Immune Response After Radiofrequency Ablation and Surgical Resection in Nonsmall Cell Lung Cancer

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The objective includes radiofrequency ablation (RFA) of a cancerous nodule results in immunogenic cell death. Tumor antigens are presented and the inflammatory environment may help stimulate adaptive and innate antitumor immunity. The objective of this study was to investigate the immune response following RFA and subsequent surgical resection in early stage non–small cell lung cancer (NSCLC). In methods, a single-session approach of computed tomography-guided tumor biopsy with immediate frozen section (and proof of NSCLC) was performed followed by RFA of the tumor in 4 patients with a solitary pulmonary nodule. Blood samples were collected before RFA and 3 days thereafter. All patients underwent radical surgical resection by video-assisted thoracoscopic lobectomy 8 days following RFA. In results, intense infiltrations of $CD4^+$ and $CD8^+$ lymphocytes were found along the perimeter of the RFA-treated tumor tissue, whereas the central tumor areas remained devoid of lymphocytes. In the peripheral blood, the frequency of proinflammatory, immunostimulatory IFNγ-secreting, and immunostimulatory $BDCA-3^+/B7-H3^-$ dendritic cells increased after RFA. Furthermore, a significant increase in T-cell proliferation was detected in T-cell assays after RFA and tumor resection. In this article, a local and systemic immune response subsequent to RFA and complete surgical resection in patients with NSCLC was identified for the first time. Treatment of patients with NSCLC with RFA and surgery leads to an activated and highly T–cell-stimulatory phenotype of dendritic cells, which may promote long-term immunity against NSCLC. The data suggest that the RFA-induced necrotic tumor debris can serve as an in situ antigen source to induce an autologous antitumor immune response.

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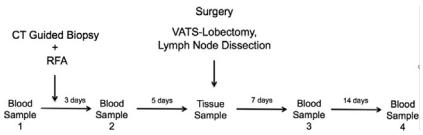
Keywords: radiofrequency ablation, non–small cell lung cancer, immune response, dendritic cells, in situ immunization



The study protocol includes CT-guided tumor biopsy with immediate frozen section (with proof of NSCLC) in a single-session approach. Radical surgical resection by video-assisted thoracoscopic lobectomy was performed eight days following RFA. Blood samples were taken at the following points of time: P-I: the day before the RFA procedure, P-II: 3 days post RFA; P-III: 7 days after surgery and P-IV: another 14 days later (i.e: 30 days after RFA).

Central Message

In this study for the first time a local and systemic immune response subsequent to RFA and complete surgical resection in patients with NSCLC was identified.Along the perimeter of the RFA-treated tumor tissue intense infiltrations of CD4+ and CD8+ lymphocytes were found after surgical resection. In the peripheral blood the frequency of proinflammatory and immunostimulatory dendritic cells increased after RFA. In T-cell assays a significant increase in T-cell proliferation was detected after RFA and tumor resection.The treatment of NSCLC patients with RFA and surgery leads to an activated and highly T-cell-stimulatory phenotype of DC. This activation may promote long-term immunity against NSCLC. The RFA-induced tumor necrosis can serve as an in-situ antigen source such as an in situ immunization.

Perspective Statement

Minimal residual disease may be the ideal target for activated of T-cells. This study-protocol combines in situ immunization with subsequent surgery and may lead the way to a non-cytotoxic adjuvant approach in the treatment of non-small cell lung cancer.

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OBJECTIVE

In recent years, percutaneous radiofrequency ablation (RFA) has emerged as a treatment option for non–small cell lung cancer (NSCLC) in nonsurgical patients.[1-3] In contrast to surgical

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resection, the tumor remains in situ after the RFA procedure, and large amounts of tumor debris are released as a consequence of cell membrane alteration, protein denaturation, and heat-induced necrosis.[4,5] In the local microenvironment, tumor antigens are presented to antigen-presenting cells subsequent to the procedure.

There is growing evidence that in situ tumor destruction may stimulate innate and adaptive antitumor immunity. In preclinical models, systemic antitumor responses based on dendritic cell infiltration and maturation as well as activation of tumor-specific T cells have been found.[6-8] In patients with lung cancer, a moderate systemic inflammatory response associated with an enhanced antitumor T-cell reactivity was reported subsequent to RFA.[9] The objective of this article was to investigate the immune response subsequent to RFA and surgical resection in patients with early stage NSCLC in a curative approach.

