https://doi.org/10.17952/38EPS.2026.P408

REENGINEERING ANTICANCER PEPTIDES VIA LIPID CONJUGATION

GIUSEPPINA GUIDA[1,2], AURÉLIE BENFIELD[1,2], NICOLE LAWRENCE[2,3], SÓNIA T. HENRIQUES[1,2]

[1]School of Biomedical Sciences, Translational Research Institute, Queensland University of Technology, QLD, Australia;
[2]ARC Centre of Excellence for Innovations in Peptide and Protein Science, Brisbane, QLD, Australia;
[3]Institute for Molecular Bioscience, The University of Queensland, Brisbane, Queensland, 4072, Australia

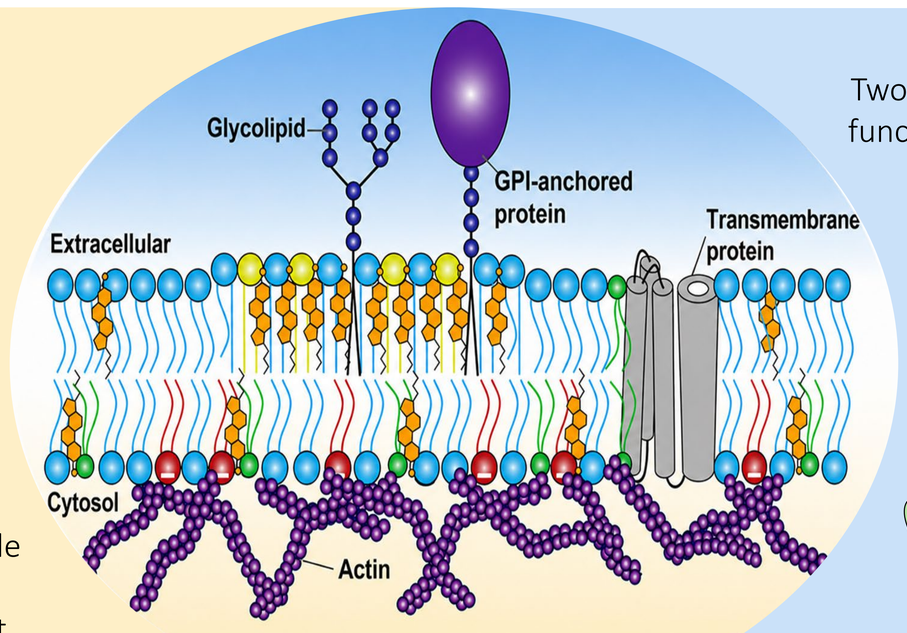
g.guida@qut.edu.au

INTRODUCTION

Peptides are emerging as a powerful therapeutic modality, combining the high specificity of biologics with the versatility of small molecules. However, their clinical translation remains limited by poor oral bioavailability due to rapid degradation, fast systemic clearance, and inefficient transport across epithelial barriers in the gastrointestinal tract.

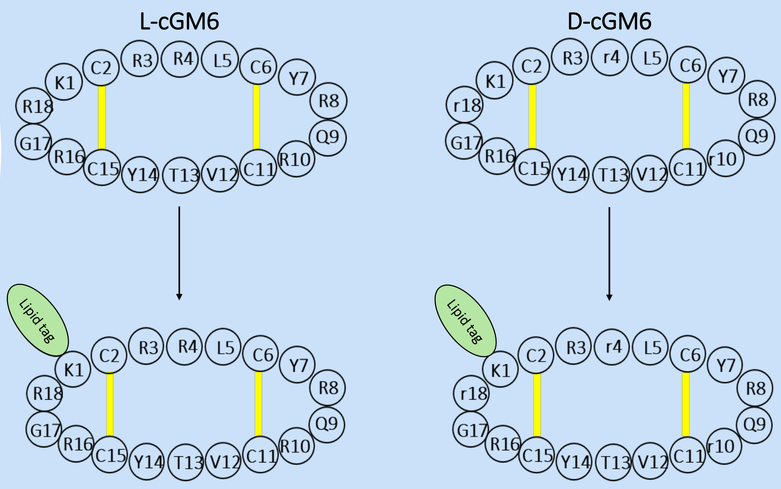
Lipidation, a clinically proven post-translational modification, has recently emerged as a promising strategy to overcome these limitations, as exemplified by therapeutics like semaglutide (Ozempic).

