Photocrosslinkable Affibodies for Therapy and Cell Engineering

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A significant challenge for solid tumor treatment is ensuring that a sufficient concentration of therapeutic agent is delivered to the tumor site at doses that can be tolerated by the patient. While antibody-based conjugates show high binding to specific cells, the eventual removal due to endolysis has hindered the efficacy of targeting agents including those used for enzyme based therapy. We have recently developed and tested the use of photo-cross-linkable affibodies that under UV light bind covalently to EGFR and demonstrate long term expression in breast cancer cells and 3D tumor spheroids up to 72 h. To utilize the photocrosslinkable affibodies in vivo at clinically relevant wavelengths, upconverting nanoparticles (UCNPs) were synthesized that could convert 980 nm light to UV and blue light. For potential therpy, we demonstrate that fusing the photocrosslinkable affibody to a prodrug enzyme cytosine deaminase followed by coupling to upconverting nanoparticles can catalyze prodrug conversion specifically at tumor sites *in vivo*. For in vivo tests, conjugates were prepared from upconverting nanoparticles and fusion proteins of affibody and cytosine deaminase enzyme (N23BP-CD). Athymic mice expressing Caco-2 tumors showed 5-fold greater N23BP-CD accumulation in the tumors than either conjugates without the CD enzyme or N23BP in the absence of NIR excitation. With oral administration of 5-FC prodrug, tumors with photoconjugated N23BP-CD yielded 2-fold slower growth than control groups, and median mouse survival increased from 28 days to 35 days. These experiments demonstrate that enzyme-decorated nanoparticles can remain viable after a single covalent photoconjugation *in vivo*, which can in turn localize prodrug conversion to tumor sites for multiple weeks.

If time remains, I will also show more recent work on using the same affibody conjugation to cells to attach DNA to produce 3D cell spheroids by using DNA-mediated assembly. We first methods to covalently modify cell receptors with affibody–streptavidin fusion proteins, where the affibody chemically crosslinks to cell expressed EGFR and the streptavidin is used to attach DNA strands. The DNA conjugated cells were then mixed with complementary DNA ‘linker strands’ to impart cell–cell interactions. When incubated in wells coated with non-adhesive polymers, cells formed dense spherical aggregates larger than 500 microns in diameter. Without either DNA on the cells or in solution as linkers, no cell spheroids were observed. After 96 h of incubation, the cultured DNA assembled spheroids were found to be mechanically stable enough to be handled easily for further analysis and confocal imaging. The findings suggest that the proposed DNA assembly method can be considered as an attractive strategy for assembling cells into stable spheroids.