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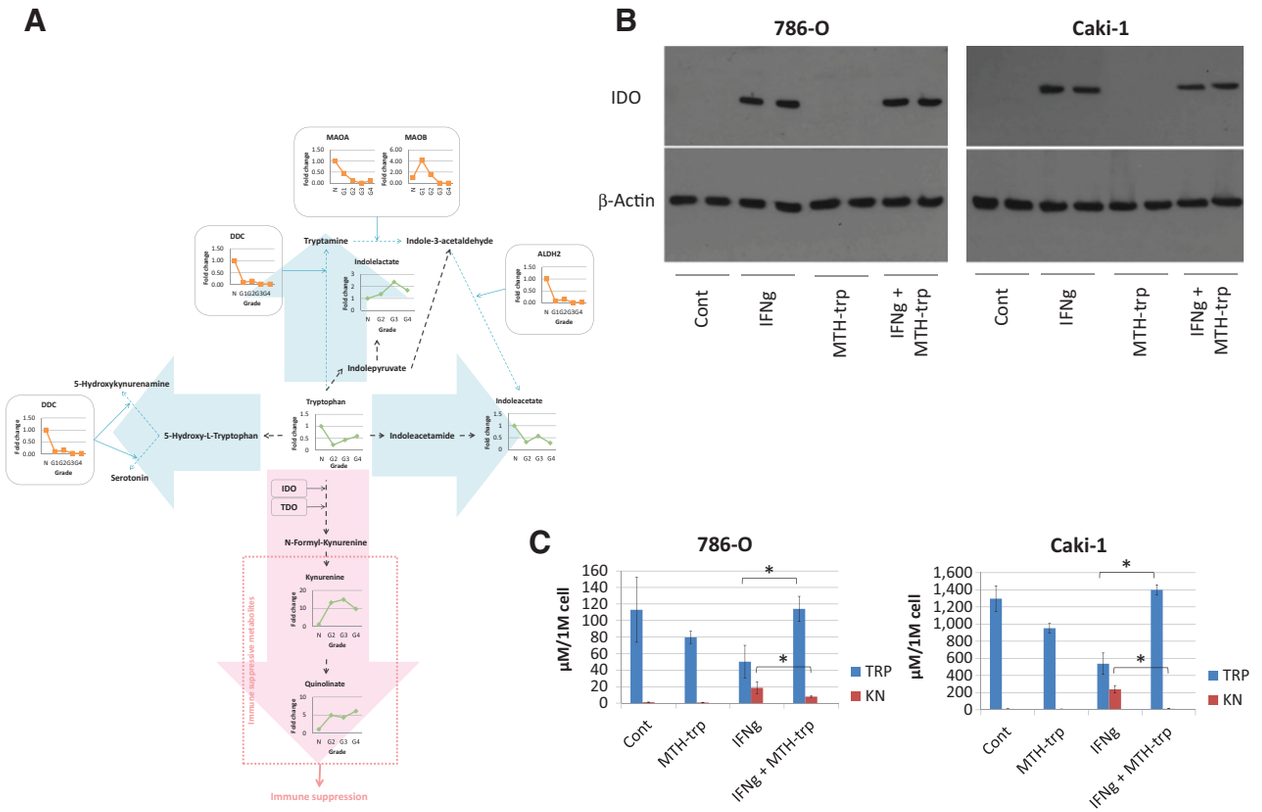

Figure 6.
Tryptophan metabolism favors a grade-dependent increase in immune-suppressive metabolites. A, combined proteomics and metabolomics data of human RCC tissue were overlaid onto a stylized KEGG-based pathway diagram. Green, metabolite; orange, enzyme; black dotted arrow, metabolism; red arrow, upregulated pathway; blue arrow, downregulated pathway; MAO, monoamine oxidase; DDC, dopa decarboxylase; ALDH, aldehyde dehydrogenase; IDO, indoleamine 2,3-dioxygenase; TDO, tryptophan 2,3-dioxygenase. B, 786-O (*VHL-mut*) and Caki-1 (*VHL-wt*) cells were plated in 6-well plates the day before treating with either human IFN-g (50 ng/mL) and/or MTH-trp (100 μmol/L) for 4 days and immunoblotted with the antibodies indicated. C, 786-O (*VHL-mut*) and Caki-1 (*VHL-wt*) cells were plated in 6-well plates the day before treating with either human IFN-g (50 ng/mL) and/or MTH-trp (100 μmol/L) for 3 days. The conditioned media were harvested and tryptophan (TRP) and kynurenine (KN) were measured by HPLC and normalized for cell number counted using the cell viability assay kit (EMD Millipore) on a MUSE (EMD Millipore). Data shown are mean ($n = 3$) and SD. *, $P < 0.05$. μM/1M cell, μM/1 million cells.

