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Investigating protein prenylation using cell-permeable peptides

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Abstract

Protein prenylation is an irreversible post-translational modification in which prenyltransferases attach an isoprenoid to a *C*-terminal CaaX-motif. This protein modification determines the localization and biological function of proteins. Targeting protein prenylation would be an exciting way to modulate their activity spectrum.

Recently, we developed cell-permeable CaaX-peptides that impaired Ras protein localization and their biological function as a molecular switch. The peptides combined a cell-penetrating peptide (CPP) with a *C*-terminal CaaX-motif of Ras proteins. They highly accumulated inside cells and exhibited pronounced toxicity to KRas mutated pancreatic cancer cells bearing a G12D mutation (PANC-1). Also, CaaX-peptides altered Ras localization causing a loss of membrane integrity and decreased KRas levels in PANC-1 cells. This likely affected other interactors as, for instance, the expression of the tumor suppressor and negative regulator of KRas, neurofibromin-1 (NF-1).

Based on these findings, my study aims to further investigate intracellular processing of CaaX-peptides and to study their influence on KRas expression and membrane localization, and how they affect distinct KRas interactors.

Design and Synthesis of cell-permeable CaaX-peptides

Design: H_2N — CPP (sC18*) — secondary signal — CaaX — OH

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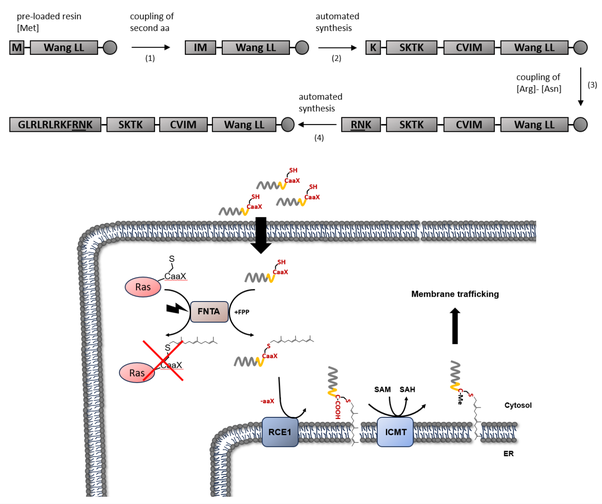
          1        10        11-160                    170       180
Kras4B:   MTEKLVVVG.....................REIRKHKEKM  SKDGKKKKKK SKTK CVIM
sC18*:                                    GL  RKRLRKFRNK
CaaX-1:                                   GL  RKRLRKFRNK SKTK CVIM
```

Name	Sequence	Mwcalc [Da]	Mwexp [Da]	Purity [%]
CaaX-1	GLRKRLRKFRNK-SKTK-CVIM-OH	2463.1	2463.645	>98
SaaX-1	GLRKRLRKFRNK-SKTK-SVIM-OH	2447	2447.568	>98
CF-CaaX-1	CF-GLRKRLRKFRNK-SKTK-CVIM-OH	2821.4	2821.992	>98
CF-SaaX-1	CF-GLRKRLRKFRNK-SKTK-SVIM-OH	2805.3	2806.022	90
Bio-CaaX-1	Bio-GLRKRLRKFRNK-SKTK-CVIM-OH	2689.4	2690.035	85
Bio-SaaX-1	Bio-GLRKRLRKFRNK-SKTK-SVIM-OH	2673.3	2673.735	80



