

Cell-penetrating doxorubicin released from Elastin-like polypeptide kills doxorubicin-resistant cancer cells

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Abstract

Background: Elastin-like polypeptide (ELP) undergoes its characteristic of phase transitioning in response to ambient temperature. ELP therefore has been used as a thermosensitive vector for the delivery of chemotherapy agents since it can be targeted to hyperthermic tumors. This novel strategy introduces unprecedented options for treating cancer, with fewer concerns about side effects. In this study, the ELP system was further modified with an enzyme-cleavable linker in order to release drugs within tumors. This system consists of ELP, a matrix metalloproteinase (MMP) substrate, a cell penetrating peptide (CPP), and 6-maleimidocaproyl amide derivative of doxorubicin (Dox). This construct may be initially targeted to the tumor by application of mild heat after administration. Within the hyperthermic tumor, then this construct is cleaved by MMP, releasing CPP-Dox, which can infiltrate tumor tissues and penetrate cell membranes.

Methods: We produced the construct in E.coli and examined its cleavage by MMP enzymes in vitro. Flow cytometry and confocal analysis were used to verify the facilitated uptake of the digested cell-penetrating Dox by breast cancer cells and Dox-resistant cells. Cytotoxicity tests further demonstrated improvements in bioavailability of cell-penetrating Dox following the enhanced cellular uptake of the cancer cells. Comparisons with the non-cleavable ELP counterpart were paralleled.

Results: This strategy shows up to a 4-fold increase in cell penetration and results in more death in breast cancer cells than the ELP-Dox. Even in doxorubicin-resistant cells (NCI/ADR and MES/ADR), ELP-released, cell-penetrating doxorubicin demonstrated better membrane penetration, leading to at least twice the killing of resistant cells than ELP-Dox and free Dox.

Conclusion: MMP-digested CPP-Dox shows better membrane penetration and induces more cancer cell death in vitro. This CPP-complexed Dox released from ELP kills even dox-resistant cells more efficiently than both free doxorubicin and non-cleaved ELP-CPP-Dox.

Full Text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed. However, the manuscript can be downloaded and accessed as a PDF.

Figures

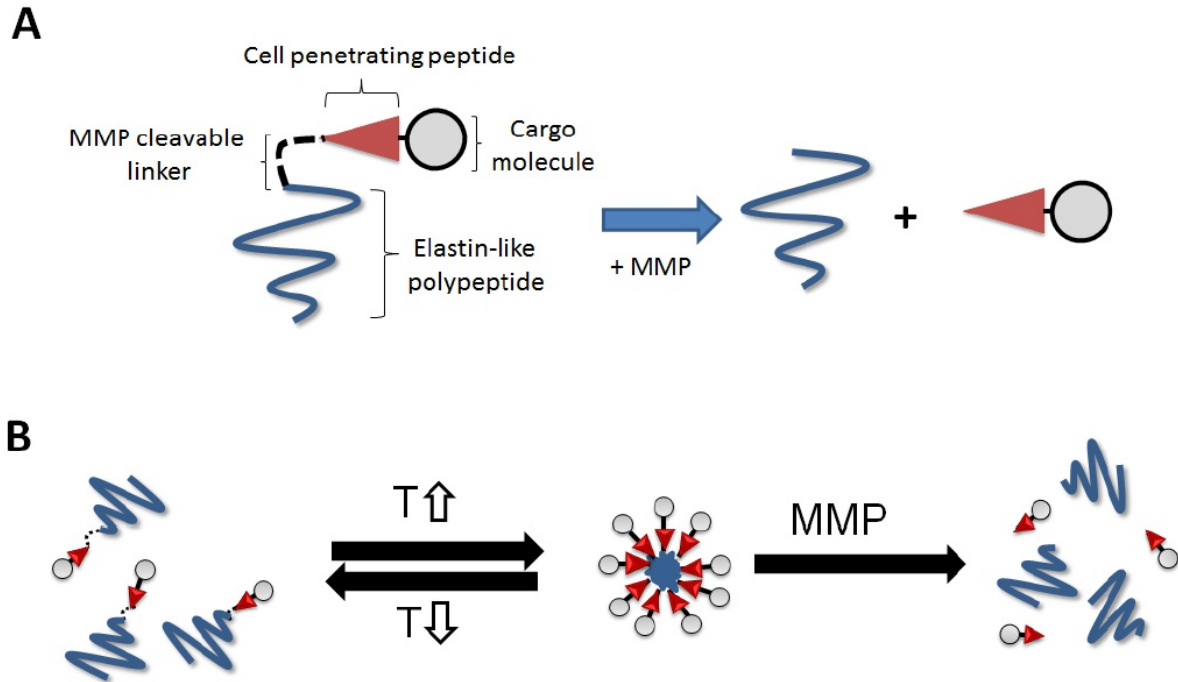


Figure 1

ELP drug delivery system A. a proposed ELP system consists of elastin-like polypeptide, MMP-cleavable linker, cell penetrating peptide and cargo molecules. The constructs are digested by MMP releasing CPP-cargo molecule. B. The ELP constructs can form aggregates and release CPP-cargo molecule in hyperthermic tumors.

