From Sequence of Tumor Liberated Protein (TLP) to Function and Potential Targets for Diagnosis and Therapy

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Abstract - From the first analysis of immuneprecipitation followed by Western Blotting (WB) Corin and TLP seem to precipitate at the same height (approximately 50KDa) and are recognized by the same antibodies. 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. 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

1 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]. 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 cardiomycocytes. 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 transfec 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.

2 Material and Methods

1. Antigens, Ac-RTNKEASI-Ahx-C-amide, Ac-Ahx-C-amide-NQRNRD, Corin
2. Antibodies, Anti-Corin antibody, Anti-TLP antibody
3. Cell Lines, Cancer cell lines: A549, H23, H82, H187 Control cell lines: MET-SA, NL-20, Primary line of fibroblasts
4. Tests, a) Immunoblotting, b) Immunoprecipitation, c) Peptide competions assay, d) Western blotting
5. Other reagents, Thrombin to cut protein, Plasmid to transfec Corin

3 Results

From the first analysis of immuneprecipitation followed by Western blotting Corin and TLP seem to precipitate at the same height (approximately 50KDa) 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 showes 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, showes 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.

### 3.1 Tables

<table>
<thead>
<tr>
<th>TISSUE MICROARRAY PROFILE (a)</th>
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<tr>
<td>NSCLC STAGE I</td>
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<td>400</td>
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### Table 2. Sensitivity and Specificity of TLP for Antibodies

<table>
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<tr>
<th>NORMAL LUNG TISSUE</th>
<th>POSITIVITY (%)</th>
<th>NEGATIVITY (%)</th>
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<tr>
<td>400</td>
<td>0 (0/400)</td>
<td>100 (400/400)</td>
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(a) Carried out by William C. Hyun, Ph.D., at the University of California San Francisco, Cancer Center, Laboratory Cell Analysis.

### 3.2 Figures

**Fig 1. In vitro and in vivo Functions of TLP**

### 4 Conclusions

Tumor Liberated Protein (TLP) is a new protein extracted from tumors in vivo and transformed cells in vitro (Fig. 1)[8]. TLP is detectable in blood as well as in cancer tissue [11];
The perspectives of TLP are the following:

- 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.

5 References


The author declares no conflict of interests.