The Use of Dendrimers in Cancer Diagnosis

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Contributor: Andreea Crintea , Alexandru Cătălin Motofelea , Alina Simona Șovrea , Anne-Marie Constantin , Carmen-Bianca Crivii , Rahela Carpa , Alina Gabriela Duțu

Dendrimers are a type of nanomaterial with unique properties, and their production can be controlled to obtain compounds with the desired characteristics. These polymeric molecules are used in cancer diagnosis and treatment through the targeted distribution of some pharmacological substances. Dendrimers have the ability to fulfill several objectives in anticancer therapy simultaneously, such as targeting tumor cells so that healthy tissue is not affected, controlling the release of anticancer agents in the tumor microenvironment, and combining anticancer strategies based on the administration of anticancer molecules to potentiate their effect through photothermal therapy or photodynamic therapy.

cancer dendrimers diagnosis treatment targeting

1. Introduction

Dendrimers are nanomaterials with unique properties used in cancer diagnosis and treatment, targeting tumor cells, controlling the release of anticancer agents, and combining anticancer strategies [1]. Cancer is a malignant condition characterized by the uncontrolled proliferation of atypical cells. Cancer progression is supported by the imbalance or damage of proto-oncogenes that encode proteins involved in the development and differentiation of tumor cells, but also tumor suppressor genes that encode proteins that produce inhibitory signals for cells in need and produce apoptosis [2]. According to the World Health Organization, cancer is the leading cause of death globally, and lung cancer is the most common cause of cancer-related death [3][4]. As the mortality rate associated with cancer is increasing, research on cancer therapies is growing, and identifying the most effective treatment method is the goal of most scientists [5][6]. The main treatment methods for this group of pathologies include surgical excision, chemotherapy, radiotherapy, and immunotherapy. The effectiveness of these therapies depends on the severity of the disease and the particular reaction of each organism. However, a major problem encountered in patients undergoing the above-mentioned therapies is that these therapies cannot distinguish between tumor cells and healthy cells in the body, resulting in the occurrence of serious adverse reactions [5][7][8][9]. These adverse reactions are most often represented by alopecia, decreased immunity through the suppression of bone marrow function, fever, nausea, vomiting, hepatotoxicity, cardiotoxicity, neurotoxicity, electrolyte imbalances, and decreased muscle tone [10][11]. One way to avoid these adverse reactions is to use targeted therapies, where drugs or mechanisms for destroying tumor cells are specifically directed to the tumor [8][12][13]. Targeted therapy is a new-generation chemotherapy that seeks to target certain proteins or genes specifically linked to a particular cancer or tumor vasculature that promotes its growth [2]. Nanotechnology is an option for such therapies and is based on the synthesis of “tools” that act as transporters of drugs to a specific tumor target [14][15][16][17].

2. Dendrimers: Polymer-Based Nanoparticles

Our planet is full of various structures that have a dendritic, branched architecture, from neuronal dendritic branches to the rich branching of tree roots. Some researchers believe that these dendritic structures have evolved over time due to the need for highly complex and efficient surfaces that allow for the processes of absorption, extraction, or distribution of different substances in the living world [18]. Inspired by his pastime as a horticulturist, Donald Tomalia, along with his colleagues, discovered hyper-branched molecules called dendrimers in the early 1980s [18][19]. The first dendrimers obtained were polyamidoamines (PAMAM) and are called starburst dendrimers. They are composed of a core of ethylenediamine or ammonia, which is surrounded by amino groups on the outside [19][20][21]. Unlike linear polymers, dendrimers are highly branched molecules that can be synthesized at the desired size and molecular weight, and whose monomeric units have the ability to self-organize [14][19][22][23].

As previously noted, the structure of dendrimers is represented by a central core, surrounded by branches, and the outermost layer of their structure is represented by a multivalent surface. The synthesis of these molecules can be done divergently or convergently [14][24][25][26]. The two synthesis methods differ in the growth direction of the dendrimer. In the case of divergent synthesis, the synthesis begins with the formation of the central molecule, the core of the dendrimer, and then this base molecule interacts with monomers, causing the structure to grow outward [27][28]. The addition of monomers to the outside of the molecule occurs for several generations in a row, adding one layer of the first-generation dendrimer. Each layer of monomers represents a generation [19][24][28][29]. Dendrimers with few layers, from generations 0, 1, or 2, have open, asymmetric structures, and as the branches become larger (dendrimers of generation 4 or higher), the dendrimers become compactly packed, taking on a globular shape. However, the synthesis of these polymer molecules cannot be infinite due to the lack of space [19][30][31][32][33]. A problem that may arise in the synthesis of dendrimers by the divergent method is the appearance of incomplete terminal groups, and this problem can be avoided by synthesis through the convergent method, in which the dendrimer is synthesized starting from the final branches. When a structure of the desired size, formed from branched monomers, is reached, it attaches to a core molecule. This method does not carry the risk of defects appearing in the final structure of the dendrimer, and purification is easy to achieve, but it cannot be used for the synthesis of high-generation dendrimers [19][34][35]. After the synthesis of dendrimers, both in their core and between the branches formed by the monomers, cavities and channels will form. The interiors of dendrimers, as well as the groups on the surfaces of these structures, can be loaded with various medicinal substances [14][19][29][36][37][38][39]. In addition, the free branches of dendrimers can be linked to other molecules in the class of nanomaterials, radioligands, or other functional molecules that reduce cytotoxicity and increase the biocompatibility of the polymer in the body. In this way, by attaching different ligand molecules, dendrimers can be targeted to different tissues [18][40]. Due to the numerous advantages of dendrimers, these polymeric molecules are increasingly being used in medicine, providing utility in the diagnosis and treatment of various conditions, directing drug substances in the body, or increasing the efficacy of other therapies [41][42][43][44][45][46][47].

