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The Role of Connective Tissue Growth Factor in Maintaining the Epithelial Phenotype of Ovarian Cancer Cells during Epithelial-to-Mesenchymal Transition

Harry Ramos
Wayne State University, he3507@wayne.edu

Sandra Galoforo
C.S. Mott Center for Human Growth and Development, sandra.galoforo@wayne.edu

Gil Mor
C.S. Mott Center for Human Growth and Development, gmor@med.wayne.edu

Radhika Gogoi
Wayne State University, radhikagogoi@wayne.edu

The Role of Connective Tissue Growth Factor in Maintaining the Epithelial Phenotype of Ovarian Cancer Cells during Epithelial-to-Mesenchymal Transition

Harry Ramos[1], Sandra Galoforo[2], Gil Mor[2], Radhika Gogoi[1,2]

[1]Wayne State University School of Medicine, Detroit, MI
[2]C.S. Mott Center for Human Growth and Development, Detroit, MI

Objective: The transition from epithelial to mesenchymal is essential for the process of ovarian cancer (OC) metastasis. The aim of our study is to evaluate the role and mechanism of Connective Tissue Growth Factor (CTGF) in epithelial to mesenchymal transition (EMT).

Methods: R182 is an epithelial OC cell line. CTGF expression +/- Transforming Growth Factor -β (TGF-β) was determined via Western blot and ELISA. R182 CTGF knock out (KO) were derived utilizing a Cas9/CRISPR-Cas9 lentivirus plasmid vector. Anoikis resistance and invasion assays were performed to characterized phenotypes of R182 wild type (WT) and KO cells. For anoikis resistance, cells were plated in triplicate in an ultra-low adhesive (ULA) cell plate with complete culture media. Using Promega CellTiter assay, cell viability was quantified by absorbance at 450 nm at 0, 24, 48, and 72 hr time points. For invasion assay, 3000 cells were suspended in 50% reduced growth factor Basement Membrane Extract. Human recombinant CTGF is added at 50 and 100 ng/mL concentrations. Cells were plated in tissue culture plate and placed in Cytation 5/Biospa. Cells were imaged at 4-hour interval for up to 6 days. Lastly, expression of mesenchymal markers including SNAIL was probed via Western blot analysis in both WT and KO cells. All experiments are being repeated in another epithelial OC cell line, R2615.

Results: CTGF is constitutively expressed in R182 with detectable levels as early as 24 hrs of culture. Expression peaked at 2 – 6 hr with TGF-β and return to baseline by 24 hrs. Loss of CTGF promoted anoikis resistance. At 72 hr in ULA plate, R182 CTGF KO cells displayed 75% viability while R182 KO only have 10% viability. Further, we demonstrated that loss of CTGF allows for OC cells to invade the matrix whereas R182 WT does not display invasion. Administration of exogeneous CTGF in KO cells suppresses invasion in a dose dependent manner where 100 ng/mL returns the phenotype similar to R182 WT level. Lastly, loss of CTGF induces SNAIL expression.

Conclusion: CTGF plays a role in maintaining the epithelial phenotype of OC cells during EMT. Loss of CTGF promotes anoikis resistance and invasion which are vital characteristics in the metastatic nature of OC. We suggest that loss of CTGF leads to increase in SNAIL expression permitting OC cells to transition to mesenchymal phenotype. Thus, loss of CTGF expression in OC cells could be a potential early target to prevent metastases in OC.

Keywords: CTGF, EMT, SNAIL, CRISPR, ovarian cancer, anoikis, invasion