https://doi.org/10.17952/37EPS.2024.P2167

Obtaining, Optimizing, and Evaluating the Activity of Anticancer LfcinB-Derived Peptides on in vitro Models against Melanoma

Karla Rodriguez[1] , Jose Mendoza[1], Javier García[1], Zuly Rivera[1], Joel Lopez[2], Ricardo Fierro[1]

[1]*Universidad Nacional de Colombia, Bogotá D.C, Colombia,* [2]*Universidad Michoacana de San Nicolás de Hidalgo, Michoacan, Mexico*

Abstract: Melanoma, a leading cause of cancer-related deaths, is expected to see a 50% increase in diagnoses and a 68% rise in mortality by 2040. This study focuses on evaluating and optimizing anticancer peptides derived from Bovine Lactoferricin and Buforin II in human (A-375) and murine (B16-F10) melanoma models. Peptide bioactivity was assessed using MTT assays and microscopy, followed by optimization through sequence modifications. Characterization was performed using RP-HPLC and LC-QTOF MS. The results of this study showed that the strategy of enhancement activity contributes to the development of new therapeutic agents for Melanoma treatment.

INTRODUCTION

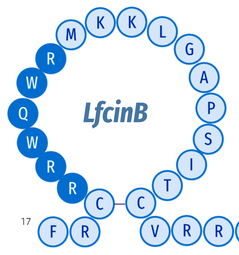
Cancer incidence in 2022

01 Lung cancer — 2 480 675 (12.4%)

02 Breast cancer — 2 296 840 (11.5%)

05 Skin cancer — 1 234 533 (6.2%)

Melanoma — 331 722 (1.6%)



- **Animal Origin:** Bovine
- **Source:** Gastric pepsin hydrolysis of LFB
- **Activity:** Equal to or higher than LFB
- **Spectrum:** Broad-spectrum homologues

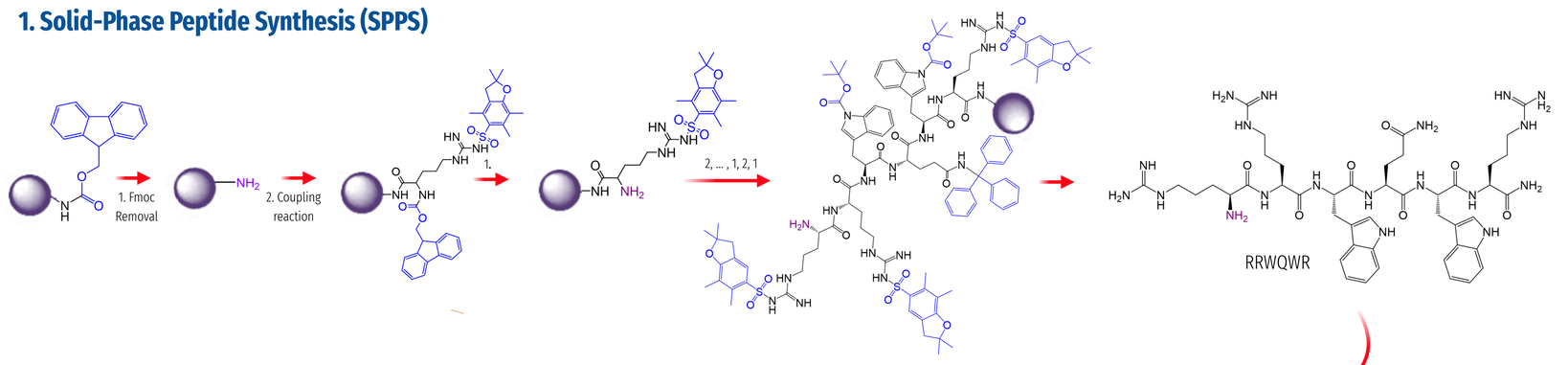
BFII

16 T R S S R A G L Q F P V G R V H R L L R K 36

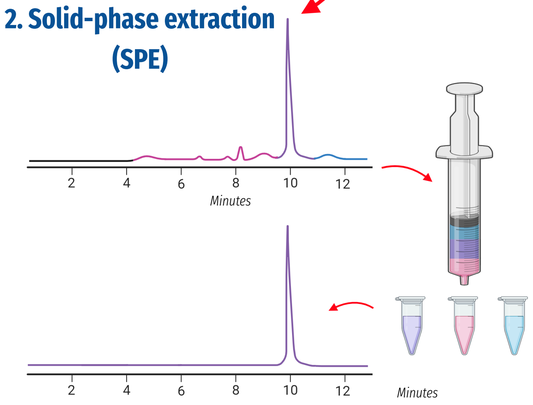
- **Animal Origin:** Asian Toad *Bufo gargarizans*
- **Source:** Proteolysis of histone H2A
- **CPPs** (Cell-Penetrating Peptides)
- **Spectrum:** Broad-spectrum PAM (Antimicrobial peptide)