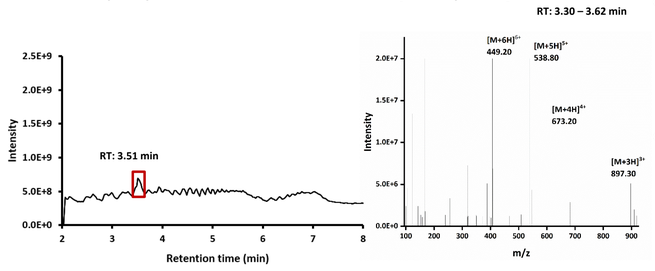
- Synthesis of CaaX-peptides derived from Ras proteins conjugated to the CPP sC18*

→ recognition of CaaX-peptides by the prenylation machinery

→ interference with Ras prenylation and subsequent signaling processes

In cellulo detection of Bio-CaaX-1

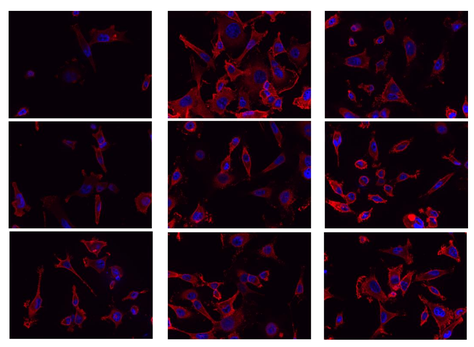
- 3 h peptide treatment (30 µM)

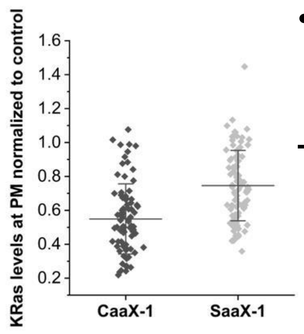


- Peptide isolation using magnetic streptavidin beads
- *In cellulo* detection of Bio-CaaX-1 by mass spectrometry

Influence of CaaX-1 on KRas membrane localization

- 3 h peptide treatment (30 µM)

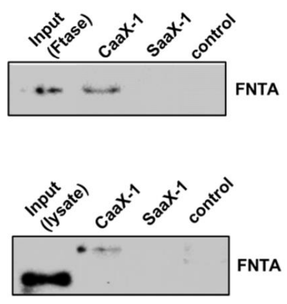




- Immunostaining using KRas antibody conjugate

→ Decrease of KRas membrane localization after CaaX-1 treatment

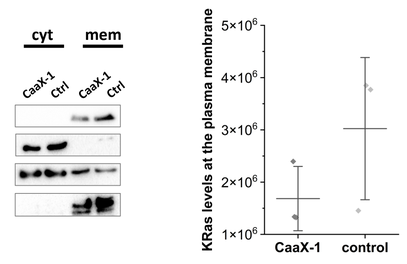
CaaX-1 interacts with FTase



- 24 h peptide treatment (30 µM)
- Pulldown assay using magnetic streptavidin beads

→ CaaX-1 interacts with FTase

- 3 h peptide treatment (30 µM)

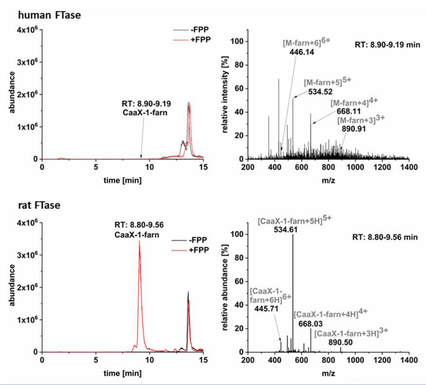


- Membrane fractionation using Digitonin (cytosol) and Triton X-100 (membrane)

→ KRas only detectable in membrane fraction

→ Decreased KRas levels in membrane fraction after CaaX-1 treatment

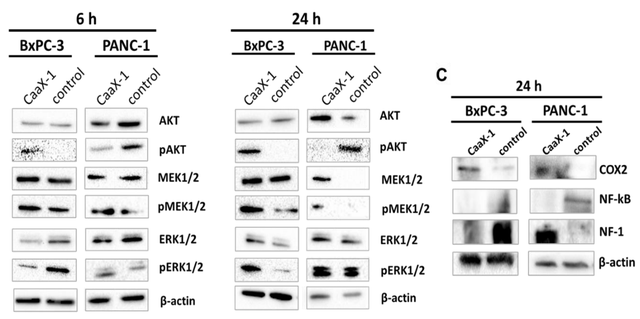
CaaX-1 is farnesylated *ex cellulo*



- 18 h reaction time
- 250 nM rat FTase + 50 µM CaaX-1 (+ 50 µM FPP)

→ CaaX-1 is farnesylated by FTase

CaaX-1 alters Ras regulators and downstream effectors



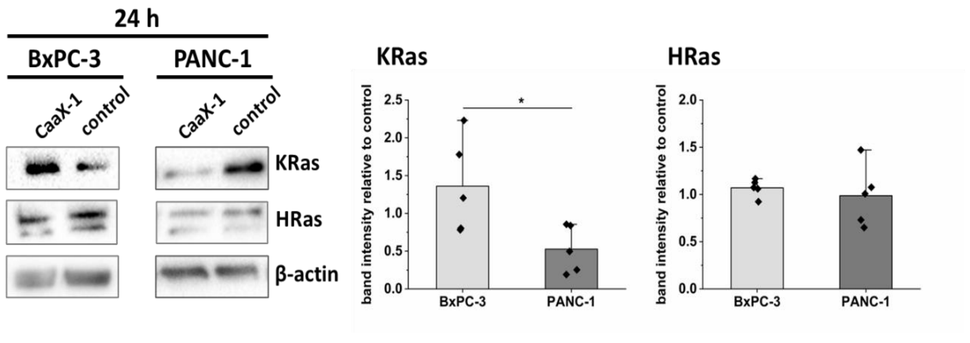
- 24 h peptide treatment (30 µM)

→ different expression levels of KRas and NF-1 potentially explain different activation of PI3K/AKT/mTOR signaling in PANC-1 and BxPC-3

CaaX-1 alters KRas abundance in PANC1

- 24 h peptide treatment (30 µM)

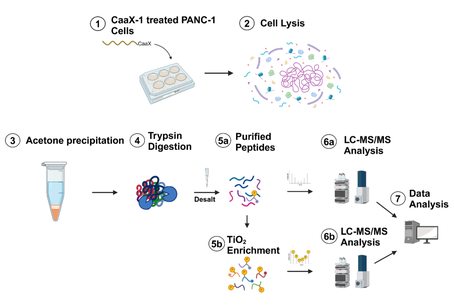
PANC-1: KRas G12D mutant pancreatic ductal adenocarcinoma
BxPC-3: KRas wildtype pancreatic ductal adenocarcinoma



→ Influence of CaaX-1 on KRas expression levels depending on Ras genotype

Outlook

- Investigating alterations of Ras signaling using a phosphoproteomic approach
- How do CaaX peptides influence nanoclustering of KRas?
- Does CaaX-1 influence the interactome of KRas genotypes?



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