In silico prediction and experimental validation of a novel anti-cancer peptide

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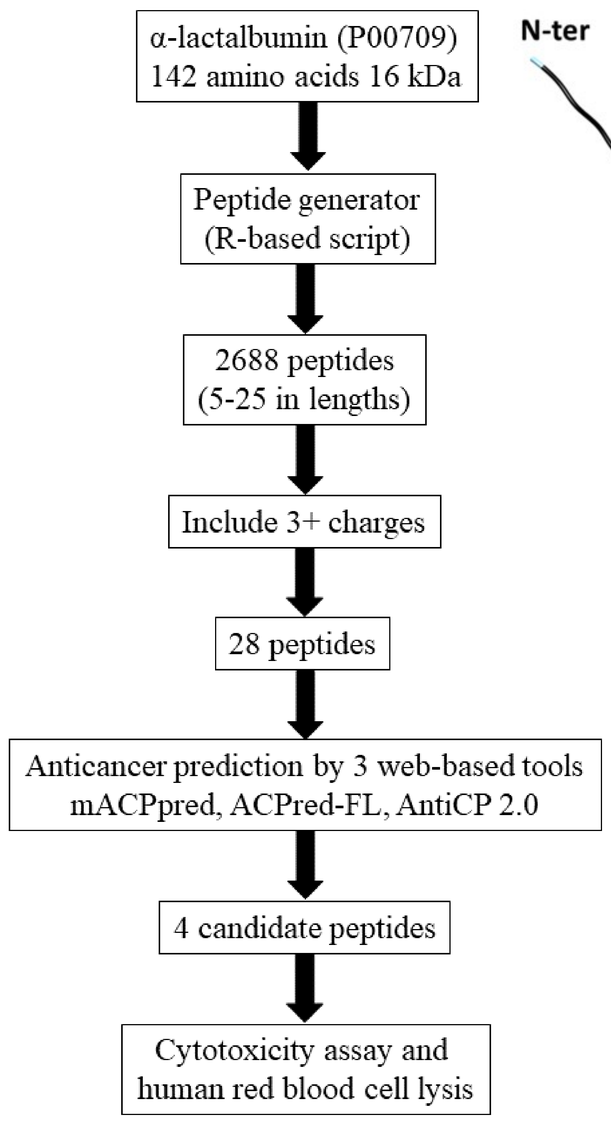
ABSTRACT

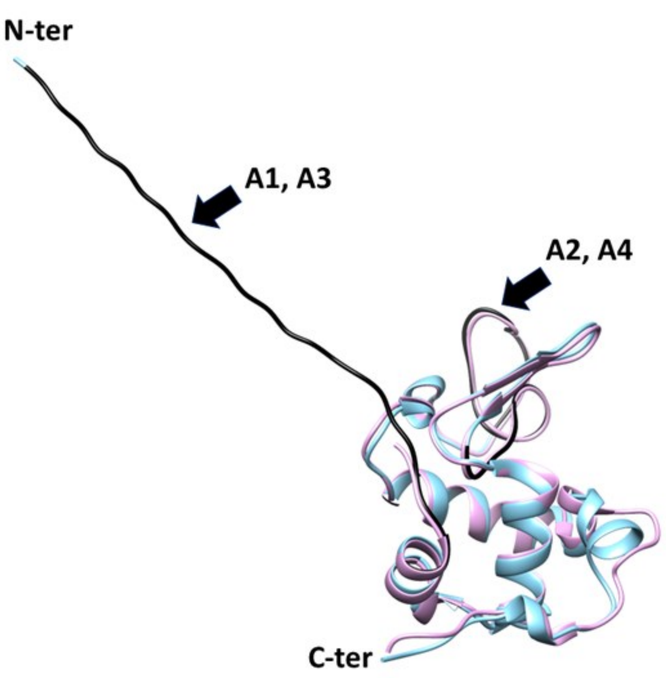
INTRODUCTION: Cancer is the leading cause of death worldwide. To improve patient outcomes, a more effective treatment agent is required. Our previous work on discovering human milk peptides that selectively kill leukemia cells has shown the approach of using mass spectrometry to identify the candidate anticancer peptides. It is limited by the small peptide library. Thus, we propose to expand the peptide library by using in silico generation of peptides from the protein of interest. **MATERIALS AND METHODS:** We in silico produced peptides from a protein with anticancer potential, alpha-lactalbumin. On-line tools were used to predict anticancer properties of the filtered peptides. Lastly, we chose four candidates for in vitro testing on cancer cells. **RESULTS AND DISCUSSION:** We in silico obtained four candidates (A1-A4) peptides with the highest anticancer scores for further in vitro testing on five cancer cell lines, SH-SY5Y, MDA-MB-231, A549, HT29, and K562. A2 was able to solubilize in media, whereas three peptides (A1, A3, and A4) precipitated. Preliminary screening revealed A2 demonstrated the most anticancer activity. To avoid the interference of peptide precipitants on cell viability, we therefore solubilized peptide in DMSO. A1, A3, and A4 exhibited dose-dependent cytotoxicity toward A549, but none of the three exhibited significant cytotoxicity toward the remaining cancer cells. **CONCLUSION:** This approach enables us to rapidly identify potential anticancer candidates prior to conducting in vitro experiments, while also expanding the pool of bioactive peptides that may be missed by mass spectrometry analysis.