METHODS

1. Solid-Phase Peptide Synthesis (SPPS)



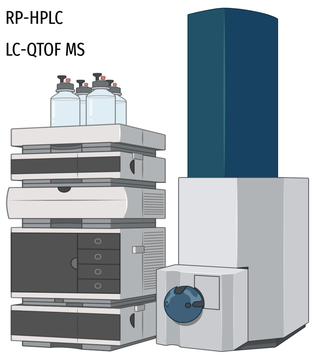
2. Solid-phase extraction (SPE)



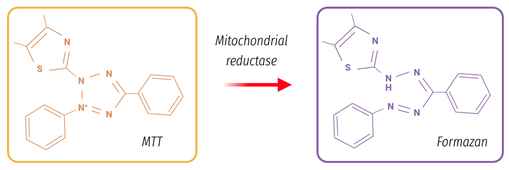
3. Peptide Characterization

RP-HPLC

LC-QTOF MS



4. MTT Viability Assay



5. Hemolytic Activity Assay

RESULTS AND DISCUSSION

Code	Sequence	IC_{50} (μM) A-375	IC_{50} (μM) B16-F10
LfcinB-1	RRWQWR	>203	>203
LfcinB-2	Ahx-RRWQWR	>182	>182
LfcinB-3	RWQWRWQWR	35	43
LfcinB-4	Ahx-RWQWRWQWR	>125	>125
LfcinB-5	$(RWQWRWQWR)_2$K-Ahx	>63	>63
LfcinB-6	RWQWRWQWO	>139	>139
LfcinB-7	RRWQWRWQWR	>111	>111
LfcinB-8	RGD-Ahx-RRWQWRWQWR	>96	>96
LfcinB-9	OOORWQWRWQWR	97	>109
LfcinB-10	RRWQWRMKKLG	>130	>130
LfcinB-11	$(RRWQWRFKKLG)_2$K-Ahx	41	46
LfcinB-12	(RRWQWR-1-Nal-KKLG)$_2$K-Ahx	>58	>58
LfcinB-13	(RRWQWR-Hphe-KKLG)$_2$K-Ahx	8	41
LfcinB-14	(ROWQWRFKKLG)$_2$K-Ahx	>61	>61
LfcinB-15	KKWQWK-Ahx-RLLRRLLR	22	166
LfcinB-16	KKWQWK-Ahx-KLLKKLLK	>96	>96
LTX-315	KKWWKKW-Dip-K	9.7	53
LfcinBOp-1	$(RRWQWRRLLR)_2$-K-Ahx	15	18
LfcinBOp-2	WKKWQWK-Ahx-RLLRRLLR	ND	88
LfcinBOp-3	WWKKWQWK-Ahx-RLLRRLLR	ND	70
LfcinBOp-4	WWWKKWQWK-Ahx-RLLRRLLR	ND	41
LfcinBOp-5	RTSKKWQWK-Ahx-RLLRRLLR	ND	52
LfcinBOp-6	KTSKKWQWK-Ahx-RLLRRLLR	ND	52

Table 1. Cytotoxic activity of peptides derived from Bovine Lactoferricin and Buforin II against Melanoma cell lines A-375 and B16-F10. Data are presented as mean ± SE. (n=3)

