EGFR-Targeted Pc4-loaded Micelles for Photodynamic Therapy of Cancer

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Statement of Purpose: Conventional cancer treatments (e.g. chemotherapy, radiotherapy) can result in systemic toxicity and negative side effects, while surgical approaches can cause invasive trauma and functional/cosmetic debilitation. A novel treatment alternative that can reduce these negative issues is photodynamic therapy (PDT). PDT involves photoactivation of a photosensitizer (PS), leading to complex energy transfer cascades, ultimately resulting in formation of cytotoxic reactive oxygen species (ROS). Harnessing such photoinduced cytotoxicity selectively within cancer cells provides a method of minimally invasive yet targeted eradication of cancer. Silicon phthalocyanine 4 (Pc 4), developed by Kenney et al, is a second generation PS that can be synthesized at high purity, has high molar extinction at tissue-penetrating deep red light and has much reduced skin photosensitivity compared to currently FDA-approved PS formulations. However, Pc 4 is a highly hydrophobic compound, and packaging it in a water-dispersible formulation for *in vivo* delivery is challenging. Formulations of Pc 4 involving oil-based surfactants like Cremophor can present Cremophor-related hypersensitivity and toxicity issues, especially for multiple doses. To address this issue, we have previously demonstrated the feasibility of loading Pc 4 in biocompatible polymeric micelles to allow for passive intracellular delivery and subsequent PDT of cancer cells, *in vitro*[1]. We further hypothesize that beyond the 'passive delivery', active targeting of the micelles to cancer cell-surface-expressed internalizing receptors can enhance the cell-selective rapid delivery of micelle-loaded Pc 4 and subsequent PDT. To test this hypothesis, we have modified the micelle surface with a small peptide having high affinity to Epidermal Growth Factor Receptor (EGFR). EGFR, significantly upregulated in several cancers, is implicated in cancer survival and spreading, and therefore is a suitable target for cancer drug delivery[2].

Materials and Methods: The EGFR-specific peptide (ESP) YHWYGYTPQNVI was custom synthesized by Abgent (San Diego, CA) with an additional cysteine residue on the N-terminal of the peptide. Maleimide-terminated poly(ethylene glycol) (Mal-PEG-OH) was purchased from Laysan Bio (Arab, AL) and co-polymerized with ε-caprolactone to form Mal-PEG-polycaprolactone (Mal-PEG-PCL). The ESP peptide was conjugated to Mal-PEG-PCL via thioether linkage to form ESP-PEG-PCL. This conjugate was incorporated with plain PEG-PCL to form micelles surface-decorated with ESP (Fig. 1a). Pc 4 was encapsulated in the micelle core via hydrophobic association, at 70% loading capacity. The Pc 4-loaded ESP-modified micelles were incubated with EGFR-overexpressing A431 human epidermoid cells *in vitro*. Pc 4-loaded unmodified micelles were used as control. At different time points, micelle internalization (hence Pc 4 uptake) in cells were monitored by imaging Pc 4 fluorescence in cells. For all studies, total 400 nM Pc 4 concentrations were used. Following this the cells were photoirradiated at 675 nm and PDT effect (cytotoxicity) was analyzed by standard MTT assay.

Results: Our results show that Pc 4-loaded ESP-modified micelles undergo intracellular uptake and enable PDT-induced enhanced cell killing at much shorter time periods, compared to the Pc 4-loaded control (unmodified) micelles (Figure 1 b and c). As an additional control, this effect was irrelevant in EGFR-deficient cell lines (e.g. MCF-7). PDT studies on A431 cells incubated with Pc 4-loaded targeted micelles showed 40% cell death within 1 hour incubation, compared to only 5% cell death with the untargeted micelles (Fig. 1b). After prolonged incubation periods (24 hr), the intracellular uptake and PDT-induced cell death were similar for both targeted and untargeted formulations, suggesting that, *in vitro*, at prolonged time periods, passive uptake and active uptake reaches equilibrium. From *in vivo* perspective, the enhanced uptake of targeted micelles at early time periods can significantly influence the PDT 'time window.'

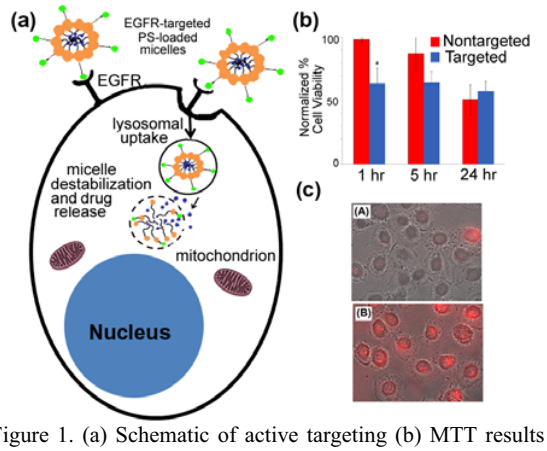

Figure 1. (a) Schematic of active targeting (b) MTT results show statistically significant difference in cell death between targeted and nontargeted micelles at 1 hr incubation and (c) studies in intracellular uptake of Pc 4.

Conclusions: We have demonstrated the ability of EGFR-targeted peptide-decorated micelles to enable rapid intracellular delivery of Pc 4 and subsequent PDT, of EGFR-overexpressing cancer cells. Refinement of this strategy can enable site-specific rapid delivery of Pc 4 to EGFR-overexpressing cancers, *in vivo*, for time-optimized targeted PDT strategies.

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References:

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2. Woodburn, JR. Pharmacol Ther. 1999; 82: 241-250.