The impact of oncogenic signaling on MHC expression in Renal Cell Carcinoma

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Introduction and Specific Aims:

The American Cancer Society estimates that there were 64,770 diagnoses of kidney and renal pelvis cancer in 2012. Of these patients, approximately 70% will have local disease with the potential for a curative nephrectomy. Unfortunately, one-third of patients who receive a curative nephrectomy will subsequently develop metastases and ultimately die from their disease (1, 2). Currently, physicians do not have good tools to predict which patients will have tumor recurrence. TNM staging is presently used to predict outcomes but this system is highly imperfect with much overlap in survival between different stages. Thus, there is a critical need to develop better predictors of metastases to help identify patients who require increased surveillance and possibly adjuvant therapy to prevent recurrence.

In addition to acquiring the capacity for aberrant growth and metastatic spread, malignant renal epithelial cells must escape host anti-tumor immunity (3). This notion is supported by reports demonstrating that some patients with advanced renal cell carcinoma (RCC) can respond to therapies that enhance anti-tumor immunity. For example, interleukin (IL)-2, which is believed to enhance the generation of anti-tumor cytotoxic T lymphocytes (CTLs), can be curative in some patients with advanced RCC (4). Additionally, because major histocompatibility complex class I (MHC) molecule expression on tumor cells is critical to their recognition by CTLs, it is not surprising that a loss of tumor cell MHC expression is associated with a worse outcome in RCC (5). Conversely, efforts to enhance tumor cell MHC expression are being sought as a means to sensitize tumor cells to host anti-immunity and immune-based therapies (6).

While targeted anti-cancer therapies were developed with minimal consideration for their immune effects, it is becoming clear that these medications can have a profound impact on immune gene expression and anti-tumor immunity (7). This suggests that oncogenic signaling not only promotes tumor cell growth but also impacts immune homeostasis. For example, our collaborator Dr. Pollack, has demonstrated that inhibitors of the epidermal growth factor receptor (EGFR) and BRAFV600E, an oncogenic form of BRAF, can enhance MHC molecule expression and induction by inflammatory cytokines such as interferon (IFN)-ɤ (8, 9). Others have shown that imatinib, an inhibitor of the receptor tyrosine kinase KIT, can potentiate anti-tumor T cell responses by reducing the expression of the immunosuppressive enzyme indoleamine 2,3-dioxygenase (10). Thus, canonical oncogenic signaling pathways not only promote tumor cell growth and survival but also have a functionally relevant impact on immune gene expression.

**Hypothesis:** The hypothesis for the experiments outlined in this proposal is that oncogenic signaling in RCC will repress tumor cell MHC expression. To test this hypothesis we propose the following specific aims:

**Specific Aim 1** – To determine the impact of oncogenic signaling on MHC-I expression in renal cell carcinoma. For this aim we will use immunohistochemistry (IHC) and automated image analysis to determine if oncogenic activation of the mitogen-activated protein kinase (MAPK) pathway and/or the AKT/mTOR pathway is associated with the repression of MHC-I expression in RCC.

**Specific Aim 2** – To define the impact of oncogenic signaling and MHC-I expression on recurrence free survival following nephrectomy for local RCC. In this aim, we will couple the IHC data generated in Aim 1 with retrospective outcomes data to determine the relationship between oncogenic signaling, MHC-I expression and recurrence free survival in RCC.
Research Design:

The working hypothesis for this proposal is that increased ERK phosphorylation is associated with a decrease in MHCI expression in RCC.

Patients: We will utilize the continuously maintained Emory kidney cancer database with over 1000 patients. We will include patients with clear cell histology and exclude patients with T4 disease, nodal disease, metastatic disease or <18 years of age. Patients from the Emory kidney cancer database have already been consented.

Clinical and Laboratory assessment: We will use tumor blocks that contain tumor and adjacent normal tissue to act as an internal control. We will perform immunohistochemistry (IHC) using commercially available antibodies and blocking peptides if available to assess activation of the MAPK and AKT/mTOR pathways and the specificity of our IHC signal. To assess activation of the MAPK signaling pathway, we will use antibodies that recognize the phosphorylated/activated form of ERK at tyrosine 204. To assess activated of the AKT/mTOR pathway, we will use antibodies that recognize the phosphorylated/activated form of the p 70 S6 kinase phosphorylated at serine 235 and 236. To assess MHCI expression we will use antibodies that recognize a common epitope on HLA-A, B and C molecules. After staining, we will utilize whole slide scanning and automated image analysis to quantify the strength of the aforementioned IHC staining, using techniques we have previously published(8). Three representative areas of tumor and of normal kidney will be selected and averaged using Aperio image analysis software (positive pixel count) as we have done previously.

Statistics:

The positive pixel count algorithm will generate a numerical value that represents the strength of the IHC signal. These numerical values will be used to determine the relationship between oncogenic signaling (of either the MAPK or AKT/mTOR pathways) and MHCI expression in RCC using the InStat software package multiple regression analysis.

Student Role: I will identify 10 patients with Fuhrman grade 2 clear cell tumors who did not have recurrence following nephrectomy, and 10 patients with Fuhrman grade 2 clear cell tumors that had recurrence following nephrectomy. The blocks of RCC tissue for the selected patients will be obtained from the core tissue bank. These blocks will contain RCC tumor with adjacent normal renal tissue to act as an internal control. The pathologist and I will remain blinded to the clinical correlation of each block. IHC and slide scanning will be done in the Winship Cancer Institute (WCI) Core Pathology Laboratory as has been done before by Dr. Pollack. I will work with Drs. Pollack and Master as well as the staff within the WCI core pathology laboratory to quantify the IHC signal using the Aperio software package. If possible, I will examine the tumor samples for peri-tumoral inflammation. I will correlate the results of peri-tumoral inflammation, a marker for increased neutrophils, with recurrence of RCC. I will also determine if there is an association between peri-tumoral inflammation and CRP levels, using identical methods we have previously published(11).

References


