for multiple conjugations of small molecules – estimated at 50 for this platinum drug – to a targeting antibody. While some efforts are underway to use dendrimeric strategies for platinum drug delivery [57], an even more demanding situation, where thousands of ligands are required per targeting antibody, is provided by boron neutron capture therapy (BNCT). Accordingly, BNCT will be discussed here as an illustrative example of how dendrimers can help overcome high hurdles in the development of innovative cancer therapies. As a brief background, BNCT is based on the nuclear reaction that occurs when boron-10, a stable isotope, is irradiated with low energy (a0.025 eV) or thermal neutrons to yield alpha particles and recoiling lithium-7 nuclei. A major requirement for the success of BNCT is the selective delivery of a sufficient number of boron atoms (@109) to individual cancer cells to sustain a lethal 10B (n, alpha)! 7Li capture reaction [58-59]. Considering that the maximal number of antigenic sites per tumor cell is in the range of 100 000, and more commonly only 1/10th that level, an a priori calculation suggests that each targeting antibody must be linked to at least 2000, but preferably closer to 5000, boron atoms. Clearly, a single TAA-targeting antibody cannot be directly conjugated at this level and conventional polymers – e.g., polylysine conjugated with @1700 boron derivatives and linked to a targeting antibody – caused the antibody to lose in vivo tumor

localizing properties [60]. By contrast, when a PAMAM dendrimer was used for polyvalent boron conjugation, the linked antibody maintained immuno-recognition (although in vivo tumor targeting remained problematic because the conjugated dendrimer had a strong propensity to mislocalize in the spleen and liver). Over the decade since these pioneering efforts were first reported, continued progress has been made to solve problems such as off-target tissue localization, which was traced to the size of the dendrimer and presence of a large number of amine groups on the surface of PAMAM, by exploiting the versatility of dendrimer chemistry. In short, the re-design of boronated, anti-body-targeted dendrimers has culminated in the successful treatment of gliomas in the rat and laid the foundation for translation of this technology into clinical tests in the foreseeable future [61].

8. CONCLUSION

Dendrimers, chemically-defined entities with tunable biological properties, have advanced over the past two decades to the point where they stand on the cusp of major contributions to the treatment of cancer in a meaningful way. Although, as has been apparent by the many instances cited throughout this chapter where gaps in knowledge still remain and that must be plugged before dendrimers are ready for wide clinical use, their extreme versatility combined with the extensive research efforts now underway are sure to add sophistication to drugs already in use as well as spur the development of entirely new classes of anticancer therapy.

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