The antitumoral potential of *Pllans-II*, an acidic PLA2 from *Porthidium lansbergii lansbergii* snake venom on human cervical carcinoma cells.

Gabriel Alejandro Montoya-Gómez[1]; Leonel Montealegre-Sánchez[1]; Nelson Rivera-Franco[2]; Andrés Castillo[2]; Eliécer Jiménez-Charris[1]

[1]*Grupo de Nutrición, Facultad de Salud, Universidad del Valle, Cali, Colombia.* [2]*Departamento de Biología, Facultad de Ciencias Naturales y Exactas, Universidad del Valle, Cali, Colombia*

1. Introduction

Cervical cancer (CC) is a malignant neoplasm originating in cervical cells, being the fourth carcinoma with more morbidity and mortality in women and occupying the ninth place in the world ranking of cancer types causing mortality (considered both sexes). About 570.000 new cases were reported in 2018, and 311.000 women die from this pathology in the world.

Chemotherapeutic treatments and radiotherapy are the most used in the advanced stages of CC. However, these concomitant treatments have various side effects, affecting patients' quality of life, leading in many cases to the abandonment of medicines, which contributes to the high death rate.

One of the significant challenges for medicine is the exploration of new intervention strategies and the implementation of more effective drugs against CC and less toxic for healthy tissues. Therefore, the scientific investigation may focus on finding molecules that exclusively affect the tumor microenvironment, particularly in the inhibition of metastasis and the induction of cell apoptosis. Many molecules with these properties have been found in snake venoms, such as phospholipases A2. These toxins are highly effective in affecting the development of tumor cells of different origins. The investigations carried out indicate that this effect is not induced mainly by the svPLA2s enzymatic activity but by interactions with cell surface receptors, affecting intracellular signal transduction pathways.

Thus, the antitumoral potential of *Pllans-II* (an Asp49-type acidic phospholipase A2 from *Porthidium lansbergii lansbergii* snake venom) was evaluated for the first time against squamous epithelial cell line of cervical cancer-Ca Ski. Cytotoxic effect, cell cycle arrest, cell death, adhesion and migration inhibition, transcriptomic analysis, and possible interactions with membrane receptors were evaluated on Ca Ski cells treated with *Pllans-II* to determine its antitumor potential on cervical cancer.

