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"Novel Treatment of Immunotherapy-Resistant Renal Cell Carcinoma Using Synergistic Effect of Interferon Gamma and LPS"

The ability of cancer cells to evade the human immune system is a strategic barrier that impedes the development of effective anti-tumor therapies. It was demonstrated previously that interferon-gamma (IFNγ) modulates the immune system's response to cancer by its ability to induce nitric oxide (NO) production in cancer cells, which has a cytostatic effect on tumor cells. However, some tumor cells do not produce NO in response to stimulation with this cytokine. In our work, we use a murine cell line of renal carcinoma (Renca) that do not respond to IFNγ stimulation. Previously, we demonstrated that these cells showed a significantly reduced expression of inducible nitric oxide 2 (NOS2), which is necessary for NO production. Other reports have shown that lack of L-arginine availability can block the induction of NO in cytokine-stimulated astrocytes. In Renca cells, the underlying mechanisms of diminished NO production are still under investigation. These data suggest that L-arginine can play a role in NOS2 translational inhibition, as evidenced by the diminished expression of NOS2 at low extracellular levels of this amino acid. While attempting to understand these mechanisms, we recently found that the production of NO can also be evoked in Renca cells by stimulation with a combination of IFNγ and lipopolysaccharide (LPS). We hypothesize that the synergistic effect of these compounds can overcome the resistance of Renca cells to IFNγ alone, regardless of L-arginine concentration.

We cultured Renca cells in media containing 1,000, 2,000, and 4,000 μM L-arginine for 24 hours. Then, we stimulated the cells with IFNγ (100 U/mL), LPS (1 μg/mL), or a combination of the two. Supernatants and cells were collected 24 hours later and tested for nitrites (Griess), NOS2 (Western blotting), and L-arginine levels (HPLC).

Our results show that variations in L-arginine concentration did not affect NOS2 expression in unstimulated cells. At 4,000 μM L-arginine, stimulation with IFNγ resulted in increased expression of NOS2 protein and nitrites. Stimulation with LPS alone or with IFNγ at 2,000 μM L-arginine did not change NOS2 expression or cause an increase in nitrite production. Interestingly, cells stimulated with IFNγ/LPS showed a significant increase in nitrite production ($p<0.001$) and an increased expression of NOS2 protein at all L-arginine concentrations, seemingly overcoming the blockade of NOS2 translation. HPLC data also show that extracellular L-arginine was decreased following IFNγ/LPS stimulation in cells cultured at 1,000 μM L-arginine; however, this relationship was not observed in cells cultured at 2,000 μM. The reason for this is not entirely clear but likely reflects the dynamic metabolism of L-arginine involved in NO production.

These preliminary results will increase our understanding of LPS signaling in NOS activation, its pro- and anti-cancer effects, and the therapeutic potential of LPS (TLR4 agonists) and IFNγ in the treatment of kidney cancer.

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