

NEW DEVELOPMENT ON TUMOR ASSOCIATED ANTIGEN WITH SPECIFIC TARGET TOWARD LUNG CANCER

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Abstract - From the first analysis of immunoprecipitation followed by Western Blotting (WB) Corin and Tumor Liberated Protein (TLP) seem to precipitate at the same height (approximately 50KDa) and are recognized by the same antibodies. In parallel the tests of immunoprecipitation by the use of cell extracts derived from lung cancer cells A549 and NCI-H23 are improved with the aim to be able of obtaining a precipitate containing only the TLP. In fact the partial aminoacid sequence of TLP shows a high homology with the sequence of human Corin (only one aminoacid is different) and is present in lung cancer under different isoforms. It is known that human Corin is expressed mostly outside the cells and the protein extract derived from the extracellular medium and from the cells transfected with the plasmid, which overexpresses Corin, shows many more bands analyzed on SDS-PAGE that are equivalent to the bands (about 50-100 KDa) observed in the WB analyzed with anti-TLP.

Keywords: TLP, NSCL, Corin, Immunotherapy, Vaccine

I. Introduction

While surgery, radiotherapy and chemotherapy are able to cure many cancers, new approaches are required to improve radical curative therapy. A possible route is to utilize the latest achievements made in research on the immunology and genetics of cancer [1]. Cancer immunotherapy [2], or the manipulation of the naturally occurring oncolytic immune reaction, is based on the observation that both in animals and humans neoplastic cell antigens stimulate the onset of specific humoral and cellular antibodies [3]. Certain difficulties that have been encountered reflect the lack of well-purified antigens and/or their ability to unblock cell immunity in the cancer patient.

Two ways are known to enhance the host's immunity: aspecific activation (BCG in primis) and specific activation (to stimulate oncolytic circulating and cell antibodies). Moreover, some researchers have performed therapeutic trials with antigens, from autologous and homologous human cancer cells, obtained by various purification procedures [4]; [5].

The first observation by Tarro et al [6] demonstrated that when TLP is extracted from a tumor, purified in the laboratory, and reintroduced into the patients

body, it boosts the immune system's cancer responsive capabilities [7]. As lung cancer accounts for the largest number of cancer deaths in the Western world, TLP may have the potential to greatly improve the cure rate and or serve as a lung cancer vaccine (Table 1) [8].

Table I. Tumor Liberated Protein from Lung Cancer and Perspectives for Immunotherapy

TLP AS A TUMOR – ASSOCIATED ANTIGEN
<ul style="list-style-type: none"> • 50 KD PROTEIN OVEREXPRESSED IN LUNG TUMORS AND OTHERS EPITHELIAL ADENOCARCINOMAS
<ul style="list-style-type: none"> • IMMUNIGENIC IN HUMANS AS EVIDENCED BY SERUM ANTIBODIES

Corin is a cardiac serine protease that activates natriuretic peptides. It consists of an N-terminal cytoplasmic tail, a transmembrane domain, and an extracellular region with a C-terminal trypsin-like protease domain. The transmembrane domain anchors corin on the surface of cardiomyocytes. To date, the function of the corin cytoplasmic tail remains unknown [9]. Corin shows high homology with TLP and is present in various isoforms in the lung [10]. 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 use 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.

II. Materials and Methods

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 1,2). 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.

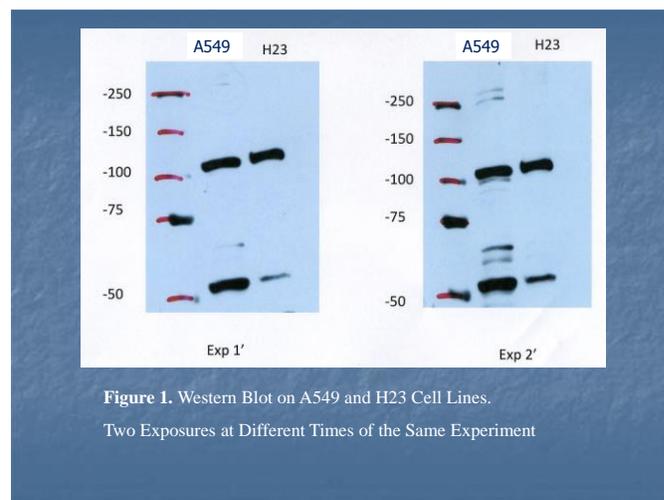


Figure 1. Western Blot on A549 and H23 Cell Lines.

