Disrupting the RANK-TRAF6 Interface: A Novel Approach for Bone Metastases Treatment

https://doi.org/10.17952/37EPS.2024.P2152

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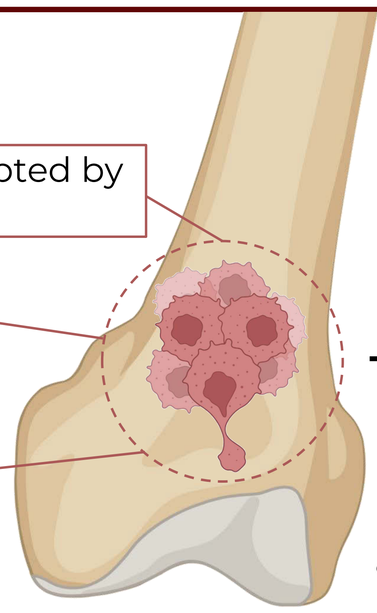
A. Background

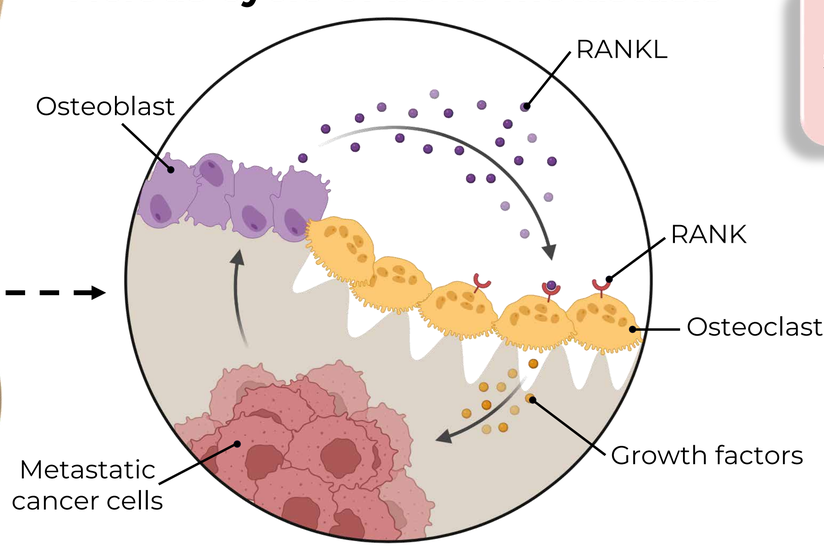
Most **cancer deaths** are prompted by **incurable metastases**[1]

Bone is one of the most frequent **metastasis** sites[1]

Bone metastasis entails **unbalanced bone remodelling**[1]



Vicious cycle of bone metastasis



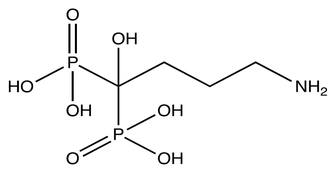
Cancer cells induce **osteoblasts** to secrete receptor activator of nuclear factor kappa B ligand (**RANKL**)[1]

RANKL binds to **RANK** in **osteoclasts** and increases **proliferation**[1]

Osteoclasts promote **resorption** and secrete **growth factors**[1]

B. Proposal

Develop **bone-seeking peptides** that **disrupt** the **RANK/TRAF6** axis and thus **hinder osteoclastogenesis** and **metastatic potential**



Bone-targeting unit → A bisphosphonate (**alendronate**) with high affinity for **bone mineral**[2]

\+ Fragment A: Cathepsin K-sensitive linkers → **Cathepsin K** is an abundant **protease** in the **bone microenvironment**[3]

\+ Fragment B: Cell-penetrating peptides (CPPs) → **Peptide sequences** able to promote **cell entry** of different cargos[4]

\+ Fragment C: RANK-derived sequences → **Bind** to tumor necrosis factor receptor associated factor 6 (**TRAF6**)[5]

