Novel Cell Penetrating Peptide for the Cellular Delivery of Molecular Imaging Probes

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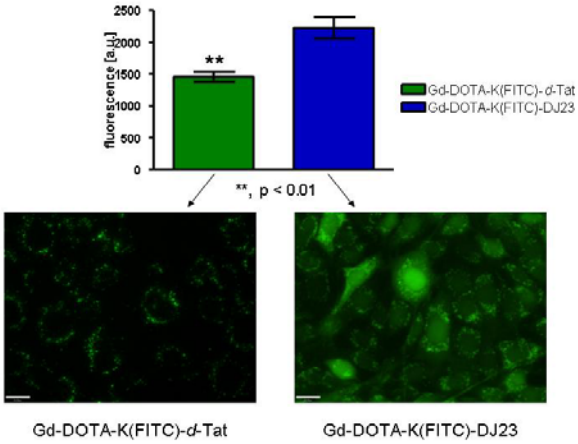
The success of intracellular targeted delivery of imaging probes or drugs depends on the efficient transmembrane delivery of the compound as well as interaction of the payload with the desired targets. Cell penetrating peptides (CPPs) are extensively used as the delivery tools. However, confinement of biomolecules into endosomes limits their use for intracellular targeting. Herein, we focus on the development of a novel cysteine rich CPP (derived from polypeptide Crotamine [1]) by Structure Activity Relationship (SAR) studies optimizing cellular uptake efficacy as well as distribution. Series of peptides were synthesized by Fmoc strategy and were N-terminally labeled with fluorescein isothiocyanate (via an additional lysine residue) for optical imaging. The optimized peptide (DJ23) is 10 amino acids long and contains aside of cationic amino acids (common for various CPPs) cysteine and tryptophane residues. It is markedly distinct showing an efficient uptake at low concentrations (≤2.5 µM) and a cytosolic distribution along with vesicular uptake unlike known for other common CPPs (e.g. Tat or Antennapedia) at these concentrations [2]. Fluorescence microscopic results highlight the importance of cysteines and tryptophans in the peptide. Additional coupling of Gd-DOTA to this peptide showed proficient uptake maintaining the cytosolic localization of the conjugate (Fig.). Furthermore, fluorescence spectroscopic data showed that the internalization efficacy at 2.5 µM increased significantly by about 50% in comparison to the conjugate containing the *d*-form of Tat peptide, known to be an efficient CPP (Fig.).

Thus, this novel peptide might prove useful for efficient transmembrane delivery of agents directed to cytosolic targets.

[1] Kerkis A. *et al*, *FASEB J.* 2004, *18*, 1407.
[2] Duchardt F. *et al*, *Traffic* 2007, *8*, 848.



Comparison of internalization efficacy of Gd-DOTA coupled *d*-Tat and the novel DJ23 in 3T3 fibroblasts

Cells were incubated with 2.5 µM of contrast agent for 18 hours.