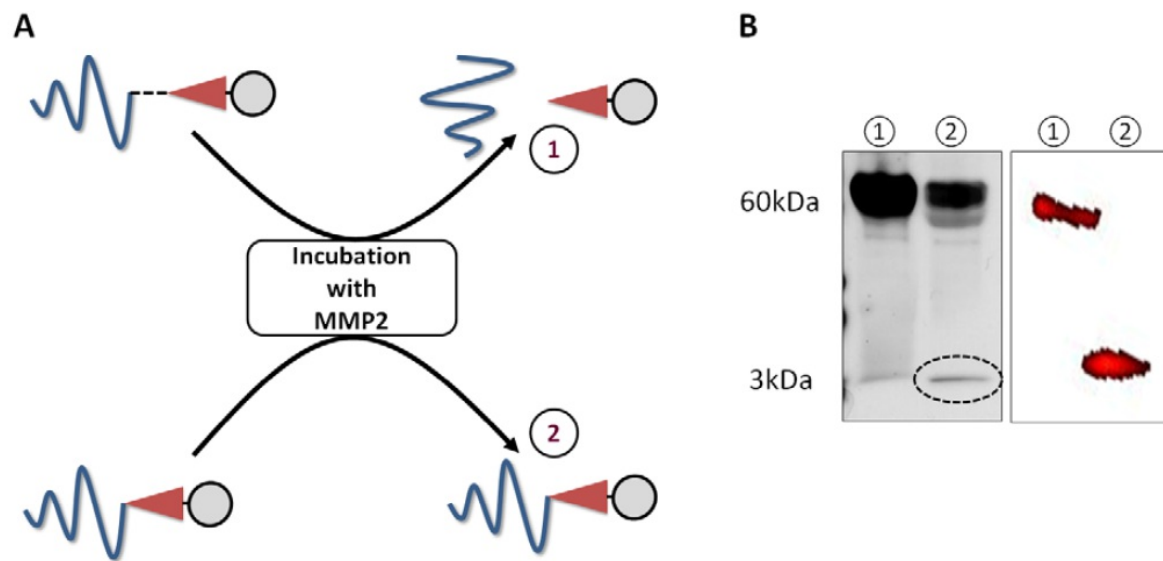


Figure 2

MMP-2 digestion of ELP-mmpL-CPP. A. ELP-mmpL-CPP-rhodamine and ELP-CPP-rhodamine were incubated with MMP-2 for 4hr in ZnCl₂ buffer (pH 7). B. MMP-2 incubation of the constructs produced ELP-CPP-Dox (63KDa, upper band) and CPP-rhodamine (3kDa, lower band). Left panel: Silver-stained gel, Right panel: fluorescence-scanned gel.