Geographic distribution of *P. lansbergii lansbergii* in Colombia

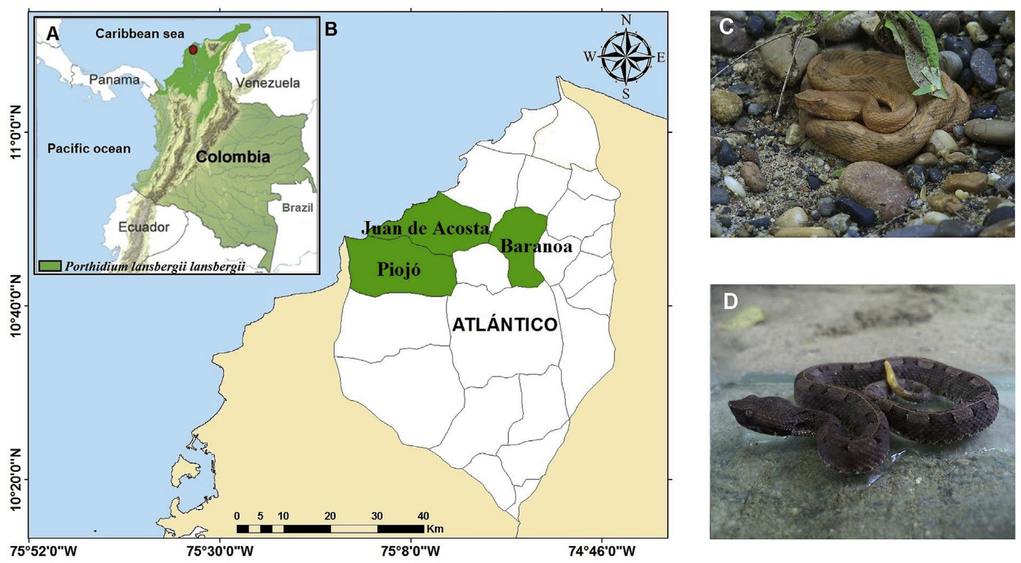
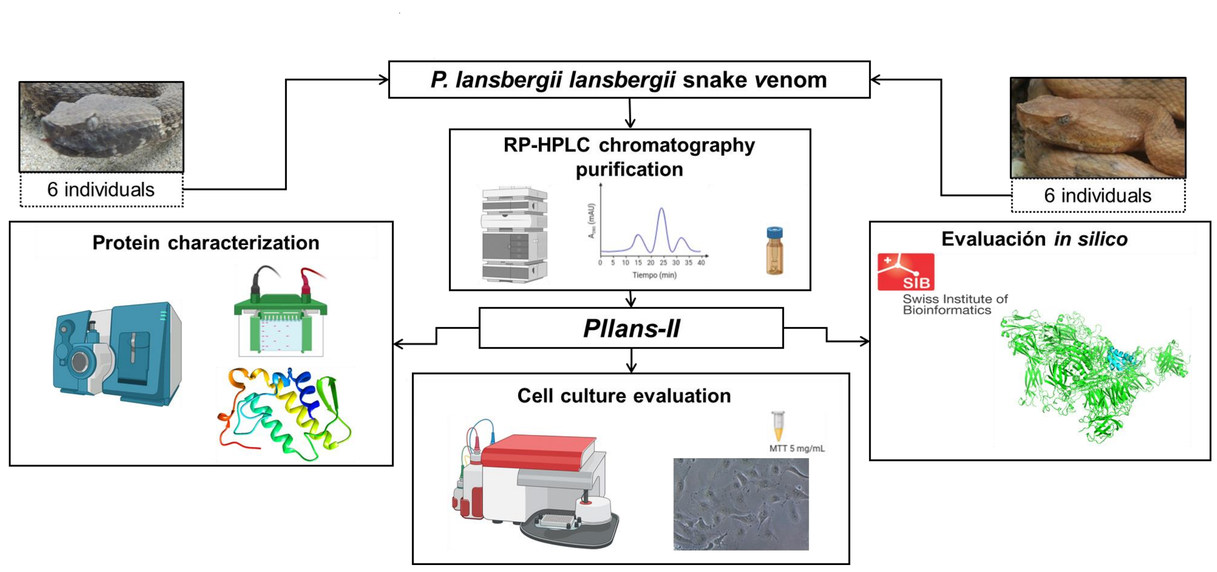


Fig. 1. Geographic distribution of the *Porthidium lansbergii lansbergii* species in Colombia (**A**) and in the Atlantic Department localities of Baranoa, Piojó and Juan de Acosta, where the samples were obtained (**B**). *P. lansbergii lansbergii* can be found in two chromatic morphotypes: yellow (**C**), and gray (**D**). Photos by Luis Manuel Solano.

2. Experimental Design



3. Results

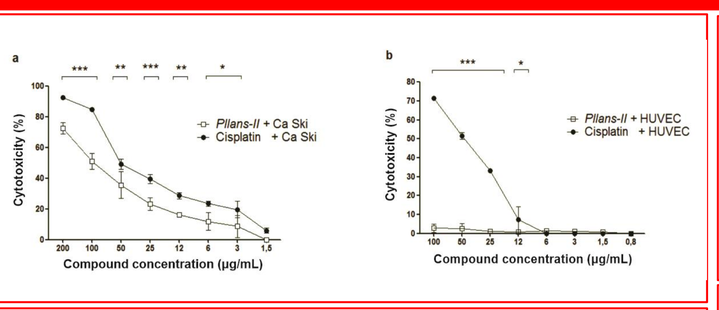


Fig. 2. Cytotoxic activity of *Pllans-II* and Cisplatin® on squamous epithelial cells of cervical cancer and non-tumorigenic cells, determined by MTT cytotoxicity assay. (a) Ca Ski cells treated with concentrations of serial dilutions (200-1.5 µg/mL) of both Pllans-II and Cisplatin®. The treatments were also performed on a non-tumorigenic line of human endothelial cells (HUVEC). In (a) and (b), statistically significant differences are observed with ***p< 0.001, **p< 0.01 and *p< 0.05

