Decreased L-Arginine Availability Blocks the Induction of NO Synthesis in Renal Cell Carcinoma Cells (Renca) as a Mechanism of Defense in Response to Interferon Gamma

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Introduction

L-arginine (L-Arg) is the only endogenous substrate of NO synthase (NOS), and it thus governs the production of NO during different disease states including cancer. L-Arg metabolism has been shown to be an important pathway in the immune escape mechanism in cancer, including renal cell carcinoma. L-Arg can be metabolized in two primary pathways: conversion of L-Arg into L-ornithine (L-Orn) and urea by arginase (ARG) and conversion of L-Arg into L-citrulline and nitric oxide (NO) by inducible nitric oxide synthase (NOS2). The presence of ARG has been shown to lead to increased tumor growth by the conversion of L-Orn into polyamines that are essential for cell growth. In contrast, the production of NO by NOS2 has been shown to limit tumor growth due to the cytostatic effects of NO in IFNγ-stimulated tumor cells. In the murine renal cell carcinoma cell line Renca, the expected production of NO was not seen in response to IFNγ. It has been shown by Western blotting that the NOS2 protein was completely absent in these cells when L-Arg availability was decreased, whereas NOS2 mRNA expression was not inhibited under these conditions. It has also been demonstrated that ARG activity is higher than baseline in Renca cells. We believe that the overactive ARG disrupts NOS2 function by depleting L-Arg levels and causing what is known as the "arginine paradox." This paradox occurs when extracellular levels of L-Arg are depleted (regardless of the intracellular level), leading to decreased levels of NOS2. The exact mechanism behind this has yet to be elucidated. Determining this mechanism, as well as investigating the potential role of glutamine in the polyamine pathway, are future goals of this project. We hypothesize that in ARG2 expressing Renca cells, the availability of L-Arg regulates the activity of NOS2 as a mechanism of resistance against IFNγ-induced cell death.

L-arginine Metabolic Pathway

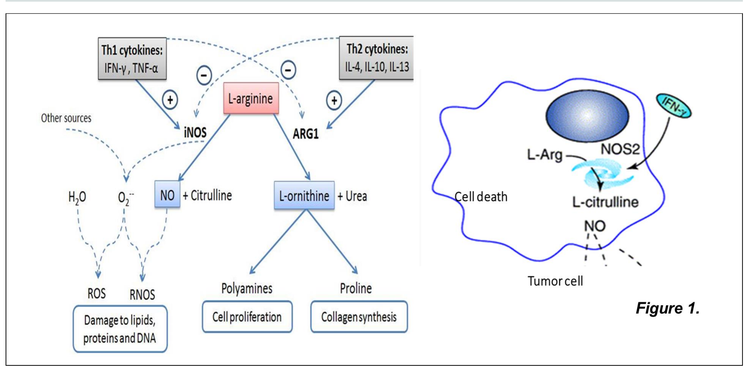

Figure 1.

Methodology

1. 300,000 cells were cultured for 48 h. in six well plates with RPMI media containing 1000, 2000, and 4000 μM of L-Arg. 10 U/ml of IFNγ was added to each well at 48 h. and cultured for additional 24 h.
2. Supernatants and cells were harvested and tested for ARG2 and NOS2 protein by immunoblot and NOS2 activity by Griess (nitrite formation). Supernatants were collected every day during the length of the experiments, which were used to determine L-Arg and L-citrulline levels by HPLC.

Results

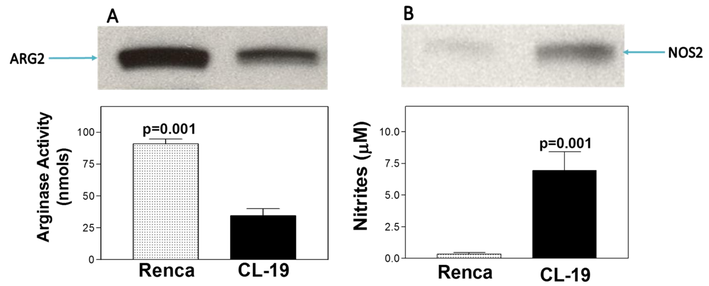

Figure 2. (A) Base line protein expression and activity for ARG2 and (B) NOS2. Supernatants and cell extracts were obtained at 48 h. Fifty μg of cell extract were tested by Western Blot and enzymatic assay. Significant differences in ARG2 and NOS2 (*P*=0.001) expression and activity is observed.

