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# Previews

## Targeting Cancer Cells with Dendrimers

**Bifunctional PAMAM-based dendrimers that selectively target cancer cells are described in this issue of *Chemistry & Biology* [1]. The targeting moiety for folate receptor is complexed to an imaging or therapeutic agent by a DNA "zipper"—a versatile strategy to create libraries of novel drugs.**

Dendritic, or "tree-like," structures widely occur in nature [2]. The dendritic network of hairs found on the Gecko foot that allows amazingly strong attachment to many types of surfaces due to van der Walls forces provides a dramatic example of dendrimers in action [3]. Clearly, synthetic methods to create dendritic molecules in the laboratory have been of great interest for many decades, for both scientific and practical reasons. A lack of facile synthetic approaches to create these molecules, however, hampered development of this field until approximately 25 years ago. In 1978, Vögtle's group reported the innovative "cascade" methodology for the synthesis of dendrimer-like molecules [2, 4], and since then, the intense efforts underway to create these macromolecules have followed two major strategies. First, in the divergent method, the growth of a dendron (a molecular tree) originates from a core site, or root; conversely, in the convergent growth process, synthesis proceeds from what will become the dendron molecular surface (i.e., from the leaves of the trees) inward to a reactive focal point at the root. Together, these efforts have resulted in the synthesis of over 100 compositionally different dendrimer families [5].

Dendrimers, being chemical entities with biological properties, provide an outstanding example of the close relationship between these two fields. As mentioned, these molecules were initially inspired by nature. The efforts of synthetic chemists to create mimics, however, have resulted in dendritic clusters with a wide variety of properties and potential applications, many of which are now distinctly nonbiological. For example, organometallic dendrimers are finding uses as quantum dots, as industrial catalysts, and in electronics [5, 6]. Other families of dendrimers remain firmly at the intersection of chemistry and biology. For instance, these molecules can be constructed to closely resemble proteins, as they are of comparable size and can be endowed with similar biologically compatible surface properties. Their surfaces, however, can also be given a significant repertoire of tunable characteristics not found on natural proteins; this feature has greatly propelled efforts toward the development of practical applications for these molecules. In particular, the ability of a dendrimer to be functionalized with far more surface groups than a protein of comparable size has given impetus to their widespread use as drug delivery vehicles.

It is no secret that drug design and delivery constitutes a formidable biomedical challenge. A potential drug must first selectively recognize and bind to a molecular target, then trigger an appropriate biological response, and all the while possess pharmacological properties that render it "drug-like." In some cases, nature has supplied appropriate small molecules—such as aspirin or penicillin—that can be used directly as drugs. Meanwhile, rational design of small-molecule drugs has, in general, proven to be remarkably intractable, especially when confronting complex diseases like cancer. Cancer epitomizes the challenges confronting drug delivery efforts: 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 kill the cell. Not surprisingly, single small molecules often function poorly at these tasks, thereby requiring the use of therapeutic cocktails in clinical practice. Proteins have shown promise in combining aspects of the different biofunctions expected of an anticancer drug. Specifically, monoclonal antibodies raised against tumor-associated antigens provide selective targeting of cancer cells; when conjugated to toxic agents, they can also kill the target cell. In many cases, however, antibody-based conjugates struggle to provide sufficiently high levels of drug. To illustrate this, consider that a cancer-specific antibody might gain access to 50,000 binding sites per cell. The widely used anticancer drug cisplatin requires internalization of at least ten times this level for efficacy. Consequently, antibody conjugates generally exclude small-molecule drugs and are limited to toxins, such as ricin, where the catalytic activity of this ribosome-inactivating enzyme allows a single molecule to kill a cell [7]. Of course, conjugation of two proteins (ricin and the antibody) does nothing to improve the poor pharmacological properties of either entity alone, and it is not surprising that after two decades of investigation such strategies remain far from widespread practical use.

The cancer cell-targeting approach described in this issue by Choi and coworkers [1] is reminiscent of the antibody-toxin/immunoconjugate strategy where distinct, but linked, entities are used to first recognize and bind and then subsequently modify a cancer cell. The current investigators' strategy, however, has great potential to improve on both the "targeting" and "payload" capsules by replacing the protein subunits with dendritic PAMAM-based clusters [8] that are covalently conjugated to complementary oligodeoxynucleotides as shown schematically in Figure 1. This approach takes advantage of the already-established biocompatibility of DNA and PAMAM, which has often been used to introduce DNA into cells for gene delivery, except that now DNA is used as a zipper between a folate-derivatized targeting capsule and its payload, an imaging agent (FITC) in this case. It also exploits the facile

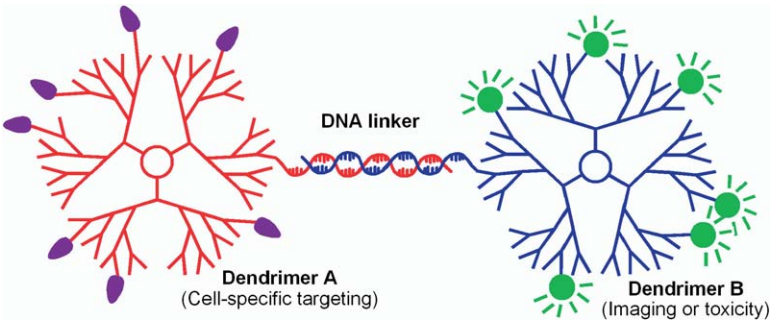


Figure 1. DNA-Dendrimer Conjugates as Potential Cancer Targeting Imaging Agents or Therapeutics