C. Approach and Results

Selected sequences

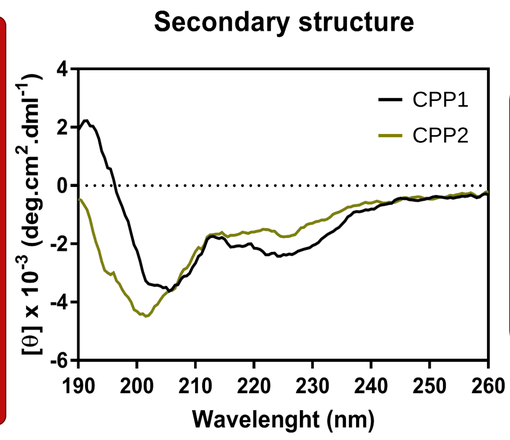
Linker1	GGPNle	**CPP1**	AAVALLPAVLLALLAP	**RANK-derived sequences**	RQMPTEDEY
Linker2	PR	**CPP2**	KLFMALVAFLRFLT		RQMATADEA
Linker3	GPR				RKIPTEDEY
					RKIATADEA

PEG – Polyethylene glycol **Ahx** – 6-aminohexanoic acid

Constructed peptides

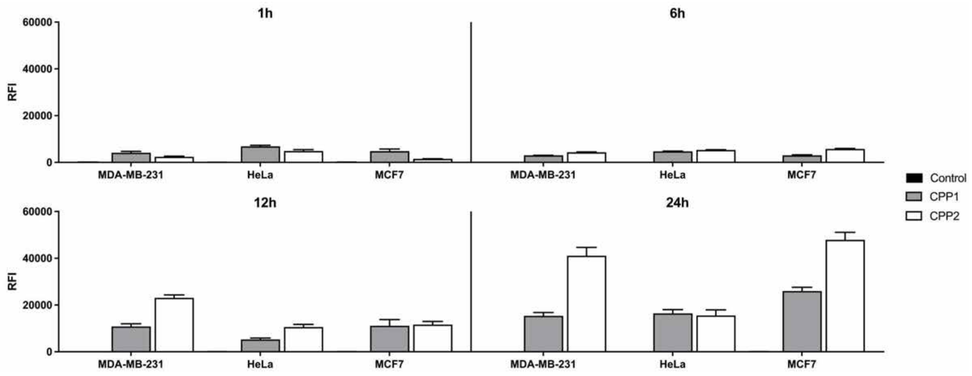
PepA	PEG2-Linker1-Ahx-CPP1-NH2	**PepF**	PEG2-Linker3-Ahx-CPP2-NH2
PepB	PEG2-Linker2-Ahx-CPP1-NH2	**3A**	CPP2-RQMPTEDEY
PepC	PEG2-Linker3-Ahx-CPP1-NH2	**3B**	CPP2-RQMATADEA
PepD	PEG2-Linker1-Ahx-CPP2-NH2	**5A**	CPP1-RKIPTEDEY
PepE	PEG2-Linker2-Ahx-CPP2-NH2	**5B**	CPP1-RKIATADEA

Circular Dichroism



The **secondary structure** of these peptides is mainly **α-helix**

Cell Internalization

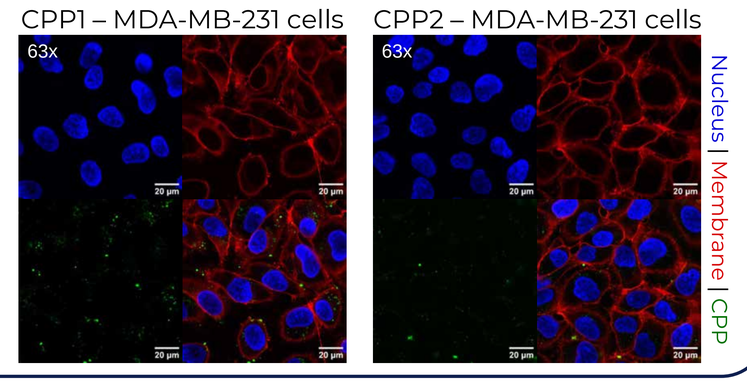


Flow cytometry

Higher internalization was observed for **CPP2** over 24 hours in **MDA-MB-231** and **MCF7** cells