RESULTS

The overall workflow of this study

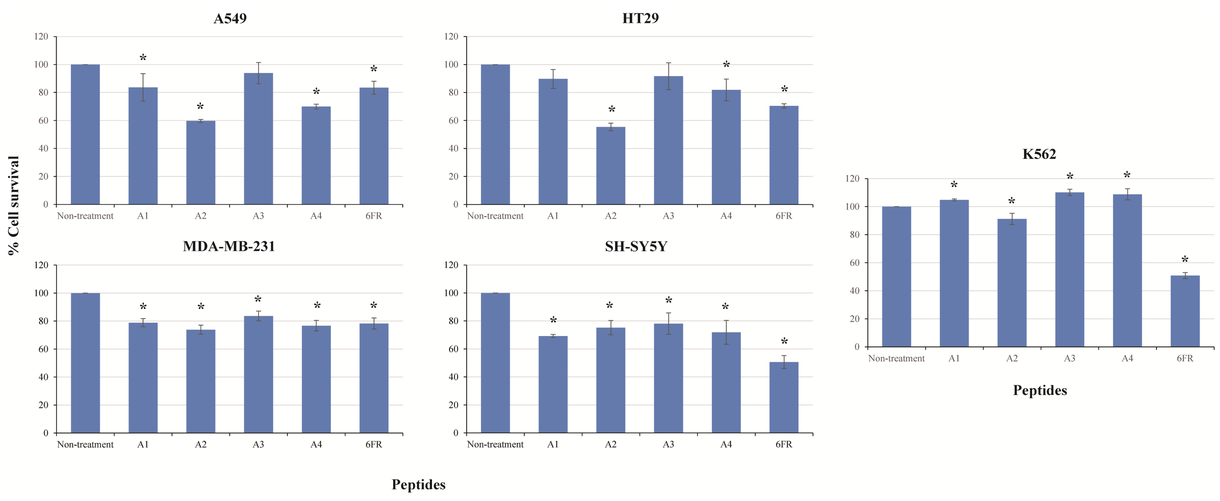


α-lactalbumin

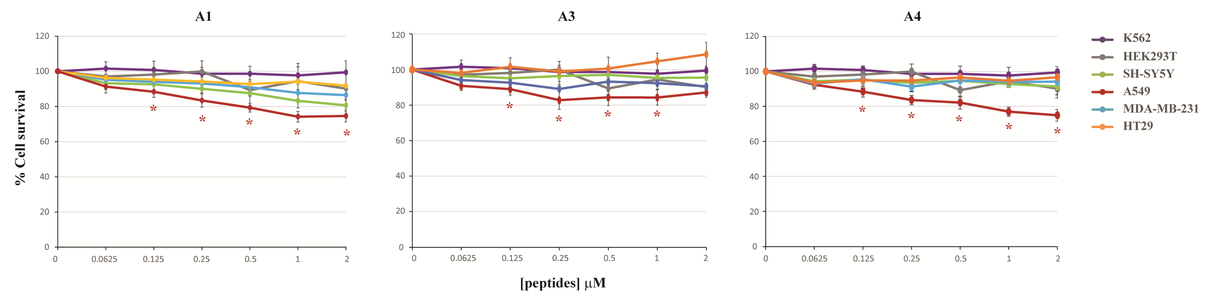


Demonstrating the locations of A1, A2, A3, and A4 peptides in the predicted full-length α-lactalbumin. The crystal (PDB: 1b9o) and predicted structure are colored pink and blue, respectively. The locations of peptides are demonstrated in black on the predicted structure. The peptide sequence of A1-A3 and A2-A4 is partially overlapped. Both structures are visualized using UCSF chimera1.15 . N-ter; N-terminal amino acid, C-ter; C-terminal amino acid.

