**Rational Design of Potent and Selective Anticancer Therapies Through Chemical Approaches and Integration of Cationic Amphipathic Oncolytic Peptides**

**Linyu Huang1**, Jeonghwan Kim1, Huy Xuan Luong1,2 and Jong Oh Kim1.

1College of Pharmacy, Yeungnam University, Gyeongsan, 38541, Republic of Korea;

2Faculty of Pharmacy, Phenikaa University, Hanoi 12116, Vietnam.

**Background and aims.** The anticancer properties of Antimicrobial peptides (AMPs) have emerged as a promising area of exploration. Inspired by the discovery of LTX315, this study focused on developing immunology oncolytic therapy via sequence modification of Mastoparan AF (MAF) – a natural venom peptide from the hornet Vespa affinis. This study aimed to elucidate the structural requirements for the anticancer activity of AMPs and to explore the potential of combining LTX315 with MAF derivatives to enhance anticancer potency and selectivity.

**Methods.** Chemical synthesis and structure-activity screening of multiple Mastoparan AF analogs. Circular dichroism spectroscopy and molecular dynamics simulations were employed to evaluate structural changes and self-association tendencies. To explore the possibility of further improving the potency and selectivity of anticancer therapy, the effect of peptide combination was investigated on cancer cells and RBCs. The mechanism underlying the enhancements of the anticancer effect of peptide combinations was clarified in the A549 cell line. Additionally, the peptides with the potential anticancer effect were subjected to a preliminary investigation of inducing the hallmarks of immunogenic cell death (ICD).

**Results.** MAF-10L exhibited significantly enhanced helicity and highest self-association propensity. MAF-10L exhibited superior anticancer activity but elevated hemolysis, which was mitigated through combination therapy with LTX315. The results of cell membrane integrity and mitochondrial function demonstrated that the combination of cell membrane leakage and mitochondria dysfunction resulted in the enhanced anticancer effect of the peptide combination compared to the treatment with single peptides. MAF induced a comparable ICD hallmarks activity with LTX-315, whereas MAF-10L exhibited enhancement effects. The results of MD simulation demonstrated its translocation mechanism, or its cytotoxic effect on cancer cells, may be attributed to its ability to insert deeply into the membrane without significant lipid interactions, ultimately leading to membrane destabilization.

**Conclusion/Discussion.** The study presents Mastoparan AF derivatives as promising candidates for anticancer therapy, showcasing the effectiveness of rational peptide design in enhancing anticancer activity. Modifications aimed at increasing hydrophobicity and cationic charge significantly improved the peptides' potency. The synergistic effects observed in combination with LTX315 highlight a promising approach to improving selectivity and reducing hemolytic side effects.

**References:**

(1) Hai Bui T P, et al (2025) Journal of Medicinal Chemistry, 68 (11), 11875-11893.

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