Protein disulfide isomerase A3–specific Th1 effector cells infiltrate colon cancer tissue of patients with circulating anti–protein disulfide isomerase A3 autoantibodies

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To investigate novel colorectal cancer (CRC)-associated antigens that could be targets of humoral or cellular responses, we analyzed the reactivity of serum from a long-surviving CRC patient (for more than 100 months of follow-up) in clinical remission, by serologic proteome analysis. Two-dimensional Western blotting (2D-WB) and mass spectrometry analysis revealed a strong reactivity of this serum against protein disulfide isomerase A3 (PDIA3). Anti-PDIA3 antibodies are not a diagnostic marker of CRC, 2D-WB and Luminex analysis revealed that they were equally present in about 10% of sera from healthy subjects and CRC patients. Kaplan-Meier analysis of survival in CRC patient cohort, after 48 months of follow-up, showed a trend of higher survival in patients with increased levels of autoantibodies to PDIA3. Therefore, the interplay between the presence of these antibodies and T-cell response was investigated. Peripheral blood T cells from CRC patients with high immunoglobulin G (IgG) reactivity to PDIA3 also secreted interferon gamma (IFN-γ) when stimulated in vitro with recombinant PDIA3, whereas those from CRC with low IgG reactivity to PDIA3 did not. PDIA3-pulsed dendritic cells efficiently induced proliferation and IFN-γ production of autologous $CD4^+$ and $CD8^+$ T cells. Finally, ex vivo analysis of tumor-infiltrating T lymphocytes from CRC patients with autoantibodies to PDIA3 revealed that PDIA3-specific Th1 effector cells accumulated in tumor tissue. These data indicate that the presence of autoantibodies to PDIA3 favors the development of an effi-

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cient and specific T-cell response against PDIA3 in CRC patients. These results may be relevant for the design of novel immunotherapeutic strategies in CRC patients. (Translational Research 2016;171:17–28)

Abbreviations: CRC = colorectal cancer; TAAs = tumor-associated antigens; IgG = immunoglobulin G; PDIA3 = protein disulfide isomerase A3; HSs = healthy subjects; Tcc = T-cell clones; SD = standard deviation; DC = dendritic cell; PBMCs = peripheral blood mononuclear cells; IFN-γ = interferon gamma; HLA = human leukocyte antigen; TILs = tumor-infiltrating lymphocytes; MHC = major histocompatibility complex

AT A GLANCE COMMENTARY

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Background

The immune response against immunogenic epitopes of tumor-associated antigens (TAAs) may induce the production of autoantibodies, which can be used as serologic biomarkers for cancers. The selection of an appropriate TAA is crucial for the design of anticancer vaccines.

Translational Significance

This study shows that a protein disulfide isomerase A3 is an interesting target for immunotherapy as the presence of autoantibodies to it is associated with an efficient and specific T-cell effector response in colorectal cancer patients.

INTRODUCTION

Colorectal cancer (CRC) is the third most common cause of cancer-related death in developed countries.[1] CRC is a heterogeneous disease, characterized by several genetic and epigenetic alterations, that when inherited and expressed in cells may foster the onset of hereditary CRC. Epidemiologic studies have indicated that about 80% of tumors in CRC patients occur as a sporadic pattern, whereas at least 20% of these tumors occur in the form of dominantly inherited.[2,3] Although recent advances in surgery, chemotherapy, and radiotherapy have led to a slight increase in the 5-year survival rate, the prognosis is generally poor, with 30%–50% of recurrence, presumably because of the presence of clinically undetectable micrometastases. New target therapeutic strategies are, therefore, being actively explored to further improve the clinical outcome.

Spontaneous humoral and cellular immune responses against tumor antigens expressed on the cell surface have been reported in some CRC patients. Because of their restricted expression in tumor tissues and not in other normal tissues, tumor-associated antigens (TAAs) are most frequently investigated in cancer immunotherapy. Current clinical trials in CRC patients involve targeting antigens that are overexpressed in tumor or oncofetal proteins, such as mucin-1, carcinoembryonic antigen, and epithelial cell adhesion molecule; results have shown that there are subgroups of patients with a positive correlation between the immune response and clinical improvement (longer survival, delayed tumor recurrence, and progression), especially when the tumor was surgically resected at early stages.[4] The immune response against such immunogenic epitopes of TAAs may induce the production of autoantibodies, which can be used as serologic biomarkers for cancers.[5,6] In fact, in vivo TAAs stimulate the activation of $CD4^+$ T cells, a crucial step for the differentiation of B cells into plasma cells and for the activation of $CD8^+$ T cells. Although it is not entirely clear how many TAAs break self-tolerance to become autoantibody targets in cancer patients, their immunogenicity may depend on their expression level and also on mutation, tissue distribution, subcellular localization, post-translation modifications, or other types of processing of proteins.[7-11] Unfortunately, many CRC-associated antigens, such as the melanoma associated antigen (MAGE) family members, failed to elicit strong humoral and cellular responses in clinical settings.[12] For these reasons, the selection of an appropriate TAA is crucial for the design of vaccines and for inducing a strong immune response.

A proteomic-based approach has been used in the study of many cancers, such as pancreatic cancer, melanoma, breast carcinoma, lung carcinoma, and renal cell carcinomas.[13-18] Serologic proteome analysis involves the discovery of new TAAs, which are able to induce circulating immunoglobulin G (IgG), using a combination of 2-dimensional electrophoresis (2DE), Western blotting (WB), and mass spectrometry (MS).[19] Using the serologic proteome analysis approach, we were able to observe that a long-surviving CRC patient displayed circulating