Cytotoxicity toward cancer cell lines

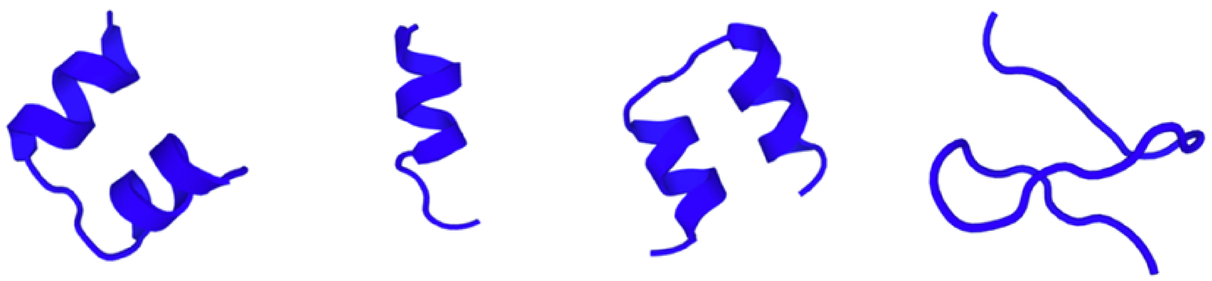


In vitro cytotoxicity toward different cancer cell lines. The cells were incubated with individual peptides at 200 mM for 24 h prior to the cytotoxicity assay using MTT. A549, HT29, MDA-MB-231, and SH-SY5Y are adherence cells, whilst K562 is a suspension cell. Data are represented as mean ± standard deviation of three technical replicates. An asterisk indicates a significantly different from the non-treatment ($p<0.05$).



In vitro cytotoxicity of peptides solubilized in DMSO toward cancer and transformed cell lines. The cells were incubated with individual peptides at various concentrations starting from 2 to 0.0625 mM for 24 h prior to the cytotoxicity assay using MTT. The mean standard deviation of two biological replicates is used to represent the data. A Student's t-test was used to compare the percentage of cell survival between HEK293T and HT29. An asterisk indicates a significantly different ($p<0.05$).

Predicted structures of peptides



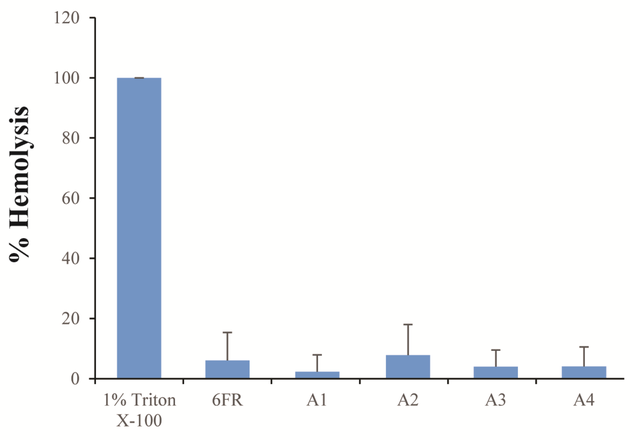
A1
coil-helix-coil-helix

A2
helix-coil

A3
helix-coil-helix

A4
coil

Human red blood cell lysis



Hemolysis activity of peptides. The human red blood cells were individually incubated with peptides for 1 h. Triton-X 100 at a final concentration of 1% was used as positive control. The mean standard deviation of three biological replicates is used to represent the data.

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SELECTED REFERENCE

Chiangjong, W., et al., HMP-S7 Is a Novel Anti-Leukemic Peptide Discovered from Human Milk. Biomedicines, 2021. 9(8): p. 981.