This study investigates how lipidation influences peptide membrane interactions in a set of gomesin-derived peptide analogues, aiming to support the development of orally bioavailable peptide therapeutics.



RESEARCH STRATEGY

Two cyclic gomesin derivatives (L-cGM6 and D-cGM6) were functionalised via conjugation with lipid chains at the lysine residue in position 1.



METHODS & RESULTS

Peptide Cytotoxicity: Comparison of cytotoxicity of cGm6 analogues against WM164 (human melanoma), HEK293 (human embryonic kidney), Caco-2 (human colon epithelium) and HIEC6 (human small intestinal epithelium) cell lines. Cell viability following 24 h peptide treatment was assessed using a resazurin-based assay, while haemolytic activity was evaluated in red blood cells (RBCs). Data represent the mean of three biological replicates (n = 3), with error bars indicating the standard error of the mean (SEM). Estimated half-maximal cytotoxic concentrations (CC_{50}) are listed in table 1.

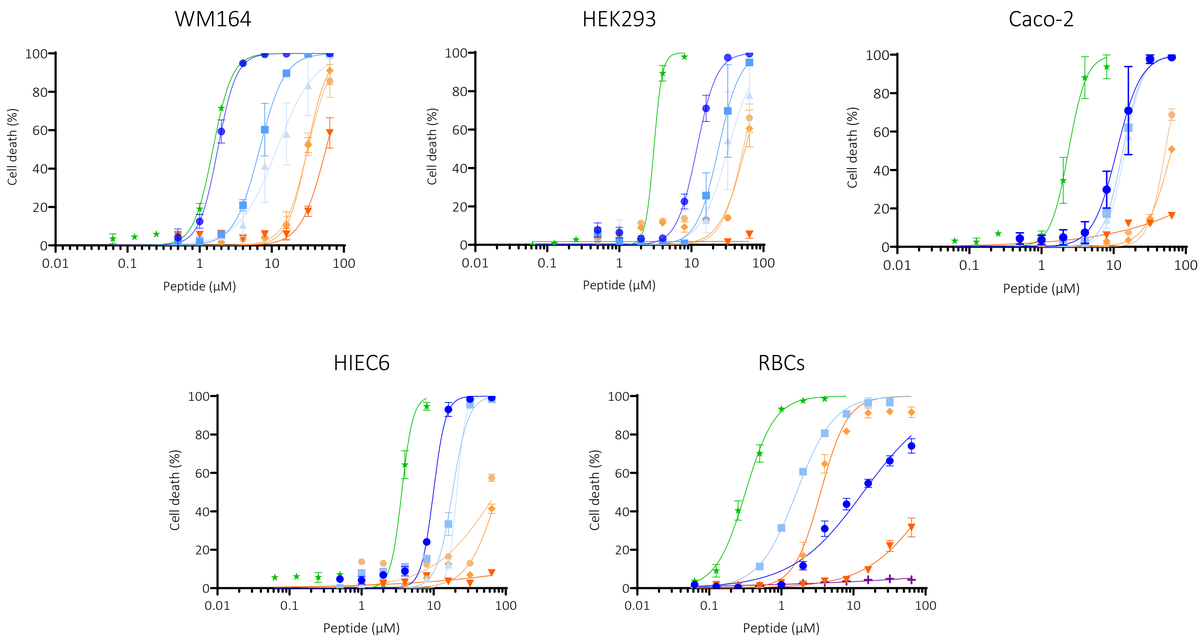
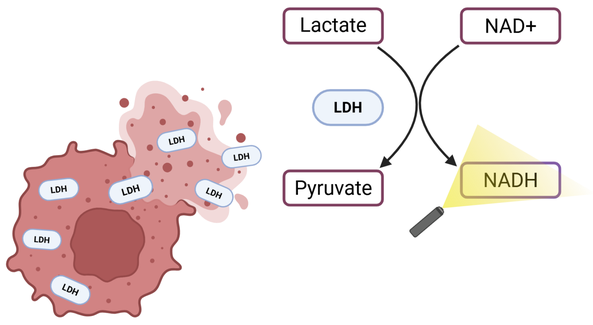
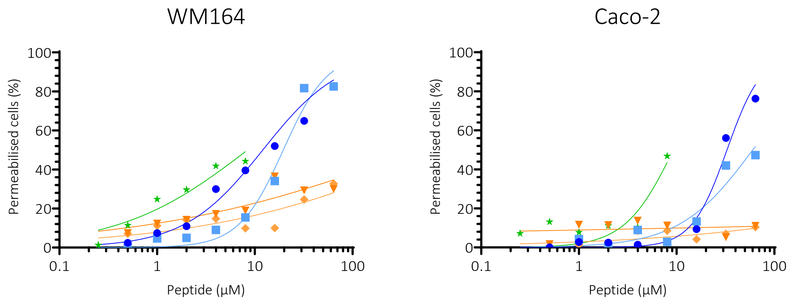


