Search in the Tumor Liberated Protein (TLP) for Specific Peptides of Non Small Cell Lung (NSCL) Cancer

Giulio Tarro, MD, PhD
Department of Biology, Center for Biotechnology, Sbarro institute for Cancer Research and Molecular Medicine, Temple University, Philadelphia, PA, USA.
Committee on Biotechnologies and VirusSphere, World Academy of Biomedical Technologies, UNESCO, Paris, France.
Foundation T. & L. de Beaumont Bonelli for Cancer Research, Naples, Italy
Correspondence to: Prof. Dr. Giulio Tarro, Via Posillipo 286, 80123 Naples, Italy
email: gitarro@tin.it giuliotarro@gmail.com

Abstract - Rabbit polyclonal immune serum against TLP was produced by immunizing rabbits with the RTNKEASIC (the 1st) and NQRNRD (the 2nd) synthetic peptides, at the Rockland Immunochemicals Inc, PA. Protein extraction was performed from three cell lines of lung carcinoma including A549 cells, accordingly to previous studies. Cell lysates were loaded into two polyacrilamide gels and then were transferred to the nitrocellulose membranes. One nitrocellulose was hybridized with the anti-TLP serum and the other one with the pre-immunization serum. Preliminary results showed two major intense bands with a molecular weigh of 50 and 100kDa, which should correspond to the monomeric and dimeric form of TLP, respectively. In conducting a competition assay (PCA) to verify the specificity of the 50 and 100 kDa bands for TLP, the antibody anti-TLP was pre-incubated with the 1st and the 2nd peptides, before its hybridization with the nitrocellulose. In fact, a reduced intensity of the 100 kDa band following the PCA assay was observed, suggesting its specificity for the antibody anti-TLP. Currently, the Rockland Immunochemicals Inc is improving the signal specificity by purifying the antiserum on chromatographic columns, through the agarose matrix and the 1st and the 2nd peptides. Moreover, next purpose will be to immunoprecipitate TLP from the cell lysate and to load it on the SDS-PAGE gel. Then, the protein band of interest will be excised from the stained gel and the peptides will be extracted from the gel slice and the aminoacid sequence will be analyzed.

Keywords: TLP, NSCL, CRC, Immunotherapy, Vaccine

1 Introduction

Long years of research were required for boosting the immune system to fight cancer [1]; [2]. In the 1890s, mixtures of dead bacteria were injected by William B. Coley into cancer patients to stimulate the immune system. According to Paul Ehrlich (1909) the immune system may suppress tumor development. In the 1960s, both in animals and men neoplastic cell antigens stimulated the onset of specific humoral and cellular antibodies [3]. In 1972 Immunogenecity of a soluble transplantation antigen from adenovirus 12 - induced tumor cells was demonstraed in inbred hamsters (PD-4) [4]. In 1975 there was the discovery of Monoclonal Antibodies, highly specific immunological tools, and in 1980 mass-production of interferon, the immunes-timulating molecule, was obtained after inserting its coding gene into bacteria. In 1986 Interferon is approved by the Food and Drug Administration (FDA) for the treatment of hairy cell leukemia. In 1997 the FDA okays the first monoclonal antibody (MA) treatment against cancer (for non-Hodgkin’s lymphoma), and in 1998 the FDA approves the MA Herceptin for the treatment of metastatic breast cancer. Basic cellular immune response to cancer [5]: 2002 – National Cancer Institute researchers prove that two kinds of immune cell – CD4+ T cells and CD8+ T cells-are required for the treatment against cancer. The CD4+ cell releases cytokine molecules that help to activate the CD8+ cells prompting them to attack other cells with the same antigen. Therapeutic Vaccine Strategies [6]; [7]: Tumor cells are removed from a patient and treated biochemically or irradiated. Then the extracts of the dead cancer cells are reinjected, boosting the immune system to attack the tumor cell. Tumor liberated protein (TLP) boosts the immune system’s cancer responsive capabilities, 1983 [8]. TLP may have the potential to greatly improve the cure rate and/or serve as a lung cancer vaccine, 1991 [9]; [10]. Detection of lower levels of TLP/antiTLP may be of clinical relevance, 1992 [11]: TLP as candidate marker for the early detection of NSCL cancer. More on therapeutic Vaccine Strategies: Tumor – associated antigens resulting from protein bits, or from synthesized peptides specific for the cancer tissue, can be used successfully as vaccine to mount a vigorous antitumor attack: Development of a vaccine approach for therapeutical and preventive application [12]. The dendritic cell is an immune cell that presents specific antigens taken from a tumor cell to two other immune cells, the CD4+ and CD8+ cells. The dendritic cells of a cancer patient are removed and loaded with antigens from the tumor. The dendritic cells grow outside the body and then are reinjected, triggering a powerful response by the T cells [13]; [14]. The FAA approves the first therapeutic cancer vaccine for advanced prostate cancer (Provenge 2010).