METHODS

Patients and Analysis of Tissue

In a total of 4 patients (between 3, 2012 and 10, 2012) with a solitary pulmonary nodule, CT-guided tumor biopsy with immediate frozen section (and proof of NSCLC) was performed in a single-session approach as previously described[10] followed by RFA (Bipolar RFA [CelonLABPower, Celon, Teltow, Germany]) of the tumor lesion. Blood samples were collected before the RFA procedure and 3 days thereafter. All patients underwent radical surgical resection by video-assisted thoracoscopic lobectomy 8 days after RFA. Radical mediastinal and hilar lymphadenectomy were performed concurrently with all procedures, including 4 compartments in the right-sided approach (paratracheal, infracarinal, inferior mediastinal, and hilar) and 4 compartments in the left-sided approach (aortic, infracarinal, inferior mediastinal, and hilar). Tumor tissue was sampled immediately after surgical resection. The tumors were bisected after resection; 1 section of the ablated tumor was snap frozen in liquid nitrogen and stored immediately at −80°C. The rest of the tumor was fixed in 4% buffered formalin solution for standard histology. In total, 2 further blood samples were taken 7 days after surgery and 14 days thereafter. The whole study protocol including the interventions and the tissue or blood samplings is shown in Figure 2A. The Ethics Committee of the University of Heidelberg approved this study (Study No. S280/2007). Each subject was comprehensively informed about the experimental nature of the treatment approach, the ablation, and the surgical procedure; written informed consent was obtained before study participation.

Histopathologic Analysis

For routine histopathology, lung and lymph node tissues were embedded in paraffin and further processed for routine hematoxylin-eosin (HE) staining. Additional sections of 1 μm thickness were obtained, deparaffined, and stained with mouse antihuman monoclonal antibodies (mAbs) against CD4 (Dianova) and CD8 (Dianova). Assays were performed according to the manufacturer's instructions.

Flow Cytometry and Isolation of Cells

Peripheral blood mononuclear cells were prepared from blood samples by Ficoll centrifugation (Biochrome, Berlin, Germany), according to standard procedures. After 3 washes with phosphate buffered

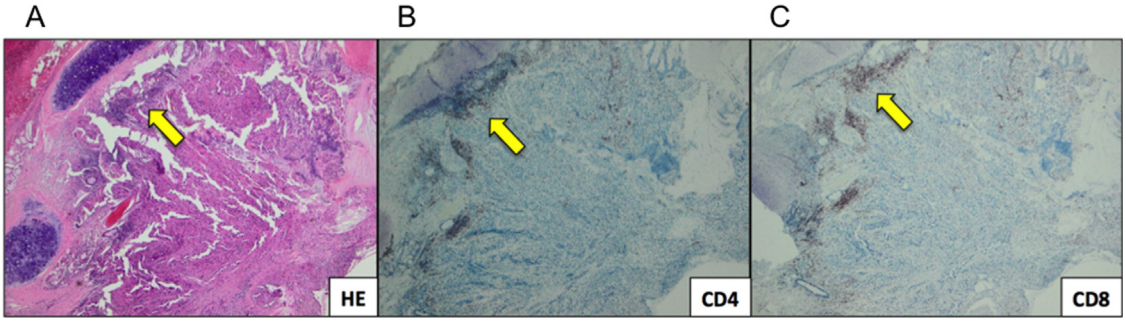

Figure 1. (A) Hematoxylin-eosin (HE) staining: cross section of the resected tumor tissue 8 days subsequent to RFA: the ablated tumor tissue is not vital, presenting with increased eosinophilia, cytoplasmatic and nuclear dissolution. In the tumor-surrounding outer areas, a dense infiltration of lymphocytes can be seen in the HE staining (marked by yellow arrow). (B) Immunohistology—CD-4 staining: CD4-positive lymphocytes are detected in the tumor-surrounding infiltration, (marked by yellow arrow). In the central parts of the necrotic tumor tissue, no CD4-positive lymphocytes can be detected. (C) Immunohistology—CD-8 staining: In the same way, CD8-positive lymphocytes are detected in the tumor-surrounding infiltration (marked by yellow arrow). In the central parts of the necrotic tumor tissue, no CD8-positive lymphocytes can be detected. (Color version of figure is available online at http://www.semthorcardiovascsurg.com.)