Table 1. Estimated half-maximal cytotoxic concentrations (CC_{50})

	CC_{50} (μM)				
Peptide	**WM164**	**Caco-2**	**HEK293**	**HIEC6**	**RBCs**
L-cGm6	1.8 ± 3.3	11.8 ± 2.6	12.0 ± 3.1	9.9 ± 0.3	13.2 ± 0.9
L-cGm6 (Palm)	6.8 ± 2.5	13.4 ± 3.1	23.7 ± 2.9	18.2 ± 0.9	1.3 ± 2.3
L-cGm6 (Stearic)	11.7 ± 1.7	14.4 ± 3.4	32.4 ± 2.2	20.1 ± 0.8	N/A
D-cGm6	56.4 ± 2.5	>64	>64	>64	>64
D-cGm6 (Palm)	31.1 ± 3.1	>64	55.8 ± 2.5	>64	2.9 ± 3.4
D-cGm6 (Stearic)	31.6 ± 2.7	52.0 ± 3.7	52.3 ± 2.9	>64	N/A
Semaglutide	N/A	N/A	N/A	N/A	>64
Melittin	1.5 ± 3.2	2.4 ± 3.4	3.0 ± 4.8	3.6 ± 0.1	0.3 ± 0.1

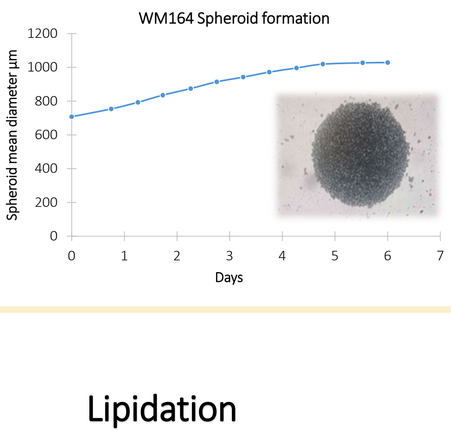
Enzymatic activity of cytosolic lactate dehydrogenase (LDH): Membrane disruption of WM164 and Caco-2 cells was quantified by measuring the enzymatic activity of the cytosolic lactate dehydrogenase released from damaged cells after treatment with peptide for 2 h at 37°C. Peptide concentration required to permeabilise 50% of cells (LC_{50}) was determined by fitting dose-response curves using binding with Hill slope equation.

