Dendrimers For Local, Targeted And Selective Treatment Of Breast Cancer

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Statement of Purpose: Systemic therapy, while life saving for many diseases, may be suboptimal for others. Local therapy can provide significant, confined concentration and retention with minimized systemic toxicity. Yet, the means by which to attain ideal release kinetics, targeting and penetration remain elusive.

We have developed a novel class of biocompatible and biodegradable adhesive materials based on dendrimers and dextrans [1] that can coat the tumor and locally release drugs in a controlled manner. Dendrimers can be functionalized with chemotherapeutic agents and simultaneously be conjugated to ligands to target specific binding sites, conferring the capability to discern between healthy and dysfunctional cells. All this together will help minimize side effects of chemotherapy while maintaining high efficacy.

In this work, I have examined a range of means for enhancing the targeting and promoting the selective penetration of dendrimers and have identified the rate-limiting factors in receptor-mediated endocytosis (RME). As a proof of concept, I have conducted different chemistries on dendrimers to study the conditions that prompt an endocytic event in breast cancer epithelial cells overexpressing the receptor EGFR. These molecules present potential for targeted delivery of chemotherapeutic agents and, when added to our adhesive hydrogel system, will be delivered in a local and sustained manner at the tumor site.

Methods: PAMAM dendrimer generation 5 (Dendritech, Inc.) was conjugated to AlexaFluor® 594 succinimidyl ester (Invitrogen) and to EGF-mimicking peptides [2] (Biopolymer Lab, MIT). Two linkers of varying lengths were used - SM(PEG)2 (17.6Å) and SM(PEG)6 (32.5Å) (Pierce). All cell lines of study (ATCC) were cultured in their recommended media. EGFR was detected with anti-EGFR XP Rabbit monoclonal antibody (Cell Signaling). For Western Blot, donkey anti-rabbit IgG-HRP was used as a secondary antibody (sc-2313, Santa Cruz) and Luminata Forte HRP Substrate (Millipore) was used for detection in a molecular imager. For dendrimer uptake experiments, 30k cells/well were seeded in 96 well-plates, incubated overnight and treated with 10uM dendrimer solutions 5 hours at 37C and 4C. Cells were fixed with 4% PFA, stained with DAPI and the uptake per cell assessed by fluorescence microscopy using ImageJ.

Results: UV-VIS spectra of dendrimer-peptide conjugates showed increased absorbance at 275nm, corresponding to a conjugation yield of over 50 peptides per dendrimer for both linkers used (data not shown). Stimulation of a number of cell receptors has been proven to improve RME efficiency [3].

We then evaluated EGFR expression of a number of cell lines with the objective of selecting a positive and a negative EGFR cell line to prove the receptor-mediated endocytosis of our dendrimer conjugates. We observed high levels of EGFR expression on MDA-MB-468, while MDA-MB-453 showed no expression of this receptor. We also selected a non-tumoral cell line (MCF-10A) to mimic the effect of our conjugates on healthy cells surrounding the tumor. The non-tumoral cell line showed basal levels of EGFR.

Incubation of EGFR+ cells with dendrimer-peptide conjugates at 37C showed higher levels of uptake than those for EGFR- cells (Figure 1x, gray solid and striped bars). On the contrary, no peptide-conjugated dendrimer uptake was observed at 4C. Levels of naked dendrimer uptake were two orders of magnitude higher than those of peptide-conjugated dendrimers both at 4C and 37C. Taken together, these data prove that our dendrimer conjugates are being uptaken by receptor-mediated endocytosis through the EGF receptor, as opposed the diffusion-driven uptake observed for naked dendrimer.

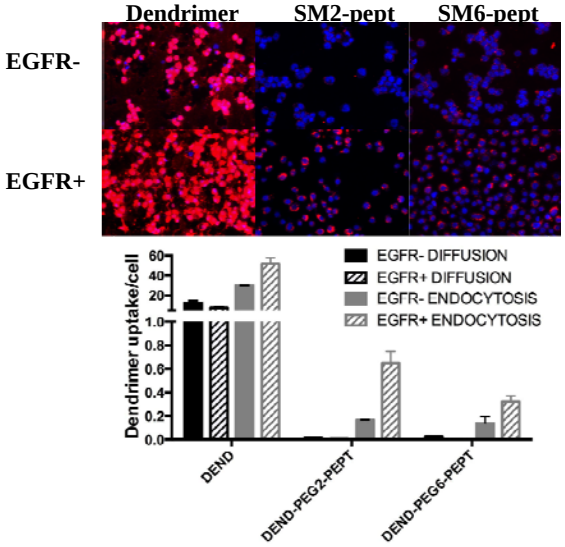

Figure 1. Fluorescence microscopy pictures (top) and quantification (bottom) of naked and peptide-conjugated dendrimer uptake by EGFR+ and EGFR- cells.

Conclusions: We have demonstrated that we can successfully conjugate PAMAM dendrimer to EGF-mimicking peptides to elicit selective RME in EGFR positive cells.

Generalization of this platform with other growth factor mimicking peptides (FGF2R, VEGFR or PDGFR) will expand the targeting capabilities of our delivery system.

Dendrimers conjugated to this repertoire of peptides and doped with chemotherapeutic drugs will be incorporated to our adhesive hydrogel network. We aim to develop a single delivery platform capable of treating highly heterogeneous tumors in vivo.

References:

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