Two Exposures at Different Times of the Same Experiment

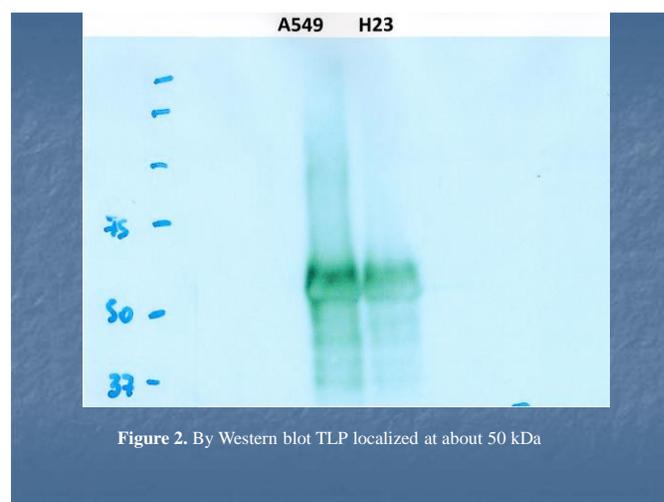


Figure 2. By Western blot TLP localized at about 50 kDa

III. Results

From the first analysis of immunoprecipitation followed by Western blotting Corin and TLP seem to precipitate at the same height (approximately 50 kDa) and are recognized by the same antibodies. Concurrently we obtained a plasmid from Prof. Qingyu (Cleveland, Ohio) that let us transfect HEK-293 cells and overexpress the human Corin with the purpose to evaluate by Western blotting (with anti-TLP and anti-Corin) whether the two proteins are really the same protein. In parallel we are improving the tests of immunoprecipitation by the use of cell extracts derived from lung cancer cells A549 and NCI-H23 with the aim to be able of obtaining a precipitate containing only the TLP. This result would allow a better sequence of the aminoterminal fragment of TLP and furthermore would allow to look in

details the homologies between TLP and Corin.

From a careful analysis of bibliography concerning both TLP and Human Corin, and from our data achieved during the present time, it seems that is coming out that Corin and TLP are really the same protein.

In fact the partial aminoacid sequence of TLP shows a high homology with the sequence of human Corin (only one aminoacid is different) and is present in lung cancer under different isoforms. From the references it is known that human Corin is expressed mostly outside the cells and the protein extract derived from the extracellular medium and from the cells transfected with the plasmid, which overexpresses Corin, shows many more bands analyzed on SDS-PAGE that are equivalent to the bands (about 50-100 KDa) observed in the Western blots analyzed with anti- TLP.

IV. Conclusions

Tumor Liberated Protein (TLP) is a new protein extracted from tumors *in vivo* and transformed cells *in vitro* (Fig. 3)[8].

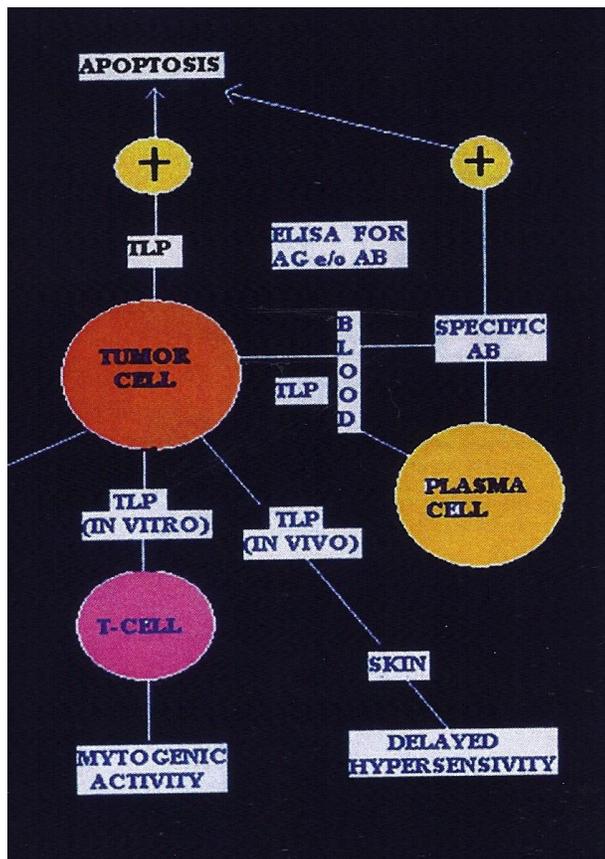


Fig 3. In vitro and in vivo Functions of TLP

TLP is detectable in blood as well as in cancer tissue [11]; [12].

TLP is a tumor associated antigen of 50 KD monomer [13]; [14].

TLP is overexpressed in lung tumor [13]; [14] and other epithelial adenocarcinomas [15]; [16].

TLP is immunogenic in humans as evidenced by serum antibodies [17].

Preliminary information on lung tissue microarray is shown in table 2.

Table II. Sensitivity and Specificity of TLP for Antibodies

TISSUE MICROARRAY PROFILE (a)		
NSCLC STAGE I	POSITIVITY (%)	NEGATIVITY (%)
400	56.3 (225/400)	43.7 (175/400)
NORMAL LUNG	POSITIVITY (%)	NEGATIVITY (%)
400	0 (0/400)	100 (400/400)

(a) Carried out by William C. Hyun, Ph.D., at the University of California San Francisco, Cancer Center, Laboratory Cell Analysis.

<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> [18].

The perspectives of TLP are the following:

- Since its sequences stimulate cytotoxic immunoresponse in humans and animal models, it is possible to design potential active and passive immunotherapies for NSCL cancer and colorectal cancers (CRC) based on TLP epitopes and humanized antibodies [19]; [20].

- Fragments of TLP can be used to stimulate immune response to attack existing tumors [9]; [21].

- At risk populations could be inoculated with TLP fragments to stimulate immune response to undetected or newly developing tumors [22]; [23].

- Therefore the ability of the immune system to recognize TLP, represents a main target for diagnosis and therapy in this field of research.

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The author declares no conflict of interests.