Marisol Mosqueda Arreola
L2
LSU Health Sciences Center, New Orleans, LA

Dr. Arnold H. Zea, PhD
LSUHSC, Stanley S. Scott Cancer Center, Department of Microbiology

"Understanding the tumor microenvironment to overcome resistance in kidney cancer"

BACKGROUND: The semi-essential amino acid L-arginine when metabolized by arginase (ARG2) and NOS2 not only plays a crucial role in the synthesis of polyamines promoting tumor growth but also the production of nitric oxide (NO) and L-citrulline which have anti-tumor capabilities. We have previously demonstrated that stimulation of renal cell carcinoma cells (RCC) with IFNγ lacking NOS2 did not affect tumor-killing compared to cells expressing it. However, a combination of IFNγ and LPS has shown to overcome tumor resistance to IFNγ leading to cell killing. Keeping this in mind, we started another phase of the project to try to understand the mechanisms by which tumor resistance is overcome. We hypothesize that L-citrulline via ASS/ASL enzymes restores L-arginine availability increasing anti-tumor activity via IFNγ-NOS2 signaling dependent on tumor heterogeneity.

OBJECTIVES: The main objective is to explore IFNγ resistant mechanisms in different RCC cell lines and screen new therapeutic drugs. We want to improve our understanding of its resistance mechanisms and to increase the efficacy of combination treatments.

METHODS: Four different murine cell lines, which express different ARG2 and NOS2 levels, were used for our experiments. The cells were cultured in RPMI media containing 1040 μM of L-arginine for 24, 48 hours and stimulated with 100U/ml of IFNγ. Toll Like receptors (TLR-agonists (10 mg) were also added to the cultures. Culture supernatants were collected at different times to test for L-arginine and L-citrulline (HPLC), and nitrites (Greiss assay). Cellular extracts were obtained and tested (30 μg) for ARG2, NOS2, ASS, ASL and GAPDH protein expression (Western blot). ARG activity was measured by enzymatic assays. MTT assay was used to quantify cell viability and proliferation.

RESULTS: At base line, the cell lines presented with different growth patterns with R0 having a higher growth rate. The growth rate appears to be independent of ARG activity. CL-19 grew like R0, but its ARG activity was higher. The nitrate production was higher in CL-19 as compared to the others, and parallels with their sensitivity to IFNγ. When stimulated with IFNγ, results were variable among the three Renca cells lines, with R2 lacking sensitivity to both IFNγ and LPS but R0 being equally sensitive to the IFNγ and LPS combination. There is a delay in Western blot and HPLC results because we had to develop a new HPLC protocol that would allow us to separate L-arginine and L-citrulline which only differ by 0.984g/mol in MW.

CONCLUSIONS: Here, we have found that RCC cell lines present with different patterns in growth, nitrite production, ARG activity, and response to stimulation with IFNγ. These changes could be correlated to the heterogeneity observed in the tumor microenvironment and tumor resistance. The role of L-citrulline, ASS and ASL as contributors of tumor resistance needs to be further investigated.