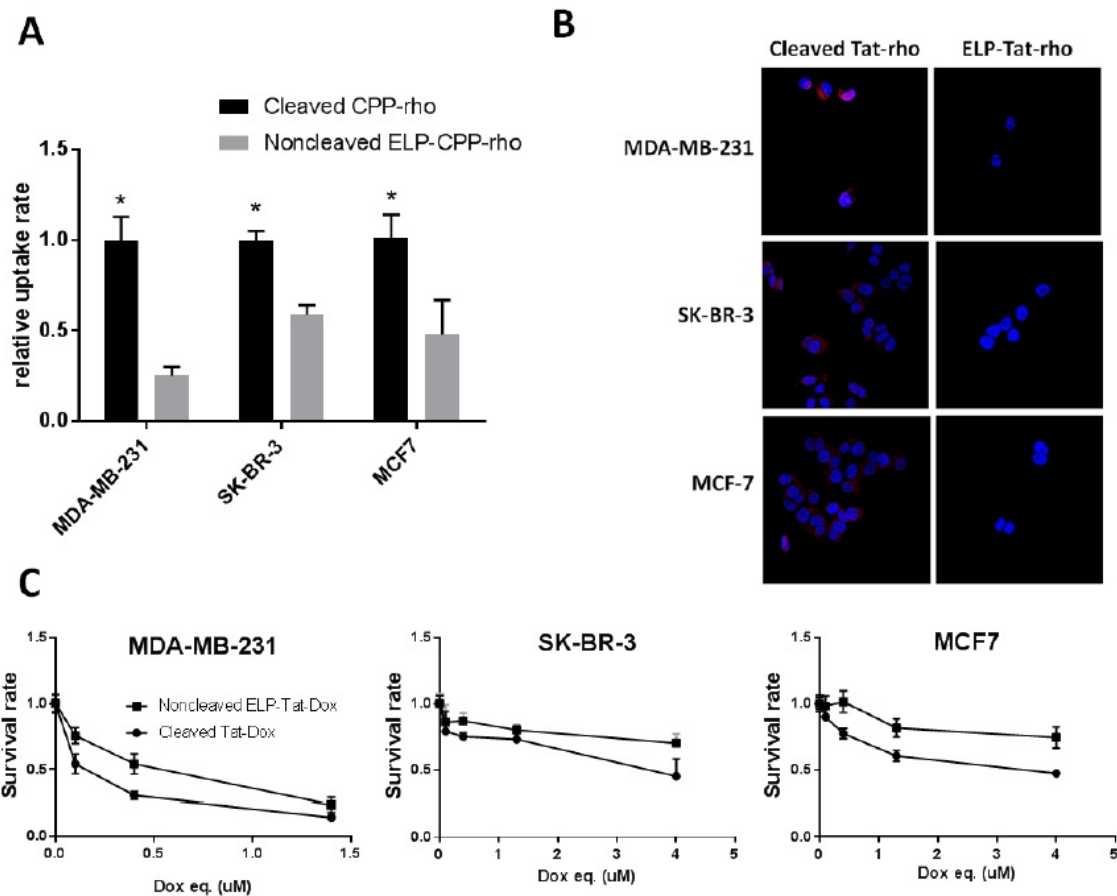


Figure 3

Cellular uptake rate of cleaved CPP-rhodamine in breast cancer cells. A. Each cellular uptake rate was measured through flow cytometry (*; $P < 0.05$). B. Merged image of Dox (red) and DAPI (blue). C. Cytotoxicity of cleaved CPP-Dox on breast cancer cells. Cells were treated with ELP-CPP-Dox and ELP-mmpL-CPP-Dox, both digested by MMP incubation.