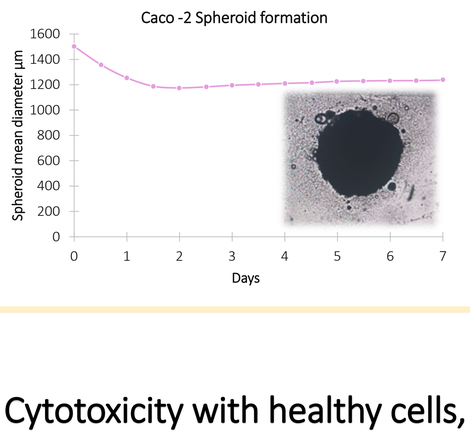


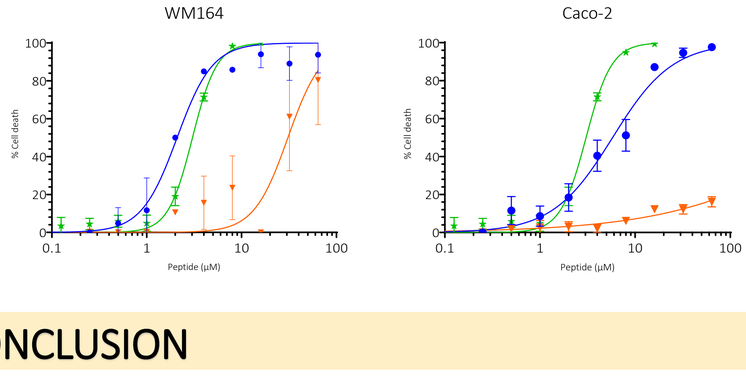


	LC_{50} (μM)	
Peptide	**WM164**	**Caco-2**
L-cGm6	12.0 ± 1.0	32.7 ± 2.4
L-cGm6 (Palm)	19.5 ± 1.9	41.8 ± 1.2
D-cGm6	> 64	>64
D-cGm6 (Palm)	> 64	>64
Melittin	8.4 ± 0.6	9.2 ± 1.7

Development of cancer spheroids to evaluate cytotoxic response to anticancer peptide: WM164 and Caco-2 spheroids were generated using the liquid overlay technique. Spheroid cell metabolic activity was evaluated daily over 6 days by measuring the fluorescent emission of resorufin with microplate reader. After spheroid formation, cells were treated with increasing peptide concentration up to 6 days in culturing condition.



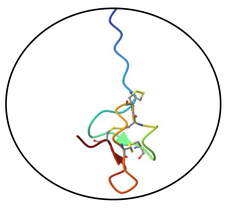




	CC_{50} (μM)	
Peptide	**WM164**	**Caco-2**
L-cGm6	1.7 ± 1.5	4.9 ± 1.2
D-cGm6	23.2 ± 0.9	>64
Melittin	2.8 ± 2.9	2.9 ± 2.0

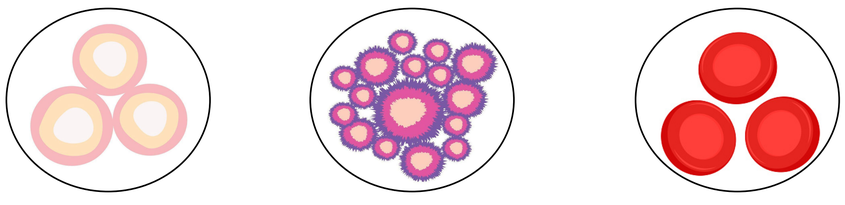
CONCLUSION

Lipidation



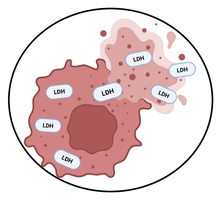
- L-cGm6 (Palm)
- D-cGm6 (Palm)

Cytotoxicity with healthy cells, cancer cells and RBCs



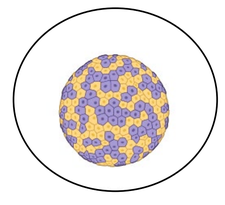
- L-cGm6
- L-cGm6 (Palm)
- L-cGm6 (Stearic)
- L-cGm6 (Palm)
- L-cGm6 (Stearic)

Membrane disruption



- L-cGm6 (Palm)

Spheroids development



- L-cGm6
- D-cGm6