Cell Targeting via Multivalent Constructs

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Statement of Purpose: A novel approach to drug targeting or imaging relies upon conjugating a drug or an imaging molecule to a ligand which binds to a cell surface receptor over-expressed by the target cells. Here we develop a construct designed to target glioblastoma cells. Glioblastoma cells up-regulate the $\alpha_6\beta_1$-integrin, thus we can target using a ligand for this integrin. (Giese, Bjerkvig et al. 2003)

Viral particles use multiple weak binding events to increase the efficiency of infection, therefore multivalency (multiple ligands per construct) can increase the overall binding avidity of these constructs and potentially increase targeting specificity. (Caplan and Rosca 2005) These constructs offer hopes of enhanced targeting due to their small size (comparable to quantum dots) which will allow better diffusion/convection through tissue and their potential to degrade into components that can be eliminated through the kidney.

Methods: The peptide of interest (TWYKIAFQRNRK) was synthesized using standard Fmoc chemistry followed by the addition of a spacer consisting of three poly(ethylene glycol) chains each 20 atoms long (Novabiochem). This synthesis scheme was repeated to create a trivalent construct, containing three peptide sequences separated by two poly(ethylene glycol) spacers. Equilibrium binding assays were performed using the trivalent and monovalent (one peptide sequence) constructs in competition with a Eu-labled monovalent construct. Binding was determined using time-delayed fluoresce measurements using Victor V plate reader (Perkin Elmer). Binding of the constructs to a glioblastoma cell line (SF 767) and normal astrocyte line (NHA) for each construct was also investigated using biotinylated constructs followed by staining with streptavdin-Texas Red (Pierce). Prior to the binding assay cells were stained using Cell Tracker dyes (Invitrogen).

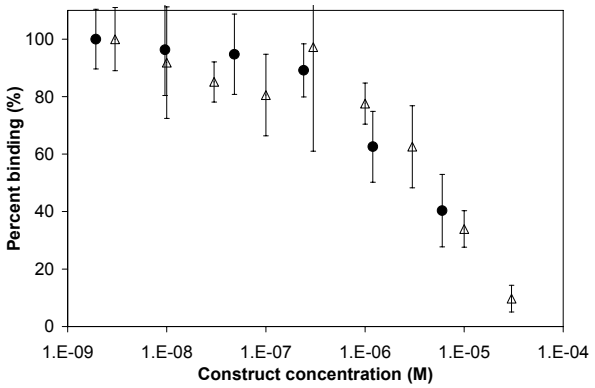

Figure 1. Competitive binding assay using monovalent (triangles) and trivalent (circles) constructs on glioblastoma cells. Error bars indicate 95% confidence intervals (n=4).

Results/Discussion: The results indicate that the multivalent constructs retain the binding capabilities. The multivalent construct exhibits a slight increase in binding avidity (comparing K_d, the 50% binding point, of each construct) (Fig.1). The increase in avidity is perhaps diminished by the entropic penalties of the flexible poly(ethylene glycol) spacers. However, it is hoped that these construct will exhibit a greater binding specificity.

The constructs show binding to cellular receptors as demonstrated by the localized binding around the cellular perimeter. This is indicative of integrin binding seen by the punctuate staining around the cells (Fig 2).

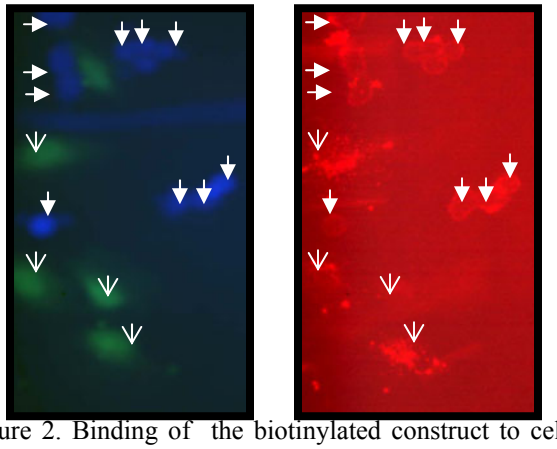

Figure 2. Binding of the biotinylated construct to cells, blue cells identify glioblastoma cells (solid arrows), green cells identify normal human astrocyte cells (open arrows), red staining marks the binding of the trivalent construct.

Conclusions: The constructs developed in this study demonstrate the capability to bind to specific cells via cellular receptors. These constructs are much smaller than conjugated antibodies or quantum dots so it is hoped that they will be more effectively delivered to an area of interest and eliminated from the body after completing their intended function.

References:

Caplan, M. R. and E. V. Rosca (2005). "Targeting Drugs to Combinations of Receptors: A Modeling Analysis of Potential Specificity." Annals of Biomedical Engineering **33**(8): 1126-1137.

Giese, A., R. Bjerkvig, et al. (2003). "Cost of migration: invasion of malignant gliomas and implications for treatment." J Clin Oncol **21**(8): 1624-36.

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