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Original article

Minimal changes in the systemic immune response after nephrectomy of localized renal masses[1]

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Abstract

Objectives: Renal cell carcinoma (RCC) is an immunogenic tumor, and multiple immunostimulatory therapies are in use or under development for patients with inoperable tumors. However, a major drawback to the use of immunotherapy for RCC is that renal tumors are also immunosuppressive. As a result, current immunotherapies are curative in $<10\%$ of patients with RCC. To better understand the systemic immune response to RCC, we performed a comprehensive examination of the leukocyte and cytokine/chemokine composition in the peripheral blood of patients with localized clear cell renal tumors pre- and post-nephrectomy.

Methods and materials: Peripheral blood samples were taken from 53 consented subjects with renal masses before cytoreductive nephrectomy and again at clinic visits approximately 30 days after nephrectomy. Samples were also obtained from 10 healthy age- and gender-matched controls. Blood samples from clear cell RCC subjects were analyzed by multi-parameter flow cytometry to determine leukocyte subset composition and multiplex array to evaluate plasma proteins.

Results: Pre-nephrectomy, clear cell tumors were associated with systemic accumulations of both "exhausted" CD8+ T cells, as indicated by surface BTLA expression, and monocytic $CD14^{+}HLA\text{-}DR^{neg}CD33^{+}$ myeloid-derived suppressor cells (MDSC). Subjects with T3 clear cell RCC also had a unique pro-tumorigenic and inflammatory cytokine/chemokine profile characterized by high serum concentrations of IL-1β, IL-2, IL-5, IL-7, IL-8, IL-17, TNF-α, MCP-1 and MIP-1β. At an early post-nephrectomy time point (~30 d), we found the systemic immune response to be largely unaltered. The only significant change was a decrease in the mean percentage of circulating $BTLA^{+}CD8^{+}$ T cells. All other cellular and soluble immune parameters we examined were unaltered by the removal of the primary tumor.

Conclusions: In the first month following surgery, nephrectomy may relieve systemic CD8 T cell exhaustion marked by BTLA expression, but continuing inflammation and MDSC presence likely counteract this positive effect. Future determination of how this systemic immune signature becomes altered during metastatic progression could provide novel targets for neoadjuvant immunotherapy in RCC.

Keywords: Carcinoma; Renal cell; Immunity; Immune suppression; Inflammation

1. Introduction

Renal cell carcinoma (RCC) is an immunogenic tumor. The preferred treatment for localized disease remains surgical resection, but at least 20% of patients with RCC

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experience a distant recurrence within 5 years [1,2]. By contrast, treatment for advanced RCC has traditionally relied upon immunostimulatory therapies such as high-dose IL-2 and interferon-alfa (IFN-α). Recently, new agents targeting molecular receptors, tyrosine kinase inhibitors, and mammalian target of rapamycin inhibitors have been introduced [3,4]. These agents produce inconsistent patient responses and do not lead to durable remission or cure. The only curative medical therapies remain IL-2 or IFN-α, but these immune therapies are effective in a minority of individuals [5,6]. In 2001, 2 landmark studies documented survival advantages in patients with metastatic RCC using cytoreductive nephrectomy in conjunction with systemic IFN-α therapy [7,8]. These findings led to additional exploration of adjuvant immunostimulatory or medical therapies in patients with metastatic renal tumors. However, in a recent study that combined high-dose IL-2, IFN-α, and sorafenib, the objective response rate was only 44% with a progression-free survival advantage of 7 months [9]. For these reasons, the use of novel immunotherapies for advanced RCC continues to be investigated, with recent clinical trials reporting promising results [10,11]. RCC escapes the host immune system and inhibits antitumor responses [12,13]. Therefore, new efforts have focused on identifying and overcoming mechanisms of tumor-induced immune suppression, with the goal of translating these findings into clinical use.

The RCC tumor microenvironment is immunosuppressive, simultaneously inhibiting the function of protective immune cells while inducing suppressive cells and cytokines. Myeloid-derived suppressor cells (MDSCs) are a key population of protumorigenic leukocytes. MDSCs are a phenotypically heterogeneous cell population characterized by their ability to suppress T-cell and natural killer cell function [14,15]. Their levels are significantly higher in patients with RCC of all stages relative to the levels in control subjects, and their relative abundance in other tumor types positively correlates with metastatic tumor burden [13,16]. There are also many proinflammatory cytokines that have potent tumor-promoting activity, including MCP-1, IL-1β, and IL-5 [17–19]. These cytokines have not been investigated as thoroughly in RCC as in other tumor types. Finally, exhaustion of the effector T cells that mediate tumor clearance is indicated by cell surface markers, such as B and T lymphocyte attenuator (BTLA) and programmed death receptor 1 (PD-1) [20,21]. Simultaneous examination of cellular and soluble immune components provides a comprehensive snapshot of the "immune signature" in a patient and may identify diagnostic or prognostic biomarkers.

Here, we examined the immune signature in subjects with RCC before and after tumor resection to determine the extent to which baseline immune responses to localized renal tumors were altered by removal of the primary tumor mass. As previous studies had shown that cytoreductive nephrectomy improved immunotherapy response rates in patients with metastatic RCC [7,8], it was possible that those improved outcomes had been partly because of a lessening of immune suppression mediated by the primary tumor. Multiple studies have characterized the immune profile of patients with metastatic RCC treated by a combination of surgery, immunotherapy, and targeted molecular therapy [11,22–24]. The immune response to localized RCC has not been as extensively examined, with no study reporting a comprehensive evaluation of both the leukocyte populations and the cytokine/chemokine profiles in these patients. An understanding of the immune response to localized tumors is needed if we are to (1) determine how tumor removal affects systemic antitumor immunity, (2) determine how progression to metastatic disease alters antitumor immunity, and (3) develop therapeutic strategies to enhance immune-mediated clearance of inoperable metastases in a greater percentage of patients. Therefore, we undertook this study to investigate the hypotheses that (1) localized renal tumors would cause detectable, systemic changes in cellular and soluble immune responses and (2) following surgical resection of localized renal tumors, the systemic immune signature would rapidly shift toward a normal baseline. We instead found that surgical resection of localized clear cell renal tumors had minimal effect on the systemic immune response at approximately 30 days after nephrectomy and that the frequencies of MDSC and proinflammatory, tumor-promoting cytokines and chemokines were unchanged.

2. Materials and methods

Approval for this study was granted by the internal review board at the University of Iowa, Carver College of Medicine.

2.1. Study subjects and protocol

Patients with renal masses scheduled to undergo resection were approached for enrollment between 2009 and 2012. Patients with metastatic disease were excluded. Peripheral blood samples were taken in the preoperative area from 53 renal mass subjects, who had provided their consent, before surgery and again at clinic visits approximately 30 days after nephrectomy. Samples were also obtained from 10 healthy age- and gender-matched controls. The demographic data and disease characteristics of subjects with renal masses and controls are listed in Table 1. Those with clear cell RCC histology were then analyzed separately according to the tumor stage. Table 2 shows clear cell RCC population characteristics. All subjects had localized disease, with 60.6% of the tumors being T1 category, 15.2% being T2 category, and 24.2% being T3 category. None of the subjects had T4 tumors. For some results, data are presented for the subjects with clear cell carcinomas a group, and other results are presented as T3 vs. T1/T2 tumor grades to provide a better understanding of how tumor grade affected results. Given the low