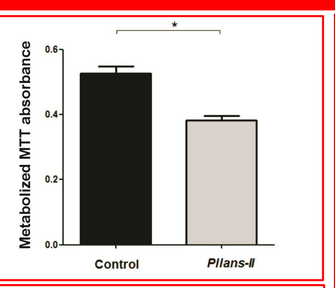


Fig. 3. Effect of Pllans-II on Ca Ski cell adhesion. Cells were incubated with 100 µg/mL or with RPMI 1640 medium (control) for 24 hours. Metabolic activity was determined by MTT assay. Statistically significant differences (*p<0.05) are observed between the treatments.

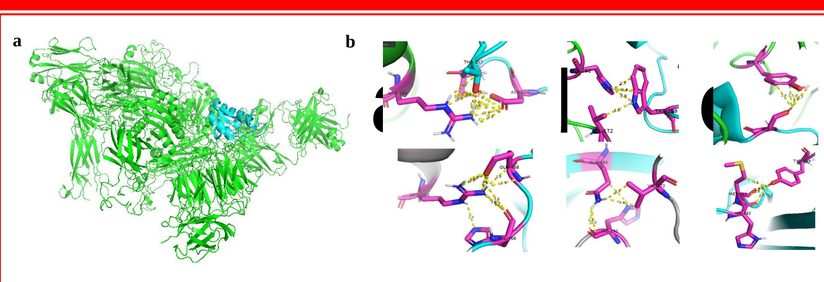


Fig. 8. (a) Molecular Docking between α5β1 integrin and *Pllans–II*. **(b)** Values obtained in the molecular Docking performed with the Cluspro server, both for the native receptor-ligand, and for the interactions between *Pllans-II* and different membrane receptors. **(c)** *Pllans-II* and Fibronectin amino acids that interact with the same amino acids of the α5β1 containing integrin. **(d)** Amino acids involved in the interactions between α5β1 containig integrin/Pllans-II and α5β1containing integrin/Fibronectin, with distances less than 3 Å. In the first three horizontal boxes for each pair of coupled proteins, it is possible to appreciate the receptor amino acids that have interactions in common with amino acids of both ligands.

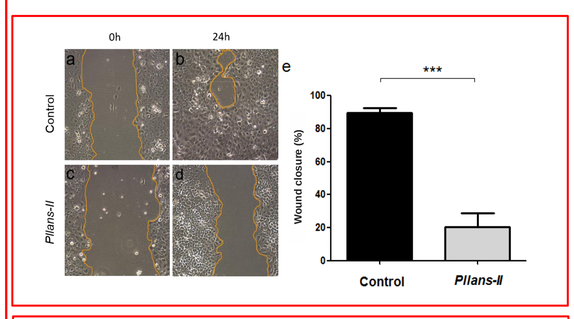


Fig 4. Migration effect of Pllans–II on Ca Ski cells by Wound healing Assay. Cells were seeded at 5 x 10^4 cells/well in 24–well plates until obtaining complete confluence. After 24 hours, the medium was discarded and the confluent monolayer was scratched with a 10 µL pipette tip to create an area devoid of cells (0 h) (a and c). After this process, cells were treated in the absence (a and a) or presence of Pllans–II at 100 µg/mL (c and d), and 24 hours after incubation, cell migration analysis were performed in an inverted optical microscope (Nikon Eclipse TS100). (e) The bar graph shows the percentage of cell migration for Pllans-II and control treatment after 24 hours of incubation. The data are expressed as means ± SD, and the procedures were carried out in triplicate. A statistically significant difference is observed with ***p< 0.001.

