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Polymers for cancer gene therapy

Browsing the library

JN Sachs, H Shen and WM Saltzman

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In gene therapy for treatment of cancer, a principal aim is to introduce genes that will kill cancer cells, while preserving healthy ones. A key component of this effort is precise control of gene expression: that is, expression of a gene product in the right cells and at the right level. Manipulations of either DNA constructs or delivery systems are the general approaches used to control transgene expression. In a recent study published in *Proceedings of the National Academy of Sciences*,[1] Anderson *et al* exploited both strategies. They identified the best polymer for gene delivery from a library, with which they were able to express a suicide gene at high levels in xenografted human cancer cells, but poorly in surrounding healthy cells. This strategy allowed the authors to reduce tumor growth in the animal with minimal systemic toxicity.

The delivery of a suicide gene to cancer cells has been a promising approach for cancer therapy for some time, and several suicide genes have been explored. Diphtheria toxin (DT) is a particularly attractive option because it works in aggressively growing tumors as well as slowly growing ones.[2] However, one major problem that must be addressed when DT is used is how to avoid deleterious effects to normal, healthy cells. One solution is to design plasmid constructs that more specifically target the cancer cells. However, such constructs are necessarily larger and more complex and one viral vector cannot accommodate all the sequences required. Thus, two or more viral vectors have to be used, which creates more challenges: in particular, for their safe and easy use in clinical practice.

An alternative approach to delivering large constructs is to use nonviral DNA delivery systems, which can accommodate much larger DNA constructs.[3] However, nonviral delivery systems have their own problems: they are inefficient and lack the specificity needed for cancer therapy. Moreover, identifying a nonviral DNA delivery system for tight control of gene expression in targeted cells is inherently difficult because little is known about exact mechanisms involved in nonviral DNA transfer. So, there are some key questions that need to be addressed before nonviral delivery systems can achieve their full potential for cancer gene therapy. Specifically: Can nonviral delivery systems be designed to achieve a sufficient level of gene expression? Can specificity to cancer cells be designed into the material?

To address these questions, Anderson *et al* used high-throughput screening of polymer libraries to identify the best polymer for DT suicide gene delivery. The authors' identification of this polymer, which they called C32, was in itself a notable achievement. However, perhaps as important are the chemical lessons that their work might teach: For example, can we understand why C32 was successful and why other synthesized polyplexes were not? What is it about the chemistry of this particular polymer that allowed the targeting, entry and intracellular trafficking necessary to transfect the tumor cells?

While targeting of polyplexes to specific cell types has often involved conjugation with target-specific ligands, the success of C32, a nonconjugated polyplex, suggests that there might be more fundamental processes that govern specificity and efficacy of nonviral gene delivery. Two well-known properties of cancer cells direct us to the lipid membrane as a key barrier. First, protons are pumped across the membranes of cancerous cells, creating an acidified extracellular milieu. Second, tumor cells show marked changes in the composition of lipids in their membranes relative to noncancerous cells. To understand and eventually exploit the effect of these two factors on transfection requires an understanding of the forces that underlie the stability of a polymer–DNA complex.[4] Local environmental factors determine the time and place at which DNA will be released from the complex: too soon or too late, and the delivery will fail.

A thermodynamic description of polymer–DNA complexes could help us understand their biological activity. For example, it is known that changing the salt conditions of the solution in which a polyplex is formed can profoundly affect transfection efficiency. Counter-ions neutralize isolated polymers and DNA in solution, so these must be stripped before a polyplex can form. There are thermodynamic consequences of this process, as associated water molecules experience a change in their molecular freedom, or entropy. This change in entropy depends on the salt solution: in a solution in which the entropy increases greatly, complexes are likely to be highly stable. Therefore, the electrostatic environment of the polyplex establishes a balance of forces that collectively determine the equilibrium between the associated and dissociated state. This is only one example of possible energetic and entropic effects on complex stability: the pH of a solution might have the same effect, by changing protonation states on the polymer. Such influences on the stability of the polymer–DNA complex could explain differences in transfection activity for these complexes towards cancer and noncancer cells.

How might the lipid composition of the cellular membrane, as well as intracellular compartments, affect the efficiency of gene delivery? A polyplex must interact favorably with the membrane in order to induce either endocytosis or the large-scale structural rearrangements that facilitate uptake. We now know that biological membranes are a good deal more complex and variable than has traditionally been represented by the fluid-mosaic model. Membranes composed of lipids with different chemical properties will respond differently to the binding of a polyplex, in ways that might alter polyplex activity. For example, lipid head group charge and size can affect the polyplex association–dissociation equilibrium.

Interestingly, both of these factors are dramatically modified in some cancer cells.

As Anderson *et al* point out, a large number of the successful polyplexes, including C32, were made from hydrophobic acrylates, and we can assume that this property facilitates critical interactions with the membrane. The changes in tumor cell lipid composition and environmental pH might be one of nature's most valuable clues in how to focus our engineered gene-therapy attack. While the engineering of nonviral gene delivery agents is increasingly heroic, as this present study demonstrates, it will not match viral evolution until we understand more about the molecular pathway that a polyplex takes. With the wealth of knowledge regarding lipid properties now available from the biophysical community, and the growing arsenal of polymer-based gene delivery particles that studies like this afford us, we might soon be able to rationally target these particles based upon electrostatic, thermodynamic and structural properties of membranes. ■

JN Sachs is at the Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT, USA and H Shen and WM Saltzman are at the Department of Biomedical Engineering, Yale University, New Haven, CT, USA. E-mail: mark.saltzman@yale.edu
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