β-HAIRPIN PEPTIDES GOING FURTHER ANTIMICROBIAL ACTIVITY AND CLASSIC MODE OF ACTION

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Introduction and objectives
Cancer is among the most common known death cause worldwide. Due to disease diversity, there’s no definitive treatment yet, and researches for new drugs are required. The β-hairpin peptides, discovered by its antimicrobial activity, have shown many different activities against several diseases, from virus to cancer, although the mechanisms often suggest membrane pore formation. This work aims to understand the cell death pathways triggered by β-hairpin peptides - Gomesin, Tachypleisn – comparing them with the effect elicited by H2O2 and Staurosporine in HeLa cells.

Materials and methods
Cell Culture: HeLa cells were seeded in DMEM high glucose with 10% FBS and antibiotics. They were plated at 10⁵ cells/mL.
Flow Cytometry: Cells were treated with peptides, Staurosporine or H2O2. We evaluated the cell viability using AnexinnV–FITC/PI assay and antibodies against phosphorilated proteins.
Microscopy: The cells were treated with each drug EC₅₀ and incubated along with PI in order to evaluate membrane permeabilization time and morphological modifications.
gPCR: Thereafter treatment, the RNA was extracted by Trizol method, cDNA synthesis and qPCR was performed using SaBioscience/Qiagen kits.

Results and Conclusion
Gomesin and Tachypleisin exhibited good potency against HeLa cells, with EC₅₀ under 10µM. Cell death was observed after 6h treatment with peptides promoting membrane permeabilization and blebbing formation. Also Caspase 3/8/9, NFKB, p38, Akt, PI3K, p44, ELK-1, SAPK, MEK, PKA and PTEN activation were verified, as well as genes involved in cell death were compared among β-hairpin peptides, Staurosporine and H₂O₂. Tachypleisin effects have similarities with H₂O₂, activating almost the same proteins and necroptotic genes, while gomesin activates only Caspase 3 and Elk-1, besides IFNG and TNFSRF10A genes. These results showed different signaling pathways activation triggered by β-haipin, Staurosporine and H₂O₂.

Acknowledgements
This work was supported by FAPESP, CAPES and CNPq.

Key Words
β-hairpin peptide, cell death pathway, cancer.