NCT00567931) presents an incentive to evaluate IDO as a novel RCC therapeutic target, which work is under way in our laboratory.

Strengths and weaknesses of current study

A strength of our study is that it demonstrates that each Fuhrman grade of tissue possesses distinct biochemical characteristics with a specific magnitude of metabolic reprogramming, a phenomenon that is logical given the markedly disparate clinical behavior of tumors of different grades. In addition, despite tumor heterogeneity observed in RCC (30), it would appear that in this era of personalized medicine, and based on the differences that we have observed, it may now be more appropriate to evaluate patient responses to drug therapies with knowledge of their initial tumor grade being part of the clinical decision-making process.

There exist potential criticisms of this study, the most significant being that the same tissues were not used for both platforms (measurement of protein and metabolite levels). This was necessary given the nature of the study in which several investigators in different institutions collaborated on the project, and for this reason, the human samples were collected separately. However, given the universally established criteria for Fuhrman grading and the use of experienced pathologists for determination of such, we have attempted to minimize potential inconsistencies from this issue. While there exists variability in any omics experiment, this possibility was minimized in this work by utilizing laboratories with abundant experience in nontargeted metabolomics and proteomics, both of which were based on mass spectrometry. To further minimize potential errors from this issue, validation of findings from each pathway was performed *in vitro*.

Although some of our findings, such as enhancement of the Warburg effect as a function of increasing tumor grade, were not surprising given the known activation of hypoxia pathways in the majority of RCCs, other pathway alterations were quite unexpected and represent novel potential therapeutic targets for further research (Fig. 7). The overarching theme of our findings regarding energy metabolism is that (i) the Warburg effect seemed to be of prime importance for these tissues, especially of high grades, at the expense of oxidative metabolism, including fatty acid oxidation and transit through the TCA cycle; (ii) the glutamine pathway functions to attenuate the high levels of reactive oxygen species that have long been known to be present in RCC (31); and (iii) tryptophan metabolism was upregulated, leading to the

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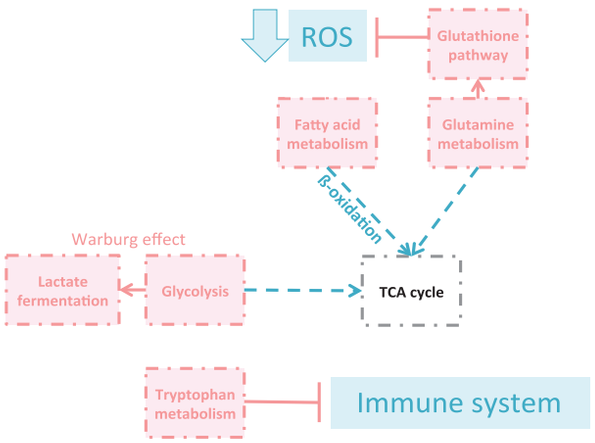

Figure 7.
Summary of grade-dependent metabolic pathway alterations in RCC. With higher grade, glycolysis was directed toward lactate metabolism at the expense of TCA cycle intermediaries. Fatty acid β-oxidation was decreased, whereas the glutamine pathway served to attenuate oxidative stress, thereby increasing cancer cell survival. Tryptophan was metabolized preferentially to immunosuppressive compounds. Red, increased; blue, decreased.

generation of immune-suppressive metabolites. These findings lead to obvious issues relevant to tumor targeting, which in theory could be designed to defeat these mechanisms.

In summary, we have for the first time utilized data from a combination of metabolomics and proteomics studies to dissect energy and immune-relevant metabolic reprogramming of RCC to a high resolution and in a grade-dependent manner. These data show markedly different biochemistry of higher grade tumors, which suggests that stratification in the clinical setting is likely to be advantageous. In addition, the changes in energy metabolism that favor alterations of the oxidative stress and aerobic metabolism pathways, as well as generation of immune-suppressive tryptophan metabolites, have profound implications for designing new targets for this disease.

Disclosure of Potential Conflicts of Interest

B. Neri is Sr. Director Diagnostic R&D at Metabolon Inc. No potential conflicts of interest were disclosed by the other authors.

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): H.I. Wettersten, A.A. Hakimi, C. Bianchi, M.E. Johnstone, D.R. Donohoe, J.F. Trott, R. Wolfert, B. Stewart, R. Perego, J.J. Hsieh
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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): H.I. Wettersten, D. Morin
Study supervision: R.H. Weiss

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