Some of the strengths of the use of dendrimers have already been noted, and **Table 1** summarizes the advantages of these polymer-based nanoparticles and the advantages of using dendrimers in cancer treatment.

Table 1. Advantages of dendrimers.

Advantages of Dendrimers	Advantages of Using Dendrimers in Cancer Treatment
Ability to synthesize molecules with desired characteristics based on the intended purpose	
High endocytosis capacity	
High capacity for drug encapsulation	
Can be administered orally, intravenously, or in combination	
Biocompatible and biodegradable	
Ability to improve the solubility of hydrophobic drugs	
Delivery and controlled release of drugs	
Possibility of conjugation with different molecules that reduce toxicity	Possibility of attaching specific ligands to target tumor tissue and reducing cytotoxicity towards healthy cells
Globular structure→ small hydrodynamic volume	
Monodisperse architecture	
Multivalent surface	Possibility of covalent conjugation with several different anticancer molecules
	Possibility of monitoring the effectiveness of the treatment
	Treatment administration avoiding the possibility of developing drug resistance
[14][19][23][24][25][37][41][42][48]	[43][44][45][46][47][49]

Although dendrimers have multiple advantages for use in biomedicine, these structures also present some disadvantages. One of these is the toxicity that dendrimers have in biological systems. Due to the terminal NH2 groups and the cationic charge on the dendrimer surface, they can interact with the negatively charged cell membranes, producing cell lysis and implicitly cytotoxicity [50][51]. It seems that PAMAM dendrimers are among the most toxic, and their toxicity depends on the dendrimer generation, the lower-generation ones being less toxic. PAMAM dendrimers whose surface is modified with anionic molecules such as carboxyl groups or with PEG polymers are less or not at all toxic. It was also proven that the modification of the surface of the dendrimer so that the amino groups are replaced with aldehyde groups led to lower toxicity. Similar to PAMAM dendrimers, PPI dendrimers also show toxicity [50][51].

The toxicity of dendrimers is generally characterized, in addition to cytotoxicity, by hemolytic toxicity and hematological toxicity. The hemolytic toxicity is caused by the interaction of the free cationic terminal groups of the dendrimers with the red blood cells (RBC), an interaction that determines hemolysis [50]. For instance, Bhadra et al.

developed fourth-generation PAMAM dendrimers for the release of an anticancer drug and found that the hemolytic toxicity of these dendrimers was around 15.3–17.3% [50][52]. Subsequently, Asthana et al. had similar results when they evaluated the same dendrimers for hemolytic toxicity and observed up to 18% toxicity [50][53]. Analyzing different generations of PPI dendrimers, a value of 35.7% hemolysis was observed for the fourth-generation dendrimers and 49.2% hemolysis for the fifth-generation dendrimers [50].

Hemolysis caused by cationic dendrimers directly influences the hematological parameters—in this case, it is a hematological toxicity. Analyzing the effect of the dendrimer PPI on blood parameters such as the number of white blood cells, the number of red blood cells, the concentration of hemoglobin, the hematocrit, and the mean corpuscular hemoglobin, a significant decrease in the number of red cells and a significant increase in the number of white blood cells were observed. Moreover, the concentration of hemoglobin, the average corpuscular hemoglobin, and the hematocrit decreased dramatically according to Agashe et al. [50][54].

Dendrimer toxicity in vivo is rarely studied, but Roberts et al. studied the in vivo toxicity of third-, fifth-, and seventh-generation PAMAM dendrimers in Swiss Webster mice and observed that only seventh-generation PAMAM dendrimers produced biological complications. The authors concluded that dendrimers do not present properties that prevent their use in biological applications. However, in order to avoid any of the possible adverse effects, it is necessary to modify the surfaces of the dendrimers so that they are more biocompatible [50][55].

