

1.3.2.2 Proteins as Ligands in Targeted Delivery

For the purposes of this thesis a protein is defined as a chain of more than 20 amino acids (aa). The smallest protein described being a 20 aa Trp cage motif (Neidigh et al., 2002). Molecules with less aa are considered to be peptides, discussed in Section 1.3.2.4. Compared to antibodies proteins are easier to produce, store, are less likely to be immunogenic and are easier to specifically modify. However, many protein receptors investigated have a widespread expression in the body, e.g. the transferrin receptor (Qian et al., 2002). Protein-targeted therapy has similar problems to antibody-based targeting with their endogenous activity presenting an additional challenge.

Using transferrin-targeted poly(lactide-co-glycolide) (PLGA) paclitaxel containing nanoparticles Sahoo et al. (2004) found a greater reduction in tumour size (PC3 in mice) and increased survival compared with un-targeted nanoparticles or a paclitaxel formulation Cremophore[®] (Sahoo et al., 2004). In contrast, although greater tumour uptake was found using transferrin-targeted palmitoylated glycol chitosan vesicles containing doxorubicin compared with that seen using un-targeted vesicles, the targeted system was less effective in terms of antitumour activity than the free drug *in vivo* (Dufes et al., 2004). This suggests that, as tumour targeting was successful, the release of drug from the carrier must have been poor.

uPAR has been successfully targeted using a diphtheria toxin-urokinase fusion protein which showed selective toxicity in leukaemic cell lines expressing > 5000 receptors/cell (Ramage et al., 2003). This fusion protein was tested in mice against glioblastoma tumours and found to significantly regress tumours (Vallera et al., 2002). These successful studies targeting uPAR are promising indicators for this study. Protein-based targeting employed in non-viral gene delivery is discussed in Section 1.5.3.

1.3.2.3 Saccharide-Targeted Delivery

The term saccharide encompasses a wide range of molecules from simple sugars to complex polymers. Two saccharides widely employed as targeting ligands are mannose (to target the mannose receptor on macrophages (Ferkol et al., 1996)) and galactose (to target the asialoglycoprotein receptor (ASGR) on hepatocytes (Plank et al., 1992)). This targeting method has seen some success with the only actively targeted polymer-drug conjugate to reach clinical trial: PK2, an HPMA-doxorubicin-galactosamine copolymer,

which has progressed to phase I/II (Seymour et al., 2002). Both mannose and ASGR expression is, however, found on both cancerous and normal cells (Hashida et al., 2001). E-selectin, a receptor exclusively expressed in endothelial cells, has been targeted using Sialyl Lewis-X-coated PLGA microparticles containing fluorescent dyes (Eniola et al., 2002). Saccharide-based targeting employed in non-viral gene delivery is discussed in Section 1.5.3.

1.3.2.4 Peptide-Targeted Delivery

Peptide-targeted delivery has a basis in nature as many peptides are used as attachment ligands by bacteria and viruses. The use of peptides as ligands for receptor-targeting has been investigated by several groups (reviewed in Shadidi & Sioud, 2003). The approach chosen for this study uses peptides identified from the binding region of uPA. Peptide ligands have a number of advantages. These include their lower antigenic potential, making them less likely to cause an immune reaction. They are also easier to synthesise and characterise. Their smaller size means multiple peptides could be attached to a single nanoparticle conferring multivalent attachment.

The use of phage display libraries to determine binding peptides for tumour targets is a powerful method that has shown much success (Nilsson et al., 2000). This method is akin to combinatorial chemistry producing large numbers of potential molecules and selecting for them by their activity, in this case binding to the cell of interest. Essentially the technique involves the recombinant insertion of a peptide motif into the coat of the phage, application to cells and harvesting of attached phage, and the process repeated to purify the most effective phage (Nilsson et al., 2000).

Arap et al. (2002) targeted prostate cancer with a short peptide sequence (SMSIARL) derived from a prostate homing phage. This phage peptide was found to be in 15 times greater amounts in mouse prostate than a control peptide, signifying that targeting had been successful (Arap et al., 2002). In a fusion between this peptide and a proapoptotic peptide they found tissue destruction in the prostate and delayed development of cancer in prostate cancer prone transgenic mice (Arap et al., 2002). Phage display experiments are usually carried out using cell culture and the results are therefore less physiologically relevant. *In vivo* selection of phage has also been developed with great success (Reviewed in Trepel et al., 2002). However, the *in vivo* panning is made in animal models and therefore may have less specificity for human