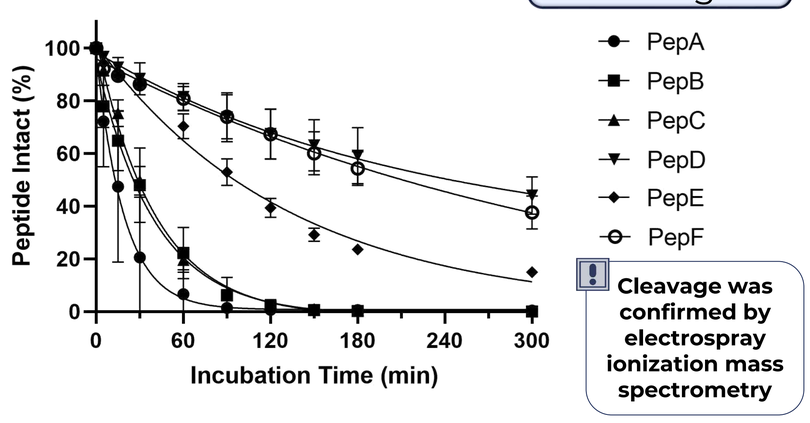
Confocal microscopy

Higher internalization was observed for **CPP1**, when in comparison with **CPP2** in **MDA-MB-231** cells



Cathepsin K Assay

Enzymatic cleavage

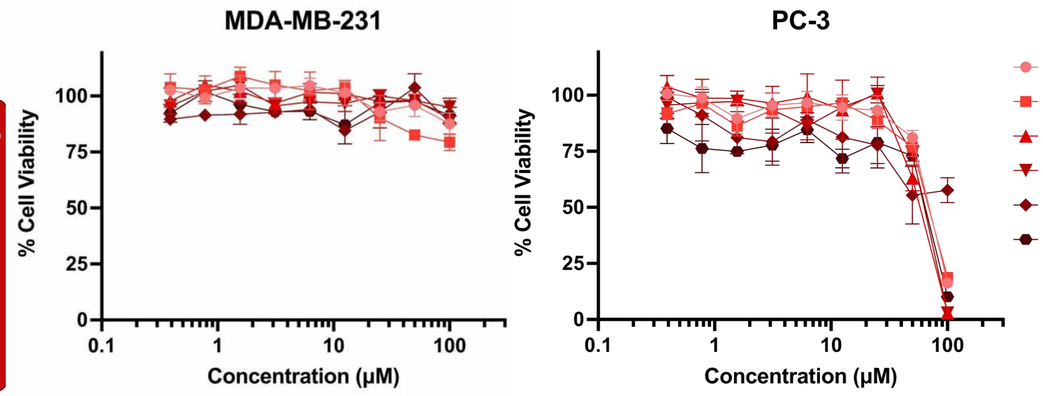


Cleavage was confirmed by electrospray ionization mass spectrometry

Cleavage kinetics strongly depends on the CPP sequence and less on the linker type

Peptides **A, B and C** exhibit a **more favorable kinetic** when compared with peptides **D, E and F**

Cell Viability



Peptide	IC50 (µM) 24 h	
	MDA-MB-231	PC-3
3A	>100	69.08±2.82
3B	>100	67.67±3.37
5A	>100	54.64±2.30
5B	>100	58.52±3.56
CPP1	>100	>100
CPP2	>100	61.64±30.73

Peptides **5A** and **5B** exhibit the **highest cytotoxicity** towards **PC-3** cells

D. Conclusions

- **PepA**, which combines **CPP1** and **Linker1**, exhibits the most favorable kinetic profile in terms of enzymatic cleavage, and will be used for assembling novel bone-seeking peptides that interfere with the RANK/TRAF6 interface.
- Peptides that combine **CPPs** with **TRAF6-binding sequences** are cytotoxic towards TRAF6-expressing prostate cancer cells.

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ACKNOWLEDGEMENTS
This work was supported by FCT through PTDC/QUI-OUT/3854/2021, UI/BD/154474/2022 and UID/Multi/04349/2020.