

2002b). It was also found that transfection efficiency was increased by an order of magnitude using TMO compared to chitosan in COS-1 (African green monkey kidney fibroblast) cells (Thanou et al., 2002). This favourable profile led to the study of trimethylated chitosans as the gene delivery vector in this thesis.

1.5.3 Targeted Non-viral Gene Delivery

Non-viral vectors are poorly efficient transfection systems when compared to viruses. It has been reported that at best, PEI polyplexes were taken up by 90 % of cells (30 mM) compared with 1 virus per cell being effective (Zaric et al., 2004). This has prompted many to investigate the possibility of incorporating targeting ligands into non-viral vectors with the aim of improving transfection efficiency.

1.5.3.1 Targeted Lipoplexes

Stealth liposomes are formed through the coating of the liposome with PEG, although this increases residence time in the body, cell uptake and therefore transfection efficiency is often lost. In an attempt to restore the activity of these stealth liposomes ligands are attached to them. When pegylated immunoliposomes carrying β -galactosidase (β -gal) were prepared containing a humanised anti-transferrin antibody and administered intravenously in Sprague Dawley rats, β -gal expression was found in the blood brain barrier, liver and spleen with little expression elsewhere (Shi et al., 2001). Although specific β -gal expression was claimed, the multiple sites at which expression was observed highlights the broad expression of transferrin receptor and raises questions over its value as a target.

Asialofetuin (a glycoprotein having tri-antennary galactose terminated sugar chains that targets ASGR) was included in a lipoplex containing the chloramphenicol acetyltransferase (CAT) gene and an increase (almost double) in CAT activity was observed in HepG2 cells (Hara et al., 1995). Similarly an anti-transferrin single chain antibody fragment bound to liposomes led to a 2-6 fold increase in transfection in H358, DU145, Hep3B and HT29 cells (Xu et al., 2002). Subsequent *in vivo* studies with p53 gene lipoplexes delivered via tail vein injection into tumour bearing mice and transfection assessed by Western blotting showed markedly enhanced p53 expression in DU145 tumours and low p53 expression found elsewhere (Xu et al., 2002). Integrin receptors were efficiently targeted using a 1,2-distearoyl-sn-glycero-3-

phosphoethanolamine-N-PEG (5 kDa) ACDCRGDCFCG-COOH DOTAP liposome with a 100-fold increase over the pegylated liposome without the RGD containing peptide. Lipoplexes were also targeted by Anwer et al. (2004) using RGD peptides and a 10-fold increase was observed in human umbilical vein endothelial cells (HUVEC) compared to non-targeted lipoplexes (Anwer et al., 2004).

1.5.3.2 Targeted Polyplexes

The idea of targeting polyplexes is not new and in 1988 Wu and Wu prepared asialoglycoprotein-PLL/DNA polyplexes to target ASGR (Wu & Wu, 1988). They found highly selective expression of a foreign reporter gene protein (CAT) in the liver demonstrating targeted gene delivery for the first time (Wu & Wu, 1988). Many efforts have been made to target PEI/DNA complexes (Benns et al., 2002, Blessing et al., 2001, Guo & Lee, 1999, Kircheis et al., 1997, Kircheis et al., 2001a, Kunath et al., 2003b, Kursu et al., 2003, Lee et al., 2002, Ogris & Wagner, 2002a, Sagara & Kim, 2002, Zanta et al., 1997) with varying degrees of success (Table 1.6). The PEI conjugates jetPEI-ManTM (linear PEI conjugated to mannose) and jetPEI-GalTM (linear PEI conjugated to galactose) are available commercially for the increased efficiency of transfection of mannose receptor or ASGR expressing cells respectively (Qbiogene, 2003).

With the aim of targeting chitosan-derived polyplexes, trimethylated chitosan polymer derivatives were further modified at the 6-O-position with chloroacetic acid and then galactose (Murata et al., 1996) and antennary galactose conjugates were prepared (Murata et al., 1997). Transfection was studied using HepG2 (human hepatoma) cells. The galactose-containing trimethyl chitosan produced a small (although statistically insignificant) increase in transfection over trimethyl chitosan, with a larger increase (although probably statistically insignificant) being seen in cells transfected with tetra-antennary galactose trimethyl chitosan (Murata et al., 1996, Murata et al., 1997). Galactosylated chitosan (25 kDa, 85 % deacetylated) was prepared by Gao et al. (2003) and tested on human hepatocellular carcinoma cell lines. Again, a small (although probably statistically insignificant) increase in transfection efficiency over the un-targeted polymer was seen (Gao et al., 2003). Competitive inhibition of transfection with 50 mM lactose showed a statistically significant decrease indicating that targeting had been achieved (Murata et al., 1997).