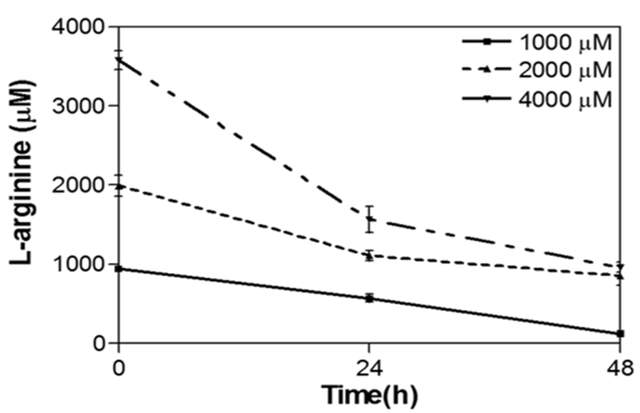

Figure 3. Extracellular levels of L-Arg measured by High Performance Liquid Chromatography (HPLC) were significantly reduced at 48 h regardless of initial L-Arg concentrations. This decrease was more noticeable when the initial L-Arg concentration was 1000 μM L-Arg values are expressed by mean ± SE from three different experiments. L-Arg availability does not affect the expression of ARG2 in Renca cells up to 72 h in culture (data not shown).

Results

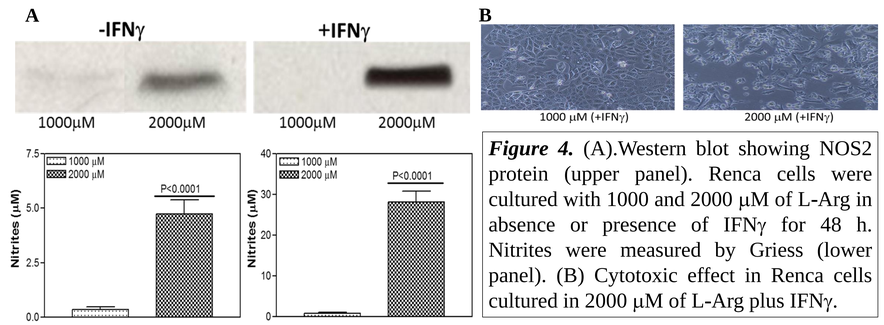

Figure 4. (A).Western blot showing NOS2 protein (upper panel). Renca cells were cultured with 1000 and 2000 μM of L-Arg in absence or presence of IFNγ for 48 h. Nitrites were measured by Griess (lower panel). (B) Cytotoxic effect in Renca cells cultured in 2000 μM of L-Arg plus IFNγ.

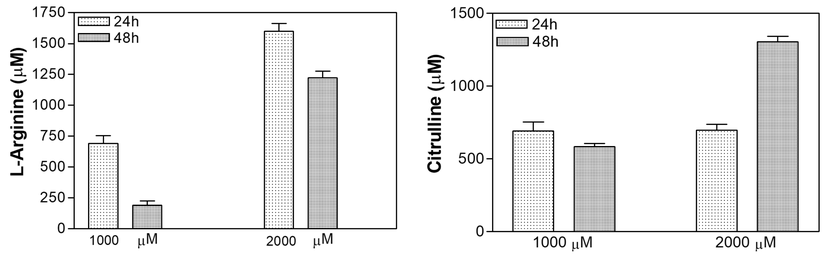

Figure 5. (A) Extracellular L-arginine and (B) L-citrulline levels in culture supernatants measured by HPLC. As expected, NOS2 activity and protein expression in Renca cells is governed by arginine transported into the cell from the extracellular medium. Values for L-arginine and L-citrulline are expressed by mean ± SE from three different experiments.

Conclusions

1. Initial data show that in resting Renca cells, NOS2 is not expressed when cultured in media containing 1000 μM of L-Arg.
2. L-Arg concentrations over 1000 μM regulate the expression of NOS2 in Renca cells, showing a proof of concept of the role of L-Arg availability in NOS2 expression.
3. These results partially explain the L-Arg paradox for NOS2 and define a distinct mechanism by which a substrate can regulate the activity of its associated enzyme.
4. Extra-intracellular L-Arg deprivation by ARG2 expression may also lead to a decreased intracellular L-Arg, possibly blocking NOS2 translation. Experiments addressing this are ongoing.
5. IFNγ stimulation of Renca cells did not alter ARG2 expression over time; therefore, L-Arg supplementation could be used to re-establish NOS2 expression in IFNγ resistant RCC.
6. The increased levels on L-Cit observed when cells are cultured in 2000 μM L-Arg suggest the possibility for some cells to use other pathways or de novo synthesis of this amino acid.

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