TITLE

Transferrin-bearing dendrimers for cancer therapy: an update

AUTHORS NAMES AND AFFILIATIONS

Sukrut Somani and Christine Dufès *

Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde,

161 Cathedral Street, Glasgow G4 0RE, United Kingdom

*** CORRESPONDING AUTHOR**

Christine Dufès

Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde,

161 Cathedral Street, Glasgow G4 0RE, United Kingdom

Phone: +44 141 548 3796

Fax: +44 141 552 2562

E-mail: C.Dufes@strath.ac.uk

PHOTOS

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Recent advances in multi-disciplinary research have resulted in the emergence of an increasing number of promising therapeutic strategies against cancer. However, the intravenous administration of these drugs is often limited by their inability to specifically reach the tumors, thus resulting in damaging secondary effects on healthy tissues. In order to remediate to this problem, a wide range of delivery systems have been investigated, aiming to specifically target the cancer cells and deliver their cargo to the pathological site. Among them, transferrin-bearing dendrimers are emerging as highly promising non-viral vectors for efficiently delivering drugs and nucleic acids to the cancer cells. The focus of this editorial is to provide an update on the therapeutic advances being made so far when using these targeted delivery systems.

Why using transferrin for targeting cancer cells?

Transferrin, a member of the family of iron-binding glycoproteins, has for main role to bind and distribute iron in the body to various target tissues overexpressing transferrin receptors (TfR) 1 and 2 [1-2]. Despite being expressed at low levels on most normal tissues, TfR1 is overexpressed on highly proliferative cells such as cancer cells (at levels higher than 100-fold compared to normal cells) [3-5]. Transferrin can also bind to TfR2, whose α-transcript product is mainly expressed on hepatocytes, but with 25-fold less affinity than for TfR1 [6]. In addition, the level of expression of TfR1 on tumors is correlated with cancer progression and tumor stage, making it a particularly promising target for the delivery of anti-cancer therapeutics to tumors [3-5].

Why using dendrimers as delivery systems for cancer therapy?

Dendrimers present unique advantages that make them highly promising for the delivery of drugs and nucleic acids to cancer cells. These highly branched polymers are composed of multiple monomers emerging radially from a central core similarly to a tree (“Dendron” in Greek) [7-11]. Thanks to their structure and spherical shape, they present a large number of surface functionalities that can be utilized for conjugating targeting ligands or drugs, as well as complexing nucleic acids. Their radial ramification of dendrons also delimitates internal cavities that have been used for the entrapment of anti-cancer drugs [7-11]. These features made them unique, extremely attractive polymers.

Recent use of transferrin-bearing dendrimers for cancer therapy

Despite their promising characteristics, transferrin-bearing dendrimers or TfR-targeting dendrimers have not been widely used so far. In a study by Han and colleagues, a PEGylated generation 5- poly (amido amine) (PAMAM) dendrimer was conjugated to the peptide HAIYPRH (T7), which is transferrin receptor-specific [12].

This dendrimer was able to co-deliver the anti-cancer drug doxorubicin (entrapped within its internal cavities) and a therapeutic plasmid encoding human tumor necrosis factor-related apoptosis–inducing ligand (TRAIL) (complexed to its surface by electrostatic interactions) (average size of the dendriplex: 200 nm). The presence of T7 peptide on the surface of the dendrimer enhanced the cellular uptake by the human liver cancer Bel-7402 cells overexpressing Tf receptors, compared to what observed with the non-targeted dendrimer. *In vivo*, the following intravenous administration of this dendriplex led to a synergy between the two therapeutic entities carried by the same dendrimer, resulting in the inhibition of 77% tumor growth on

mice bearing subcutaneous Bel-7402 human hepatoma. This impressive therapeutic effect was much higher than that observed with the drug only or the non-targeted dendrimer [12].

Another PEGylated PAMAM dendrimer has been studied for the treatment of C6 glioma. The targeting strategy in this study was different compared to the previous study. In this case, the dual targeting Tf- wheat germ agglutinin (WGA) was intended to target the TfR1 present on the blood-brain barrier, not on the tumor: TfR1 is expressed at low levels in most normal tissues, but is highly expressed on the vascular endothelium of the brain capillaries among normal tissues [13]. As a result, TfR1-targeting on mice without peripheral tumors would therefore reach the brain, whereas a similar targeting on mice bearing peripheral tumors would mainly reach the tumors. Tf- and WGA- bearing PEGylated generation 4-PAMAM dendrimer loaded with doxorubicin (average size of the drug-loaded dendrimer: 199 nm) was able to decrease the viability of C6 glioma cells in a brain microvascular endothelial cells (BMVEC)/C6 glioma co-culture model. The viability of C6 glioma cells was 14.5 % in comparison with 21.3 % for PAMAM-PEG-Tf-DOX, 23.7 % for PAMAM-PEG-WGA-DOX and 22.4 % for free DOX [14].

