Advances in Radiation Oncology (2017) ■■, ■■–■■

advances
in radiation oncology

www.advancesradonc.org

Research Letter

Comprehensive assessment of circulating immune cell populations in response to stereotactic body radiation therapy in patients with liver cancer

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Received 27 April 2017; received in revised form 7 June 2017; accepted 3 August 2017

Abstract

Stereotactic body radiation therapy (SBRT) can positively influence an antitumor immune response by inducing necrotic cell death. SBRT also been shown to eliminate tumors outside the radiation therapy field through an immune-mediated process known as the abscopal effect. Recent advances in immunotherapy may provide new therapeutic approaches for patients with liver cancer. Therefore, understanding the immune status of patients with cancer will likely guide how immunotherapy might be used in combination with SBRT. We hypothesized that we would observe changes in circulating blood immune cell populations of patients who received SBRT for liver tumors. Therefore, we assessed 110 immunophenotypes in the peripheral blood of 10 patients with liver cancer or metastases to the liver pretreatment and 2 posttreatment time points. Patients with liver cancer and metastatic patients both exhibited several immunophenotypic abnormalities at baseline compared with a group of healthy volunteer controls. In longitudinal studies, SBRT caused a specific reduction in $CD3^+$ T cell counts and immature $CD56^{br}CD16^-$ NK cell counts. The immune profiling and potential identification of circulating biomarkers shown here could lead to the design of

Sources of support: This study was funded with institutional internal sources.

Conflicts of interest: M.P.G. and A.B.D. report associated intellectual property and 2 patents pending: treating patients based on immune subtypes issued and methods and materials for assessing immune system profiles issued. L.R.R. reports receipt of personal fees from Bayer and grants from BTG, Gilead Sciences, and Wako Diagnostics outside of the submitted work. S.B., S.S.P., D.A.G., and C.L.H. have nothing to disclose.

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https://doi.org/10.1016/j.adro.2017.08.003

combinatorial approaches with SBRT and immunotherapy to optimize the timing of treatment and direct the most effective immunotherapy with SBRT.

Introduction

Primary liver and metastatic liver tumors represent a major source of the cancer burden in the United States and worldwide. In the United States in 2016, approximately 28,000 new cases of primary liver tumors (hepatocellular carcinoma [HCC] and intrahepatic cholangiocarcinoma [CCA]) were diagnosed, and this number is expected to continue to rise with the increasing prevalence of hepatitis C infections and nonalcoholic fatty liver disease.[1] The liver is also a common organ of spread for many malignancies, most notably colorectal cancer, in which resection of limited liver metastases may be curative. Surgical resection remains the optimal treatment for patients with resectable liver tumors. Recently, stereotactic body radiation therapy (SBRT) has emerged as an effective local treatment modality for primary and metastatic liver tumors. Although local control rates of 80% to 90% have been achieved, the majority of patients treated with SBRT ultimately develop intrahepatic or extrahepatic (systemic) recurrence outside of the irradiated volume.[2-4] Systemic therapy only has a modest impact on survival for patients with primary or metastatic liver tumors.

SBRT induces necrotic tumor cell death, a prerequisite for eliciting an antitumor immune response. Although a detailed understanding of SBRT's effects on the immune system is incomplete, reports of partial or complete eradication of tumors outside of the radiation therapy (RT) field (defined as the abscopal effect) suggest that SBRT is capable of priming and expanding tumor-reactive T cells within the irradiated tumor and among the draining lymph tissues. These activated, tumor-specific T cells are thought to migrate to and eliminate nonirradiated tumors. Preclinical models have established that the abscopal effect is T cell dependent.[5,6] Recently, immune checkpoint blockade at the level of immune priming (CTLA-4 blockade) or effector function (B7-H1/PD-1) is being investigated in many clinical trials and is yielding promising results. Whether a distinct RT regimen, such as SBRT, synergizes with immune checkpoint blockade and elicits a potential systemic curative response is an important question. The combination of anti-CTLA-4 and RT is capable of inducing abscopal effects in patients with melanoma.[7,8] B7-H1 or PD-1 blockade in preclinical RT models increased the rate of tumor regression and reflected results characteristic of PD-1 blockade in conjunction with other therapies in patients with advanced cancer.[6] However, the impact of PD-1 blockade on SBRT-mediated abscopal effects and synergism with SBRT is largely unexplored.

For patients with liver tumors undergoing SBRT, further knowledge of the effect of SBRT on immune cell populations may help define the potential role of the combination of SBRT with immunotherapy, such as adoptive immunotherapy and/or checkpoint inhibition. Therefore, we tested whether SBRT induces changes in peripheral blood leukocytes using multiparameter flow cytometry on fresh, unmanipulated whole blood samples from patients with liver cancer and patients with liver metastases. A total of 110 immune cell phenotypes encompassing all major peripheral blood cell populations were assessed in this cohort. Immunophenotypic differences after SBRT as well as differences between healthy volunteers (HVs) and patients with cancer are described.

Methods and materials

Patients

Ten patients receiving SBRT for liver metastases (n = 4), CCA (n = 1), and HCC (n = 5) consented and were enrolled with the approval of the institutional review board. Patients were enrolled in accordance with the following inclusion criteria: age ≥18 years and Eastern Cooperative Oncology Group performance status score of 0 or 1; life expectancy >6 months; diagnosis of liver metastases, CCA, or HCC; received SBRT; and able to undergo blood draws. Other patient characteristics are listed in Table 1. The SBRT treatment technique used at our institution has been previously described.[9] All patients had a single tumor treated. The median maximal tumor dimension was 3.8 cm (range, 2.2-4.9 cm). The target volume consisted of the liver tumor with a margin of 5 to 7 mm for setup uncertainty. The prescription dose was 50 to 60 Gy in 5 fractions or 54 Gy in 3 fractions delivered on consecutive weekdays. Each patient received a single course of treatment. A total of 11 age-matched HV samples were collected, and an additional 29 samples from a previous study were included for analyses.[10]

Immunophenotyping by flow cytometry

Unmanipulated whole blood samples were stained directly with antibodies. Flow cytometry was performed with 7 flow protocols on the 3-laser, 10-color Gallios Flow Cytometer (Beckman Coulter, Brea, CA). All 10-color procedures, antibodies, flow protocols, instrument settings, analysis software, dot plots, and gating strategies have been previously described.[10,11]