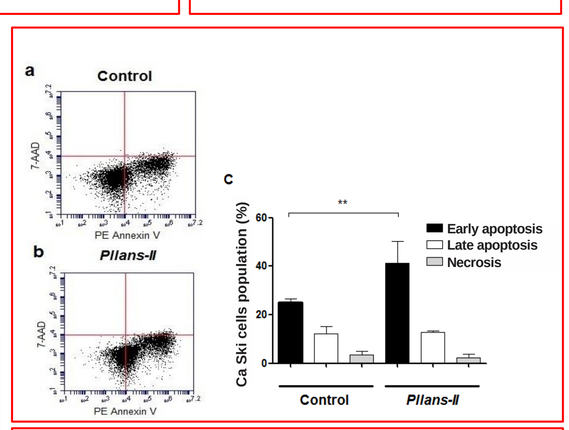


Figure. 5. Cell death in Ca Ski cells by Pllans-II treatment. The distribution of apoptotic and necrotic cells was analyzed by flow cytometry, both for control (a) and for treatment with the protein (b). (a-b) The dot plot revealed the percentage of cells in early apoptosis (lower right quadrant), late apoptosis (upper right quadrant) and necrosis (upper left quadrant). (c) The bar chart shows the percentages of cells at each stage. Statistically significant differences (**p< 0.01) are observed between the protein treatment and the control.

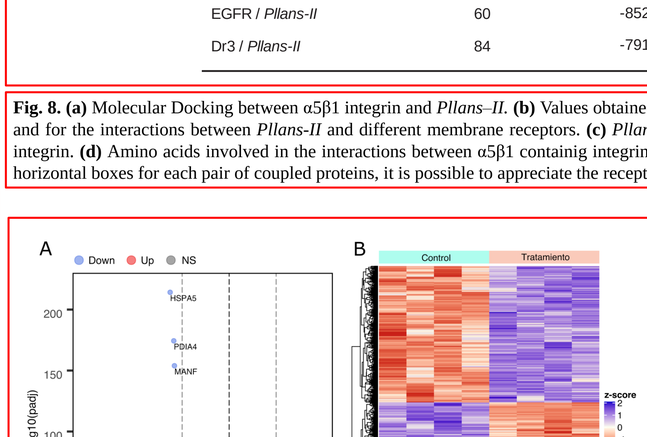


Fig 9. Analysis of differentially expressed genes (DEG) from CaSki cells treated with pllans-II and control. (A) Volcano-plot: the dots represent individual genes. The red dots represent genes with significantly higher expression in controls than in treatment (down-regulated), while the blue dots represent genes significantly more expressed in the treatment group (up-regulated). The gray dots indicate genes that are not differentially expressed. (B) Hierarchical SDR cluster heat map analysis. The heat map shows changes in gene expression for each of the treatment and control samples. The blue and red colors represent respectively the increase and decrease in expression, while the white color indicates no change.

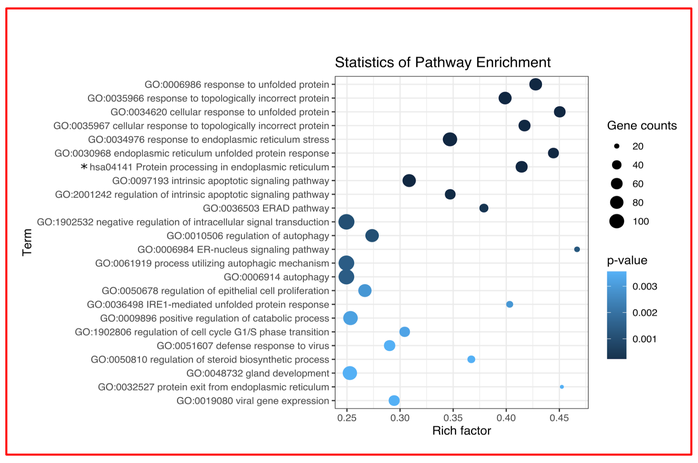


Fig 10. Pathway enrichment analysis. The term (Y-axis) represents the identification code and the pathway for the GO and KEGG databases. Asterisk (*) indicates the pathway found significant for KEGG. The enrichment factor (X-axis) represents the relationship between the number of differentially expressed genes and the total number of genes in a given pathway. The area of each colored circle is proportional to the number of genes involved in each pathway, the color indicates the p-adjusted value.