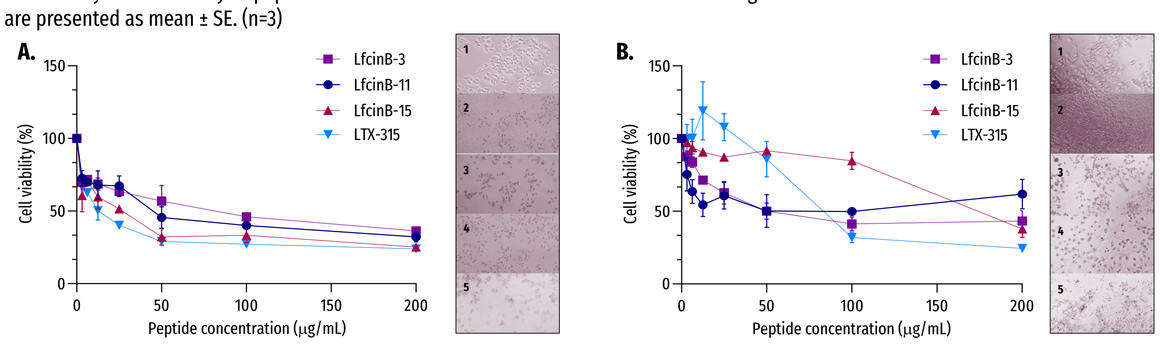
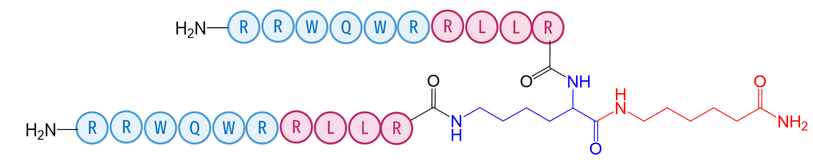


Figure 1. Cytotoxic Effect of Peptides with Enhanced Activity Against Melanoma Cell Lines: (A) A-375 and (B) B16-F10. Data are presented as mean ± SE. (n=3) . Microscopic images (100x) show: (1) negative control (no treatment), (2) LfcinB-3, (3) LfcinB-11, (4) LfcinB-15, and (5) LTX-315.

CONCLUSION

Bioactivity screening showed that the peptides derivated of LfcinB and Buforin II have cytotoxic activity against melanoma cell lines derivated from human and murine sources (A-375 and B16-F10 respectively). Consequently, a variety of optimization strategies, such as sequence conjugation and W-tagging, were explored. Peptide design focused on identifying suitable locations for modifications that would maintain or enhance cytotoxic activity. Peptides analogous to RRWQWR and RLLR, exhibited equal or improved cytotoxic activity in melanoma cell lines, generated through modifications involving functionalization with these sequences, the KTS/RTS tripeptide or W-tagging at the N-terminus. The peptide LfcinBOp-1: $(RRWQWRRLLR)_2$-K-Ahx exhibit a potent cytotoxic activity at 2 hours of treatment in both cell lines, with no significant differences in IC_{50} values. Furthermore, it was shown that the inclusion of the W_n or the KTS/RTS motif generated sequences with increased anticancer effect against the more resistant cell line, murine melanoma cell line. These results suggest that these strategies could be considered in development of peptide sequences with cytotoxic activity against melanoma.

Peptide Chimera Dimer Containing Minimal Motifs from LfcinB and BFII (LfcinBOp-1)



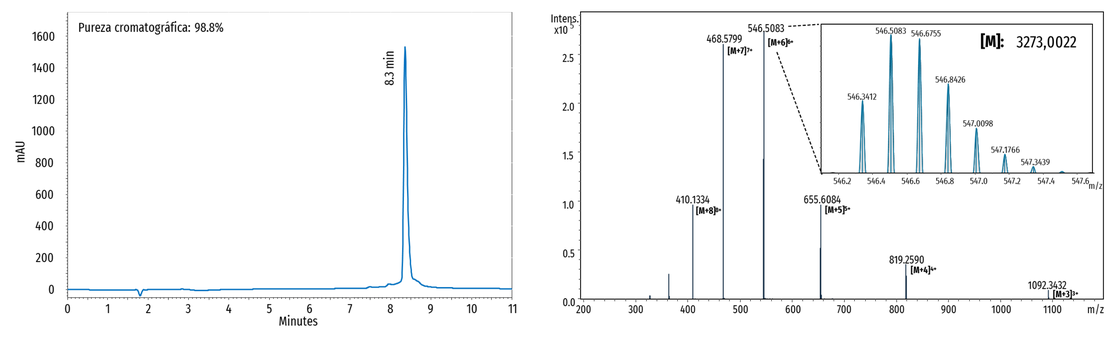


Figure 2. Characterization of the peptidic chimera dimer containing minimal motifs from LfcinB and BFII by RP-HPLC (left) and LC-ESI-QTOF Mass Spectrometry (right).

