ECE-1c activates Akt and FAK thereby promoting growth and invasion of colon cancer cells.

Huerta H¹, Silva-Pavez E¹, Niechi I¹, J.P Muñoz², Aguayo F², Fernandez C³, and Tapia J.C¹.

¹Cell Transformation Laboratory ICBM; ²Virology Program ICBM; ³Department of Anatomopathology HCUCH, Faculty of Medicine, University of Chile.

Introduction and objectives. Endothelin Converting Enzyme-1c (ECE-1c) is an integral membrane Zn-metalloprotease that synthesizes Endothelin-1 (ET-1) through its catalytic domain. In many cancers, ET-1 levels are increased, promoting malignant characteristics like augmented proliferation and angiogenesis. Furthermore, recent studies propose to isoform ECE-1c as a key mediator of cell invasiveness. Therefore, we studied the role of ECE-1c in colon cancer cells and the dependency of ET-1 for the ECE-1c-promoted malignancy.

Material and Methods. We overexpressed the full-length ECE-1c or the construct NT-ECE1c (first 100 N-terminal residues of ECE-1c fused to GFP) to investigate the role of its N-terminal region in activation of FAK and Akt in DLD-1 human colon cancer cells. BQ-123 and LY-204002 were used to inhibit ETaR and PI3K, respectively. Cell migration was evaluated by transwell and wound healing assay. Anchorage-independent cell growth was measured by soft agar and viability was measured by MTS assay. Cell invasion was evaluated by matrigel and localization of NT-ECE-1c by confocal microscopy.

Results. ECE-1c promoted activation of FAK and Akt, as well as increased migration, invasion and anchorage-independent growth. Overexpression of ECE-1c in ET-1 silenced cells showed similar increase in migration compared to control. Inhibition of ETaR and/or PI3K in ECE-1c overexpressing cells showed no effect in proliferation, while mock cells showed decreased proliferation. This suggested an ET-1-independent mechanism by which ECE-1c promotes cell viability and migration. Surprisingly, NT-ECE-1c expression promoted an opposite effect in kinases activation, which correlated with decreased levels of the anti-apoptotic protein survivin and subsequently cell viability.

Conclusions. ECE-1c promotes activation of FAK and Akt thereby enhancing malignant characteristics via a mechanism both dependent and independent of ET-1 in colon cancer cells.

Acknowledgements. FONDECYT grant 1120132, CONICYT Ph.D. fellowship 21130753

Key Words: ECE-1c, cancer, migration/invasion