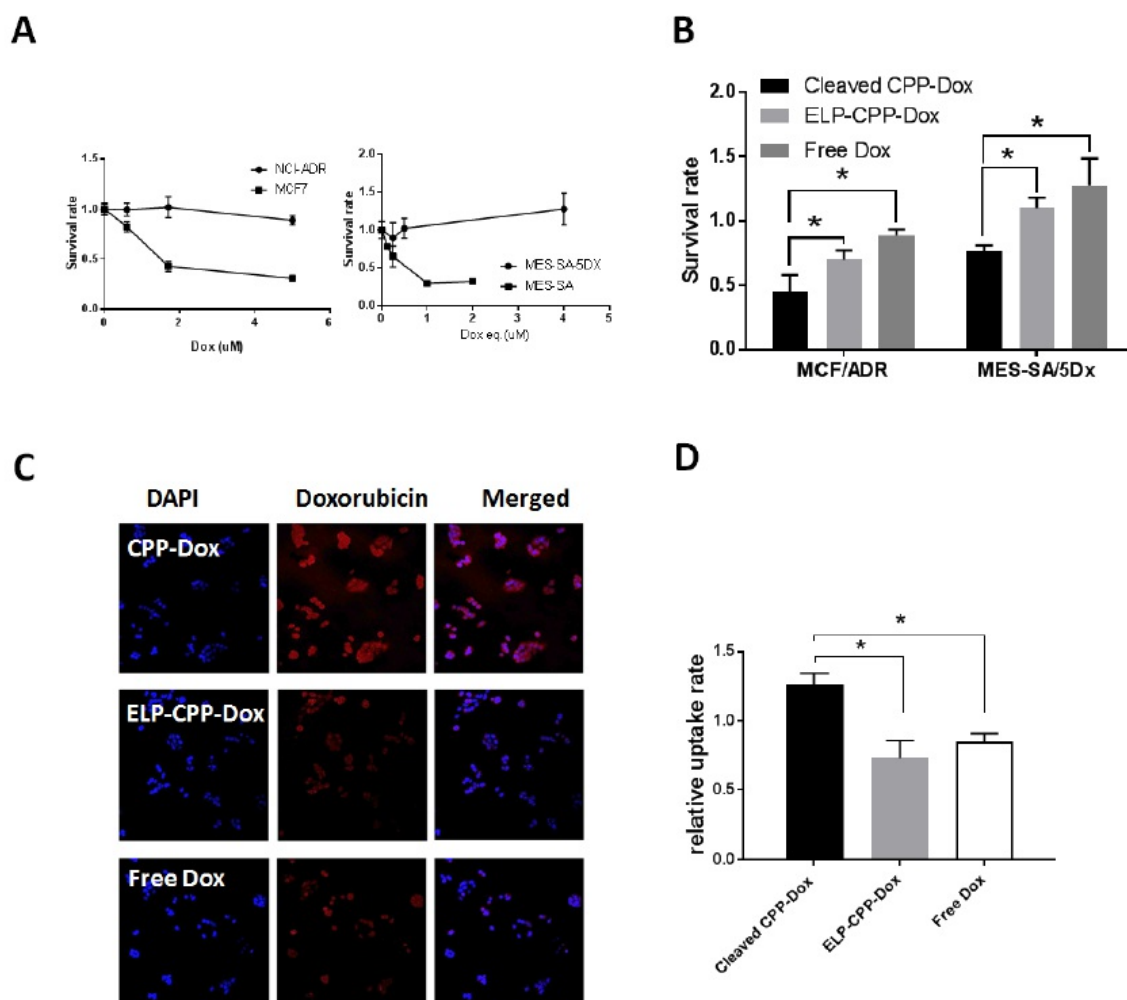


Figure 4

Cytotoxicity of CPP-Dox against Dox-resistant cancer cells. A. The cytotoxicity results represent Dox-resistance of MCF/ADR and MESSA-5DX. B. Cytotoxicities of CPP-Dox and ELP-CPP-Dox in MCF/ADR and MES-SA/5DX at 4uM Dox equivalence. C. Confocal microscopic images show that CPP-Dox penetrated into MCF/ADR. D. Flow cytometry, 60% increased uptake in CPP-Dox in comparison with ELP-CPP-Dox and free Dox. *: $P < 0.05$

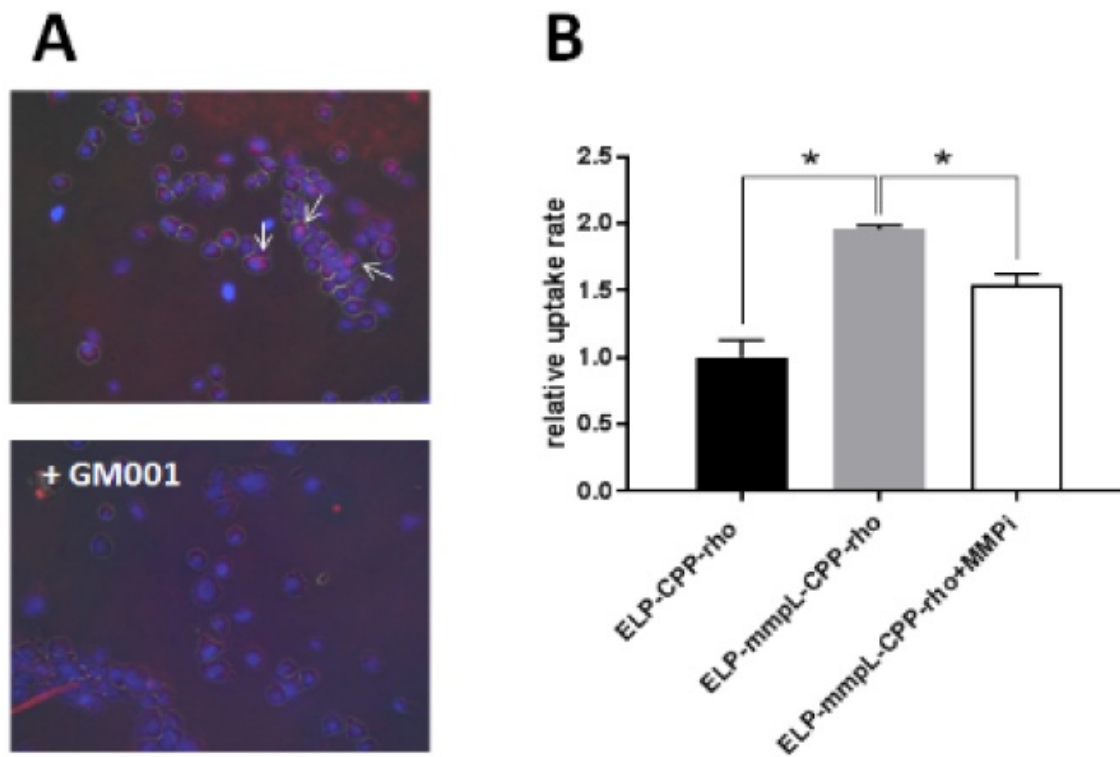


Figure 5

Cellular uptake rate of CPP-rhodamine in MMP-expressing HT-1080. A. Localization of CPP-rho (fluorescence microscopy, 20X) B. Flow cytometry showing increased uptake in cleaved CPP-rho in cells.