A pH-sensitive dual-targeting PEGylated generation 4-PAMAM dendrimer was also synthesized by conjugating Tf on the surface of the dendrimer and entrapping tamoxifen and doxorubicin (average size of the drug-loaded dendrimer: 117 nm) [15]. The resulting dendrimer exhibited high BBB transport ability across an *in vitro* BBB model and displayed an accumulation of doxorubicin in avascular C6 glioma spheroids.

We recently used another dendrimer, generation-3 diaminobutyric polypropylenimine (DAB) as an alternative of PAMAM for cancer therapy. As demonstrated by Kabanov

and colleagues [16], one of the advantages of this dendrimer is that it binds to DNA via electrostatic interactions involving only the peripheral amine groups, leaving the tertiary amine groups in the dendrimer free to act as a “proton sponge” in the endosome. We recently demonstrated that Tf-bearing DAB dendriplex (average size: 287 nm) resulted in gene expression mainly in the tumors after intravenous administration [17]. Consequently, the intravenous administration of this dendrimer complexed to a therapeutic DNA encoding tumor necrosis factor (TNF) α resulted in the disappearance of 90% of A431 human epidermoid carcinoma tumors, compared to the 40% complete tumor regression observed with the non-targeted dendriplex [17].

It was less efficacious on prostate cancer cells, resulting in tumor suppression for 60% of PC-3 and 50% of DU145 prostate tumors [18]. In a parallel study, we wanted to evaluate the therapeutic potential of a plasmid DNA encoding p73, a member of the p53 family of transcription factors, following complexation to DAB-Tf. We demonstrated that, indeed, systemically administered p73-encoding DAB dendriplex resulted in a prolonged inhibition of tumor growth and tumor suppression for 10% of A431 and B16-F10 tumors in mice [19]. Crucially, the treatments with the various tested dendriplexes were well tolerated by the animals.

Future perspective

TfR–targeting dendrimers have demonstrated to be efficacious delivery systems for therapeutic genes and drugs to cancer cells and have already led to significant improvements in the systemic delivery of therapeutic entities to their site of action. Still, the question of absolute targeting remains unanswered (and may still be so for a long time?). So far, we do not have 100% of drugs or genes delivered exclusively to

the tumors, using Tf or any other targeting ligand. Various strategies are currently investigated to improve the level of tumor targeting, with more or less success. For example, we recently investigated if the replacement of Tf by Lactoferrin (Lf), an iron-binding member of the Tf family which has intrinsic anti-cancer properties [20], on the surface of DAB, would lead to improved tumor targeting and improved therapeutic efficacy. The conjugation of Lf to DAB dendrimer increased gene expression in the tumor while decreasing the non-specific gene expression in the liver, but with tumor targeting effects that were not improved compared to that obtained with DAB-Tf [21]. The intravenous administration of Lf-bearing DAB dendriplexes encoding TNFα (average size: 260 nm) led to suppression of 60% of A431 tumors and up to 50% of B16-F10 tumors [21].

Other studies have investigated the use of anti-TfR monoclonal antibodies for enhancing tumor targeting. However, unlike Tf, these antibodies do not appear to have more affinity for tumor-overexpressed TfR1 than for non-specific TfR2 [22].

The use of dual targeting as another strategy to increase tumor targeting of Tf-bearing dendrimers appears to be promising and is currently under investigation. As described above, this strategy has already proven its efficacy for its targeting of the blood-brain barrier and should hopefully demonstrate the same improvement on cancer cells.

Despite the need of “fine tuning” cancer targeting, the encouraging advances related above demonstrate that the targeting of Tf receptor still remains a huge potential for the development of more specific and safer gene and drug-based therapeutics. Clinical trials using dendrimers in cancer therapy are still pending, but dendrimers have already been successfully introduced in the clinic as anti-viral agents. It is hoped that continuous research in this area would enable the synthesis of “next

generation” Tf-bearing dendrimers-based nanomedicines, expected to provide many exciting therapeutic opportunities and to pave the way for better treatment options against cancer.

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