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UDC 577.2.616-006

Serological Analysis of the Repertoire of Human Cancer Antigens and Autoantigens

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Received September 25, 2003

Abstract—The spectrum of human antigens allows a monitoring of various pathological processes such as autoimmune disorders and tumorigenesis. Serological analysis of cDNA expression libraries (SEREX) is now used to search for new cancer-associated antigens, which are potential diagnostic markers or targets for immunotherapy of cancer. The results obtained for several solid tumors are reviewed. Groups of antigens detectable by SEREX, causes of immunogenicity of autoantigens, and prospective implication of antigens in diagnostics and monitoring of cancer are discussed.

Key words: autoantigens, cancer immunodiagnostics, expression libraries, cDNA

INTRODUCTION

Analysis of the repertoire of autoantigens may contribute to the understanding of mechanisms of various pathological processes, including autoimmune disorders and tumor transformation. Antigens changed in the course of tumor transformation may serve as diagnostic markers and targets of immune therapy.

To identify the antigens recognized by T cells, it is necessary to culture cytotoxic T cells of a patient [1]. The most convenient method to directly identify the antigens recognized by B cells is serological analysis of cDNA expression libraries (SEREX). This method has initially been proposed for a search for tumor antigens [2]. With SEREX, earlier unknown antigens have been identified in melanoma, breast cancer, colorectal cancer, Hodgkin's lymphoma, lung cancer, esophageal cancer, and several other tumors [2–5].

Here we summarize the results of our search for tumor antigens by SEREX.

EXPERIMENTAL PROCEDURES EMPLOYED IN OUR WORK

Sera were obtained from the Division of Combined Therapy, Blokhin Cancer Research Center and the Proctological Division, Moscow Hospital no. 24. To construct cDNA expression libraries, we used RNA

isolated from colorectal cancer cell lines LoVo and SW-480 and pooled testicular RNA of healthy subjects. With these preparations, cDNA was synthesized and cloned in the λ -ZAP expression vector as recommended by Stratagene (United States). Immuno-screening was carried out as described earlier [5, 6]. Allogenic screening was performed using serological mini-arrays of recombinant tumor antigens (SMART) [9]. Phage cDNA clones were converted to pBCKMV phagemid forms by *in vivo* excision as recommended by Stratagene. Plasmid DNA was purified and sequenced with fluorescent terminators by Sanger's method on an ABI Prism automated DNA sequencer (Perkin Elmer, United States).

SEREX ANALYSIS OF VARIOUS CANCER TYPES AND PATTERNS OF THE SEROLOGICAL RESPONSE

In SEREX, a cDNA library of a tumor is constructed with a phage expression vector. Recombinant proteins expressed on lytic infection are transferred onto a nitrocellulose membrane and incubated with autologous serum precleared of antibodies against bacterial and phage proteins. Clones displaying an immune response with a high IgG titer are selected and screened individually. Tumor specimens obtained by surgery are known to be contaminated with B cells and plasmacytes. As a result, such libraries contain

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