Uptake of pro-apoptotic peptide amphiphiles by SJSA-1 cells *in vitro* induces specific cell death

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Statement of Purpose: Self-assembled micelles composed of peptide amphiphiles (PAs) have recently been shown to actively target specific tissues following introduction to the systemic circulation[1,2]. Experiments with mixed micelles, incorporating two different PAs, demonstrated that despite their inherent instability[3], these structures were able to reach their targets intact and deliver their payload. What is not clear is how these nanostructures interact with cells following delivery to the tissue of interest. To answer this question we studied the interactions of PAs with cells *in vitro*. We selected a peptide derived from the MDM2-binding region of tumor suppressor p53. In tumor cells that overexpress MDM2 and in this way inactivate wild type p53, this peptide can induce apoptosis by inhibiting the p53-MDM2 interaction.

Methods: In order to study PA micelle-cell association we synthesized fluorescently labeled PAs (Figure 1) and employed fluorescence microscopy in conjunction with flow cytometry to investigate internalization by cells. We used SJSA-1 osteosarcoma cells (*wt* p53), MDA-MB-435 (mutated p53) as well as HeLa and NIH 3T3 cells. The use of different culture conditions and incubation with inhibitors allowed us to investigate the mechanisms of internalization. Finally, we studied cell viability in response to PA intracellular delivery.

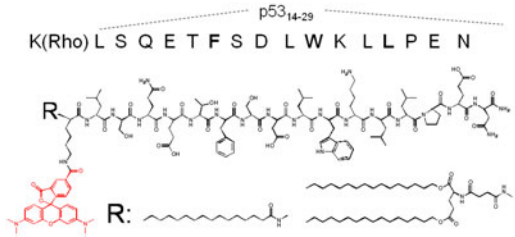

Figure 1. Structure of fluorescent $p53_{14-29}$ peptide amphiphiles used in this study

Results: Attachment of a lipid-like tail on peptide $p53_{14-29}$ and fluorescent labeling was achieved using standard solid phase peptide chemistry. This modification caused peptide internalization in a variety of cell lines as evidenced by higher cell-associated fluorescence compared to unmodified peptide (Figure 2). Microscopy revealed a punctuated intracellular fluorescence pattern, indicative of endosomal entrapment. Inhibition studies using energy depleted cells, Na^+/H^+ exchange impairment and cholesterol depletion revealed clathrin-mediated endocytosis as the principal mode of PA entry, and co-localization studies with tranferrin confirmed this finding. Using micelles of different fluorescence intensities we were able to determine that PAs internalized as monomers instead of intact micelles[4].

Endosomal disruption in hypertonic medium (sucrose 0.3 M) caused redistribution of the PAs, with diffuse fluorescence suggesting cytoplasmic localization. Even though increasing sucrose concentration had deleterious effects on both SJSA-1 and MDA-MB-435 cells, specific p53-MDM2 inhibition by $p53_{14-29}$ PAs was demonstrated by exhibiting decreased cell proliferation only in the cell line with *wt* p53 (Figure 3).

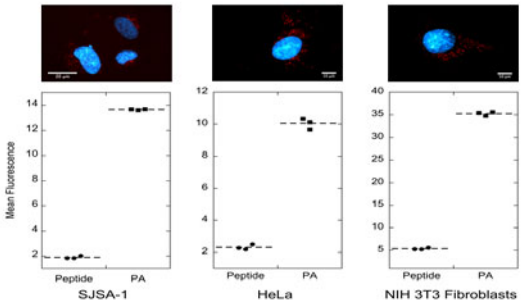

Figure 2. $p53_{14-29}$ peptide amphiphiles internalize in different cell lines (top: epifluorescence microscopy; bottom: flow cytometry/comparison with unmodified peptides)

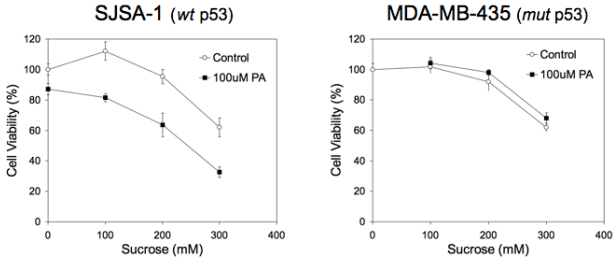

Figure 3. $p53_{14-29}$ peptide amphiphiles induce cell-specific decrease in viability following internalization and endosomal disruption.

Conclusions: Covalent attachment of a hydrophobic tail on a pro-apoptotic, cell-impermeable peptide facilitated its uptake in cells. The generality of this approach was demonstrated by using a panel of different cell lines. Based on our mechanistic studies we hypothesize that monomers in equilibrium with the self-assembled micelles first insert into the plasma membrane and then are taken up through constitutive endocytosis. The importance of this finding relates to the intracellular fate and trafficking of PAs. In the present work, the targeted protein-protein interaction occurs in the cytoplasm and nucleus and therefore endosomal disruption was necessary for specific effects. We envision cases where our strategy could be applied in peptides that are active in different intracellular organelles that could be targeted through appropriate lipid-tail selection. Furthermore, these PAs could be co-assembled with targeting PAs to form targeting micelles as recently demonstrated[1,2].

References:

[1]Karmali PP. Nanomedicine 2009; 5:73
[2]Peters D. PNAS 2009; 106: 9815
[3]Kastantin M. Langmuir 2009; 25: 7279
[4]Missirlis D. Biochemistry 2009; 48: 3304