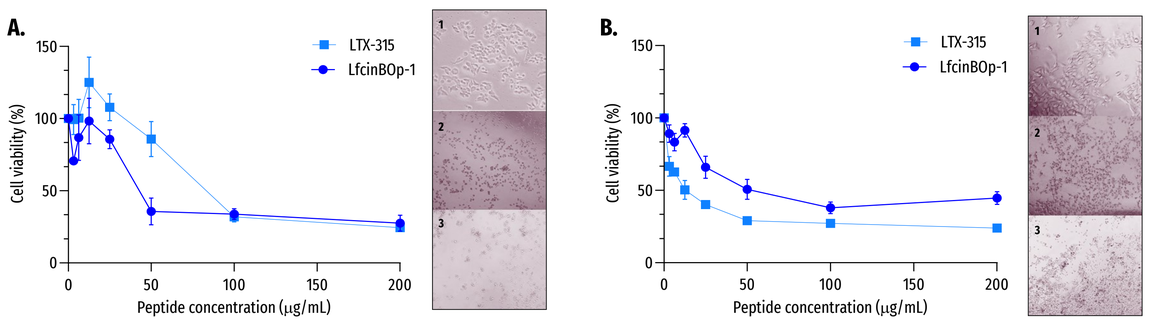


Figure 3. Cytotoxic Effect of the LfcinBOp-1 Peptide Against Melanoma Cell Lines: (A) A-375 and (B) B16-F10. Data are presented as mean ± SE. (n=3). Microscopic images (100x) show: (1) negative control (no treatment), (2) LfcinBOp-1, and (3) LTX-315.

Code	Sequence	%Hemolisis
LfcinBOp-1	$(RRWQWRRLLR)_2$-K-Ahx	18.9*

Table 2. Hemolytic Activity of the LfcinBOp-1 Peptide. *Percentage of Hemolysis at IC_{50} Concentration in A-375 Cells (15 μM)

REFERENCES

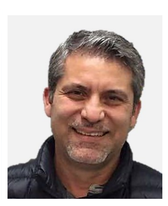
- Arnold, M., Singh, D., Laversanne, M., Vignat, J., Vaccarella, S., Meheus, F., Cust, A. E., de Vries, E., Whiteman, D. C., & Bray, F. (2022). Global Burden of Cutaneous Melanoma in 2020 and Projections to 2040. JAMA Dermatology, 158(5), 495–503. https://doi.org/10.1001/jamadermatol.2022.0160
- Arias, M., Hilchie, A. L., Haney, E. F., Bolscher, J. G. M., Hyndman, M. E., Hancock, R. E. W., & Vogel, H. J. (2017). Anticancer activities of bovine and human lactoferricin- derived peptides. Biochemistry and Cell Biology = Biochimie et Biologie Cellulaire, 95(1), 91–98. https://doi.org/10.1139/bcb-2016-0175
- Lee, H. S., Park, C. B., Kim, J. M., Jang, S. A., Park, I. Y., Kim, M. S., Cho, J. H., & Kim, S. C. (2008). Mechanism of anticancer activity of buforin IIb, a histone H2A-derived peptide. Cancer Letters, 271(1), 47–55. https://doi.org/10.1016/j.canlet.2008.05.041
- Solarte, V. A., Conget, P., Vernot, J.-P., Rosas, J. E., Rivera, Z. J., García, J. E., & Arango-Rodríguez, M. L. (2017). A tetrameric peptide derived from bovine lactoferricin as a potential therapeutic tool for oral squamous cell carcinoma: A preclinical model. PloS One, 12(3), e0174707. https://doi.org/10.1371/journal.pone.0174707
- Kozar, I., Margue, C., Rothengatter, S., Haan, C., & Kreis, S. (2019). Many ways to resistance: How melanoma cells evade targeted therapies. Biochimica et Biophysica Acta. Reviews on Cancer, 1871(2), 313–322. https://doi.org/10.1016/j.bbcan.2019.02.002
- Duong, D. T., Singh, S., Bagheri, M., Verma, N. K., Schmidtchen, A., & Malmsten, M.(2016). Pronounced peptide selectivity for melanoma through tryptophan end-tagging. Scientific Reports, 6, 24952. https://doi.org/10.1038/srep24952


PhD. Zuly Rivera


PhD. Javier García


Jose David Mendoza


PhD. Ricardo Fierro


PhD. Joel López

37th EPS European Peptide Symposium

14th IPS International Peptide Symposium

UNIVERSIDAD NACIONAL DE COLOMBIA

SAMP

UNIVERSIDAD MICHOACANA DE SAN NICOLÁS DE HIDALGO