Economical Synthesis and Evaluation of Cancer Targeting Constructs

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Statement of Purpose: By conjugating ligands specific for over-expressed cellular receptors with carrier entities such as polymers, multivalent constructs may be created enabling more specific targeting of tumor cells relative to non-tumor cells. Previous work in our group by Rosca et al. has demonstrated that specific targeting of glioblastoma cells via the $\alpha_6\beta_1$ integrin can be attained; however, the construct assembly requires lengthy peptide synthesis and purification steps. Here we demonstrate the development of a multivalent construct with a one-step conjugation of aminooxy-functionalized peptides to a pre-made polymer backbone. This synthesis scheme has the potential to make multivalent targeting more economical and easily customizable.

Methods: Multivalent polymer constructs consisting of a poly(3,3'-diethoxypropyl methacrylate) (PDEPMA) backbone conjugated to an aminooxy-functionalized dodecapeptide (TWYKIAFQRNRK) were synthesized. The conjugation involves the reaction of the deprotected polymer side chain aldehydes with the aminooxy group of the peptide, thereby forming a selective oxime bond (Li et al. 2006). Conjugation of PDEPMA with the aminooxy-terminated dodecapeptide was intended to obtain 10, 20, and 30 percent side chain substitutions. To ensure water solubility, the remaining free polymer side chains were reacted with aminooxy-functionalized tetra(ethylene glycol) (TEG) subsequent to each peptide reaction. Competitive binding assays (Rosca et al.) were performed using glioblastoma (SF767) and normal astrocyte (NHA) cell lines for each construct. Fluorescent binding assays were performed on both cell types and visualization of either construct or free peptide binding was observed.

Results/Discussion: H^1-NMR results indicate that multivalent constructs were successfully synthesized resulting in relative increases in peptide substitution for each intended substitution percent. Figure 1 shows the spectra for 30% peptide conjugation to PDEPMA. Aldehyde peaks characteristic of unreacted polymer are no longer observed at 9.5-10.5 ppm, instead sharp peaks in the 6.6-7.7 ppm range indicate oxime bond formation along with the presence of aromatic groups from tyrosine, tryptophan, and phenylalanine in the peptide (Fig 1d). Similarly, observation of the peptide peaks in the 1.1-1.5 ppm methyl group region (Fig 1b) is indicative of the CH_3 groups of threonine, isoleucine, and alanine. The relative trends of the increase in peptide in both the aromatic region, and in the upstream methyl group portion of the spectra indicate a relative increase in peptide presence relative to polymer (Fig 1a and c), which was further confirmed via absorbance spectroscopy measurements. Competitive binding assay results show that the 10, 20, and 30% peptide substituted multivalent constructs have at least equal avidity as peptide alone. When fluorescently labeled constructs are used to visualize binding to cells cultured *in vitro*, results show that for an optimal concentration (5μM), binding is attained primarily at the periphery of the SF767 cells. In contrast, minimal, rather punctate binding is observed to NHA cells (Fig 2b). Further, for peptide alone at the same concentration (5μM) (Fig 2c), no specific binding is observed, likely resulting from the higher dissociation constant of the peptide, k_r.

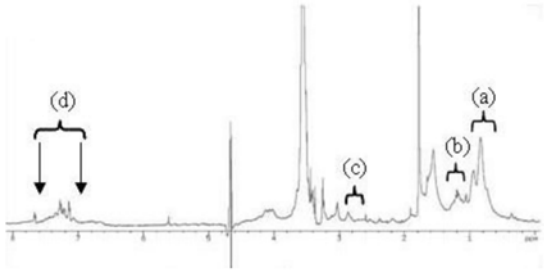

Figure 1. NMR results for polymer-peptide conjugate with intended peptide substitution of 30%.

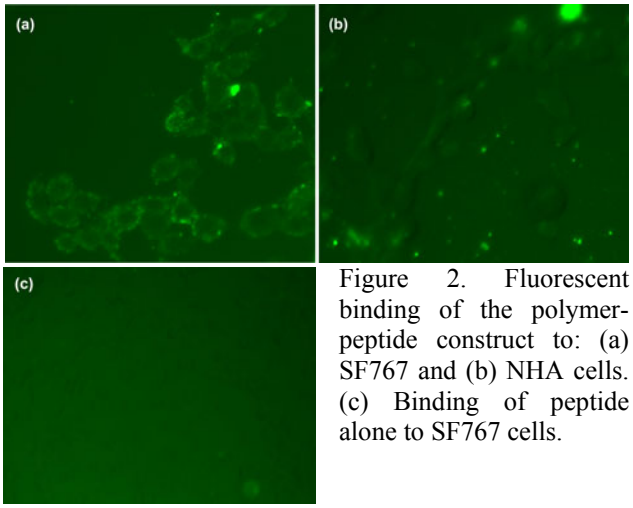

Figure 2. Fluorescent binding of the polymer-peptide construct to: (a) SF767 and (b) NHA cells. (c) Binding of peptide alone to SF767 cells.

Conclusions: Using a rapid, potentially inexpensive, and customizable method to synthesize multivalent constructs provides the ability to specifically target tumor cells via up-regulated cellular receptors. This could potentially enable an oncologist to biopsy a tumor, find a suitable receptor target, have an aminooxy-functionalized peptide synthesized and conjugated to an off-the-shelf polymer, which could then be used for imaging or therapeutics.

References: Li, R. et al. (2006). J Poly Sci: Part A: Polymer Chemistry 44(17): 5004-5013. Rosca, E.R. et al. (2007). Biomacromolecules 8:3830-35.

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