Besides the disadvantage of the toxicity of dendrimers, the possibility of these structures being immunogenic was also investigated, but it seems that immunogenicity was not identified—it was very weak. Agashe et al. investigated the immunogenicity of 5.0 G PPI dendrimers in Balb/C mice using ELISA to monitor the antibody titer and concluded that the dendrimers did not elicit any detectable humoral immune response under the experimental conditions. Thus, dendrimers are treated by the host's immune system as "native" and this is an advantage for their use in drug delivery [50][54].

Due to their properties of encapsulating and transporting different molecules, but also the possibility of modifying their surface so as to present a high degree of biocompatibility, dendrimers can be used in cancer diagnosis.

3. The Use of Dendrimers in Cancer Diagnosis

Cancer encompasses a group of invasive diseases, which is why the early diagnosis of these diseases is extremely important in order to initiate effective treatment as early as possible. Cancer diagnosis can be established through many methods, two of which are magnetic resonance imaging (MRI) and computed tomography (CT) [56][57][58][59]. MRI is a technique used to obtain anatomical images of the internal organs and the vascular tree [19][60][61]. The use of contrast agents to obtain these images significantly improves their quality, and the administration of contrast agents can be done using dendrimers [1][56][62][63]. Contrast agents commonly used in MRI are paramagnetic metal cations of gadolinium, such as Gd(III)-N,N′,N″,N‴-tetracarboxymethyl-1,4,7,10-tetraazacyclododecane (Gd(III)-DOTA) and Gd(III)-diethylenetriamine pentaacetic acid (Gd(III)-DTPA), or other derivatives thereof. However, due to their low molecular weight, these agents can diffuse from blood vessels and

have a short circulation time in the body, and differentiated targeting towards diseased and normal tissues is inefficient. This disadvantage can be minimized by attaching gadolinium cations to the surfaces of dendrimers [19][30][37][56][62][64][65].

The efficiency of using dendrimers conjugated with contrast agents has been demonstrated by Bourne et al., who tested the efficiency of gadolinium-conjugated dendrimers in imaging the pelvic blood vessels of rabbits. They obtained much clearer images and much better contrast between blood vessels and the soft tissues around them when using gadolinium dendrimers [19][66]. Furthermore, PAMAM dendrimers labelled with Gd have been used to evaluate the development of lymphatic vessels in tumors [56][67][68].

Other authors have noted that these dendrimer systems could be used as molecular probes to amplify the signals of contrast agents when they reach a tumor microenvironment. This is achievable due to the ability to modify the surface properties and compositions of dendrimers by attaching specific antibodies or ligands targeting tumor receptors [64][69][70][71]. Dendrimers can also be covered with a transducer film functionalized with receptors for certain biological analytes. When the analytes bind to specific receptors, the transducer film will be mechanically stimulated and will produce a signal. In this way, cancer biomarkers can be detected [72][73][74][75].

PEG-covered PAMAM dendrimers have been used for subsequent conjugation with antibodies, folic acid, or biotin molecules, serving for specific capture of circulating tumor cells [72][76][77]. Additionally, the surface of the dendrimer can be covered with DNA/RNA or biotinylated antibodies to detect cancer antigens [72][78][79].

Thus, dendrimers can be used in many ways for the diagnosis of cancer, with the possibility of combining multiple utilities of these macromolecules for a single investigation, such as dendrimers used both in targeting the tumor and improving the images obtained by MRI.

In addition to the uses mentioned so far in cancer diagnosis, dendrimers can also be used for immunodiagnosis. Immunodiagnosis is based on the generation of signals that can be easily visualized when there is an antigen–antibody interaction between certain target molecules. The use of antibodies in immunodiagnostics allows the attachment of a single group that emits a fluorescent signal when the antigen–antibody complex has been formed, but, using dendrimers, a large number of signal molecules can be attached (for example, fluorescein) and the fluorescence signals are greatly improved; thus, immunodiagnosis is influenced by the density of molecules capable of emitting light signals [80].

A study analyzed the fluorescent signals emitted by antibody–fluorescein conjugates compared to the fluorescent signals emitted by antibody–dendrimer–fluorescein complexes and it was proven that the intensity and clarity of the fluorescence signals was significantly stronger when dendrimers were used [81].

The aim of another study was to synthesize a complex immunodiagnostic device based on dendrimers; PAMAM dendrimers with a ferrocene core were used, i.e., dendrimers that were coated with cysteamine-modified gold electrorides. The molecules obtained had the function of providing the analytical redox signal generated by the

ferrocene fragments and immobilizing the prostate-specific antibody (PSA) with the help of the primary amino group on the surface of the dendrimer. The best results were obtained using first-generation dendrimers, with these complexes detecting PSA from 10 pg/mL to 100 ng/mL [82].

As mentioned previously, one of the greatest advances in the field of cancer is the use of molecules that can precisely target the tumor microenvironment, and dendrimers, in addition to their use in diagnosis, can also be used in the targeting and treatment of tumors.

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