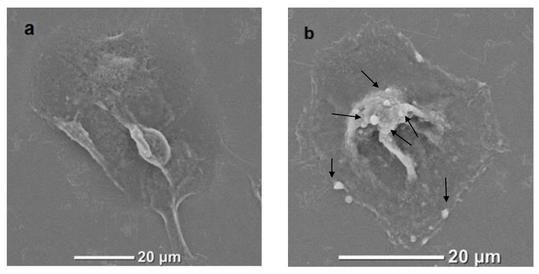


Figure. 6. morphological changes induced by Pllans-II on Ca Ski cells, detected by scanning electron microscopy (SEM) (A) Ca Ski cell treated with RPMI 1640 culture medium. (B) Pllans-II treated Ca Ski cell, in which the formation of apoptotic bodies (marked with arrows) and size reduction is evident.

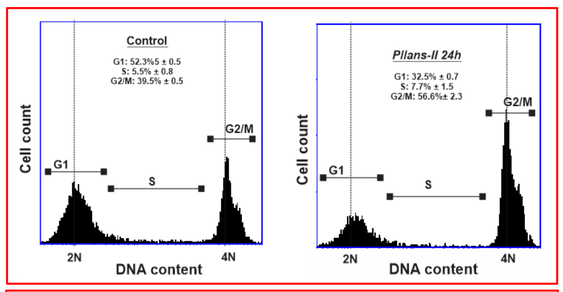


Fig. 7. Effect of Pllans-II on the cell cycle of Ca Ski cells. DNA content in each phase of the cell cycle was analyzed by flow cytometry. Pllans-II induced a significant decrease in the number of 2N cells in G1 phase, and an increase in the number of cells in G2/M phase compared to the control, after 24 hours of incubation. Statistically significant differences are observed with ***p< 0.001.

4. Conclusion

Pllans-II, an Asp49-type acidic phospholipase A_2 from *Porthidium lansbergii lansbergii* snake venom, displayed for the first time antitumoral potential against the squamous epithelial cell line of cervical cancer Ca Ski. *Pllans–II* presented a dose-dependent cytotoxic effect on cancer cells and an insignificant effect on healthy endothelial cells HUVEC. *Pllans–II* also inhibited the adhesion and migration ability of cancer cells and induced cell cycle arrest in the G2/M phase and apoptosis on Ca Ski cells. Transcriptomic analysis revealed that cell death was related to endoplasmic reticulum stress by interfering with α5 and β1–containing integrins. These results demonstrate that *Pllans–II* has an antitumor potential on cervical cancer and represents a possible biotechnological tool for designing anticancer prototypes.

5. References

- Bray, Freddie, et al. "Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries." *CA: a cancer journal for clinicians* 68.6 (2018): 394-424.
- A.V. Osipov, Y.N. Utkin, Antiproliferative effects of snake venom phospholipases A2 and their perspectives for cancer treatment, in: L. Cruz, S. Luo (Eds.), Toxins and Drug Discovery, Springer, Dordrecht 2015, pp. 1–15
- Jiménez–Charris, Eliécer, et al. "Antitumor potential of Pllans–II, an acidic Asp49–PLA2 from Porthidium lansbergii lansbergii snake venom on human cervical carcinoma HeLa cells." *International journal of biological macromolecules* 122 (2019): 1053-1061.

6. Acknowledgments

The authors thank Eliécer Jiménez Vargas and Raul Charris Morales for their help in the collection of venom samples. This work was supported by the School of Basic Sciences, Faculty of Health, Universidad del Valle.