Differentially functionalized dendrimers covalently conjugated to complementary deoxy-oligonucleotides can readily form duplex combinatorial nanoclusters that possess cancer cell-specific ligands hybridized to an imaging agent or drug. Cell-specific target ligands (e.g., folic acid) are appended to dendrimer A, and dendrimer B is conjugated with an imaging agent (e.g., fluorescein) or drug.

duplex DNA formation for the generation of hybrid nanoclusters, thus circumventing the tedious synthesis of multiply-functionalized dendrimers. The current work is limited to "proof-of-concept" experiments, and a long road lies ahead to actual use at the clinical level. For example, potential obstacles include nonspecific cellular uptake of PAMAM-based cationic dendrimers, membrane destabilization, and toxicity [6]. These limitations, however, are already being addressed by further chemical modification of the dendrimer surface [9] or substitution with anionic, polyester dendrimeric clusters [10], leaving viable the exciting possibility that this technique can be applied to many types of cancer by mixing-and-matching various targeting ("A" in Figure 1) and modifying ("B") clusters.

In the future, bifunctional dendritic molecules can be readily adapted to exploit surface markers other than the canonical folate receptor [9, 11] or the numerous approaches that target tumor-associated antigens [12]. One area of rapidly expanding investigation is the abnormal glycosylation associated with the cancer cells. Dendrimeric scaffolds provide a unique platform to control the multimeric carbohydrate presentation needed to enact the "cluster glycoside effect" [13, 14], which is crucial for targeting diseased tissues in malignant diseases [2, 6]. Finally, additional "chemical biology" strategies can come into play, such as the expression of metabolic analogs into sialic acids, sugars that are often overexpressed on cancer cells to provide a "chemical handle" for targeted delivery of a second agent [15]. In this case, the dendritic presentation of complementary chemical functional group could enhance binding of the second agent to the cell surface.

To complement the targeting cluster and complete the activity of a DNA-assembled PAMAM cluster, the action modules represented by "Dendrimer B" (in Figure 1) fall into two main categories; one category consists of imaging agents such as the FITC conjugate used in the current cell-based work. A natural extension of this approach will be to substitute gadolinium chelators for use as MRI contrast agents [16] for the detection and diagnosis of cancer. The actual killing of 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 simple cell labeling. Many options are available to ensure successful delivery of the prodrug into the cell. These include activation by low pH found in endosomal vesicles, installation of enzyme-cleavable ester linkages onto the drug, disulfide bonds that are liberated in the reducing environment of the endoplasmic reticulum, or sensitivity to ultrasound. Clearly, a wealth of different forms of bifunctional clusters can be envisioned. The ability to design and produce arrays of dendrimers assembled by DNA zippers constitutes an important step in the development of these versatile nanoclusters as imaging and therapeutic agents.

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**Selected Reading**

1. Choi, Y., Thomas, T., Kotlyar, A., Islam, M.T., and Baker, J.R. (2005). Chem. Biol. *12*, this issue, 35–43.
2. Boas, U., and Heegaard, P.M.H. (2004). Chem. Soc. Rev. *33*, 43–63.
3. Autumn, K., Liang, Y.A., Hsieh, S.T., Zesch, W., Chan, W.P., Kenny, T.W., Fearing, R., and Full, R.J. (2000). Nature *405*, 681–685.
4. Buhleier, E., Wehner, W., and Vöglte, F. (1978). Synthesis (Mass.) *405*, 155–158.
5. Esfand, R., and Tomalia, D.A. (2001). Drug Discov. Today *6*, 427–436.
6. Ozkan, M. (2004). Drug Discov. Today *9*, 1065–1071.
7. Olsnes, S. (2004). Toxicon *44*, 361–370.
8. Maiti, P.K., Çagin, T., Wang, G., and Goddard, W.A. (2004). Macromolecules *37*, 6236–6254.
9. Quintana, A., Raczka, E., Piehler, L., Lee, I., Myc, A., Majoros, I., Patri, A.K., Thomas, T., Mulé, J., and Baker, J.R. (2002). Pharm. Res. *19*, 1306–1310.
10. Ihre, H.R., Padilla De Jesús, O.L., Szoka Jr, F.C., and Fréchet, J.M.J. (2002). Bioconjug. Chem. *13*, 443–452.
11. Leamon, C.P., and Reddy, J.A. (2004). Adv. Drug Deliv. Rev. *56*, 1127–1141.
12. Zhang, J.-Y. (2004). Cancer Detect. Prev. *28*, 114–118.
13. Yarema, K.J., and Bertozzi, C.R. (1998). Curr. Opin. Chem. Biol. *2*, 49–61.
14. Lundquist, J.J., and Toone, E.J. (2002). Chem. Rev. *102*, 555–578.
15. Mahal, L.K., Yarema, K.J., and Bertozzi, C.R. (1997). Science *276*, 1125–1128.
16. Kobayashi, H., and Brechbiel, M.W. (2003). Mol. Imaging *2*, 1–10.