S6-1 *[Oct. 20 (Fri) 13:30~14:20/Hall A]*

The Use of Synthetic polymers in Gene Delivery using Viral and Non-Viral Systems

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There are two main types of synthetic vector for gene delivery those based essentially on cationic lipids, and those based on cationic polymers. The former have the advantage of good ability to disrupt membranes and mediate relatively efficient delivery of DNA, while the latter are more selective in transfection and are more suitable for intravenous administration. Cationic lipid/DNA complexes are usually sequestered by the first capillary bed they encounter, normally the pulmonary bed following intravenous administration, and this severely hampers their ability to reach dispersed systemic cellular targets. Polyelectrolyte DNA complexes, on the other hand, show less tendency to occlude capillary beds following intravenous injection, often passing through the lungs into the oxygenated blood and then dispersing throughout the body in the arterial circulation.

We have been evaluating the possibility of introducing a surface layer of hydrophilic polymers onto the surface of polyelectrolyte complexes in order to induce a level of stabilisation, preventing their interaction with cells and proteins (Dash et al., 2000). Probably the most significant advance is the demonstration that coating the surface of polyelectrolyte DNA vectors with multivalent hydrophilic polymers endows them with extended plasma circulation profiles. Whereas non-coated poly(L-lysine) (pLL)/DNA complexes are cleared immediately into the liver, pLL/DNA complexes coated with reactive polymer based on N-[2-hydroxypropyl]methacrylamide show extended circulation and accumulate within subcutaneous tumours. We are continually improving formulation of polymer-coated complexes, but presently can achieve a 30 min circulating level of DNA of over 75%, with a clearance half life 90 min.

Careful selection of coating conditions and methods can easily endow the complexes with various surface properties, depending on the requirements of the

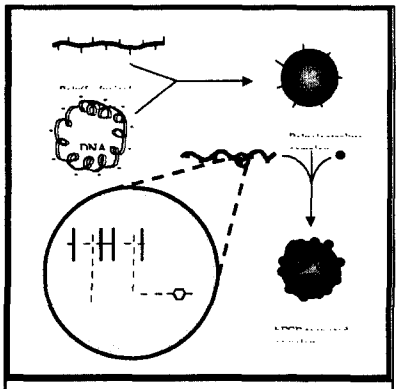

Figure 1: Coating and targeting of

precise application, and they can be targeted by linkage of targeting agents onto the polymer coating (Figure 1)

Ligand-targeted complexes demonstrate specific uptake into receptor-positive cells (Fisher et al., 2000). Ligand-targeted pLL/DNA complexes also show high levels of receptor-mediated transfection, resistant to inhibition by serum. This suggests that such targeted complexes may be suitable for application in vivo, where the presence of plasma is known to abolish infection using many agents.

Polymer-based retargeting of adenovirus:

Recent studies in our group have led to the rather unexpected observation that adenoviruses coated with a non-biodegradable polymer can be retargeted to enter cells via novel receptors, and mediate highly efficient transgene expression. Adenovirus normally enters cells by binding via the knob domain of fibre protein to the Coxsackie and Adenovirus receptor (CAR) and then internalising through an interaction of the penton base protein with av integrins. Widespread distribution of CAR has led to a requirement for less-promiscuous infection, however attempts to produce target-specific virus have so far focussed on genetic strategies or using bi-specific antibodies, both approaches that do not avoid neutralisation by pre-existing anti-adenovirus antibodies. We have recently developed a non-genetic approach to retargeting adenovirus: The virus is first coated with a multivalent hydrophilic polymer based on poly-[N-(2-hydroxypropyl)methacrylamide] (pHPMA), preventing CAR-binding and recognition by neutralising antibodies, and then surface-modified with new ligands to endow the required tropism. To date we have retargeted virus using basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF) and transferrin. In each case CAR-binding is blocked by the presence of the polymer coat, the virus attaches to cells via the new ligand and is able to enter cells and express encoded transgenes as efficiently as the unmodified virus using CAR in fully permissive cells.

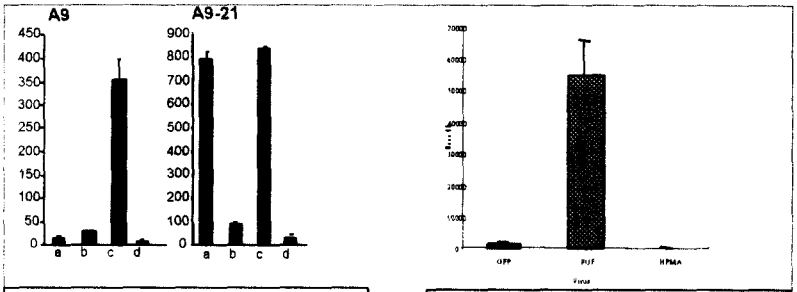

Figure 2. Infection of A9 and A9-21 cells with AdGFP, either unmodified (a), polymer-coated (b) or retargeted with bFGf (c) or VEGF (d). 48h p.i.

Figure 3. Infection of SKOV3 cells with unmodified AdGFP ("GFP"), bFGF-pc-AdGFP ("FGF"), pc-AdGFP ("HPMA"). $1x10^5$ cells, $6x10^8$ particles. 48h p.i

Figure 2 demonstrates ablation of CAR-mediated binding and transduction of A9-21 cells (which express CAR) by polymer coated adenovirus (pc-virus), and restoration via bFGF retargeting, but not via VEGF. In contrast A9 cells (CAR-negative) show no transduction by the unmodified virus, although retargeting with bFGF yields good transgene expression. Figure 3 shows similar data, obtained using the human ovarian cell line SKOV3. These pc-viruses show great versatility for cell-specific targeting, and we are presently developing them for systemic targeting to disseminated cancer cells.

References

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- Fisher, K.D., Ulbrich, K., Strohalm, J., Ward, C.M., Mautner, V., Blakey, D. and Seymour, L.W., A versatile system for receptor-mediated gene delivery permits increased entry of DNA into target cells, enhanced delivery to the nucleus and elevated rates of transgene expression, *Gene Therapy*, 7, 1337-1343 (2000).