Previously, we identified a ~100 kDa protein, which is part of a protein complex named tumor liberated proteins (TLP), as a promising blood marker for early
diagnosis of lung cancer [12]; [15]. In particular, this protein proved to have high specificity and sensitivity for stage I patients with NSCLC. TLP might also represent a predictive marker of cell transformation since it is expressed in interstitial lung fibrosis. Moreover, TLP showed a specific immunogenic activity, suggesting its possible use as an anticancer vaccine. Indeed, it is able to induce delayed hypersensitivity reactions and to promote blastogenesis in cultured lymphocytes from patients presensitized with TLP.

Research is ongoing to obtain the complete sequence of TLP, by proteomics approaches, in order to achieve adequate antigen preparations that might be used to generate assays for early diagnosis and, possibly, a specific anticancer vaccine [16].

2 Results

According to the partial sequencing of TLP, two peptides were synthesized: TLP peptide 1: Ac-RTNKEASI-Ahx-C-amide TLP peptide 2: Ac-Ahx-C-amide-NQRNRD A mixing of the two peptides was administered to two rabbits in order to obtain a serum for subsequent analysis. Therefore different sera samples were taken at various dates. The capability of sera to recognize TLP was analyzed by Western blotting using protein extracts of lung cancer cell lines (A549, H23, H82, H187) and control lines (MET-SA, NL-20 and primary line of fibroblasts). The signal obtained by anti-TLP antibodies was found to be not very specific.

In order to improve the specificity of the anti-TLP antiserum a Peptide Competition Assay was carried on. In this assay, the antibody is preincubated with the peptides before its use in the immunoblotting.

The immunoblotting experiment is conducted in duplicate, one with the antibody preincubated with the peptide and the other one with the control antibody. The results show a better signal quality and on the basis of these data, a request has been made to the company responsible for the production of the sera to purify the antibodies on a series of resins conjugated with the peptides TLP1 and TLP2.

The serum obtained after purification was found to be more specific, in particular a sample specifically recognized the band of 100 kDa and 50 kDa protein, presumably corresponding to the TLP. However in numerous subsequent analysis the data has not been confirmed. For this reason the company has been requested a new specimen of purified anti-TLP serum.

In parallel several immune precipitation assays were carried out using cell extracts of A549 and H23 lines in order to obtain a precipitate containing only the TLP protein (Fig 1). This would allow complete sequencing of the protein TLP and would also exclude the possibility that TLP and Corin are the same protein. Corin shows high homology with TLP and is present in various isoforms in the lung.

From the first analysis of the immunoprecipitation followed by Western blotting TLP (Fig. 2) and corin seem to localize at the same height (around 50kDa) and are recognized by the same antibodies.

We are currently trying to get enough staff of immunoprecipitated TLP in order to make the protein sequence and at the same time we would like to immunoprecipitate fragments of the two proteins (TLP and Corin). If the fragments from cutting with thrombin proved to be the same the data would support the hypothesis that TLP and Corin are the same protein. At the same time we are arranging to get a plasmid that allows us to transfect and over-express human Corin with the purpose to assess by Western blotting (with anti-TLP and anti-Corin antibodies) whether the two proteins are actually the same protein or are different proteins [17].
3 Conclusions

TLP is a tumor-associated antigen and a 100 kDa protein overexpressed in lung tumors and other epithelial adenocarcinomas [12]. It is immunogenic in humans as shown by serum antibodies [18]. Since TLP is a fragment of a protein identified in extracts of human NSCL cancer [19]; [20] and colorectal cancers (CRC) [21]; [22] and its sequences stimulate cytotoxic immunoresponse in humans and animal models, it is possible to design potential active and passive immunotherapies for NSCL cancer and CRCs based on TLP epitopes and humanized antibodies [23]; [24].

Therefore, TLP is a platform technology that can be used for: - a cancer diagnostic test to measure TLP levels in serum [12]; [15]; - a cancer therapy monitoring test - might measure changes in TLP levels in response to therapy [11]; [15]; - a cancer therapy - fragments of TLP can be used to stimulate immune response to attack existing tumors [10]; [25]; - a cancer vaccine: at-risk populations could be inoculated with TLP fragments to stimulate immune response to undetected or newly developing tumors [26]; [27].

We can use sequence information to express proteins, and then screen against phage antibody libraries for “pull down” for single chains of antibodies and test antibody against cell lines, colon and lung tissue microarrays.

Finally, the ability of the immune system to recognize TLP, thus enabling development of a vaccine approach for therapeutic application, represents a main target of this field of research.

The author declares no conflict of interests.

4 References


