



Opinion

Volume 1 Issue 5 - August 2017
DOI: 10.19080/JTMP.2017.02.555574

J Tumor Med Prev

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Tumor Liberated Protein (TLP)



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Submission: August 08, 2017; **Published:** August 28, 2017

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Background

A tumor Associated Antigen (TAA) was isolated from Non Small Cell Lung (NSCL) cancer and named TLP, by Tarro [1]. The immune is to chemical analysis revealed a cytoplasmic localization of TLP in small granules or in larger accumulation forms. In some specimens it was also detected in the lumen of atypical glands and in the bronchial secretions, suggesting that TLP could be considered a secretory product of the neoplastic cells. Tarro have demonstrated that when TLP is extracted from a patient's tumor, purified in the laboratory and reintroduced into the body, it boosts the immune system cancer responsive capabilities. The partial sequence analysis of this protein (the complete sequence is currently not identified) led to the synthesis of the corresponding peptides that have been used as antigens for producing sera in rabbits. According to Tarro [2] four TLP-peptides were sequenced:

- A. Rtnkeasi
- B. Gsaxftn
- C. Qrnrd
- D. Gppevqnan

Based on this information, Tarro [1] have produced a polyclonal antibody against TLP by using the peptide RTNKEASI and detected in sera of 53.1% of NSCLC patients (N = 534) with 75% being positive in the early stage (stage I) and dropping to 45% in the late stage (stage IV). 0% positivity was instead detected in patients with tumors other than NSCL cancers, in the surrounding normal tissues or in inflammatory cells [3,4]. Although these previous studies are interesting to date there are very limited data on the full length TLP protein, RNA and gene sequences to draw any firm conclusion regarding the diagnostic value of this marker.

Analysis of TLP Protein by Mass-Spec After Specific Immune Precipitation

The first aim of this project will be to identify the complete protein sequence of TLP. The analysis of the diverse published putative TLP-peptides by using the NCBI-BLAST software program showed partial homology with other proteins such as corin, SEC

24 suggesting that they could form a complex with TLP or have parts in common with TLP protein. A partial homology with one of the peptide sequences was also found with the carbonic anhydrase IX, a known cancer marker. These results imply that it is of paramount importance to produce highly TLP-specific antibodies in order to be able to specifically immune precipitate TLP and associated proteins and determine their sequence by mass spectroscopy. In order to exclude immunological cross-reactions of these antibodies with proteins unrelated to TLP we aim to produce a monoclonal antibody and polyclonal antibodies by using the peptide RTNKEASI which was previously used by Tarro [1] successfully to generate a TLP-specific antiserum. This Abs will be used to examine by histochemistry and immunoblot analysis its specific reactivity with the TLP protein specifically in lung cancer tissues and not with other proteins. As controls we will analyse the same tissues with the same antibodies preincubated with the control peptide as well as respect to the presera. With the newly produced polyclonal and/or monoclonal Ab specific for TLP we will then immune precipitate TLP from cell lysates prepared from TLP-positive lung cancer tissues and analyse the precipitated TLP and proteins eventually complexed with it by mass spectrometry.

This analysis should allow an ambiguous identification of the TLP amino acid sequences, and if TLP exists as a multiprotein complex, also that of one or several of its interaction partners. We will then search in available protein data banks whether or not TLP and any of its associated proteins have been identified previously or are novel proteins. Depending on the result of this analysis we will or will not thereafter attempt to isolate cDNAs coding for TLP and associated proteins. If TLP or any of the associated proteins correspond to known proteins for which cDNAs, antibodies and recombinant proteins exist, we will try to get our hands on these reagents or produce them by recombinant DNA technology and functionally analyse them by appropriate molecular biological techniques [5].

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DOI: [10.19080/JTMP.2017.02.555574](https://doi.org/10.19080/JTMP.2017.02.555574)

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