Tumor Liberated Protein (TLP) and human corine in lung cancer cells: Homology or different isoforms in the sequence at the cytoplasmic surface

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According to the partial sequencing of TLP, two peptides were synthesized: TLP peptide 1: Ac-RTNKEASI-Ahx-C-amide and 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-5A, NL-20 and primary line of fibroblasts). 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. This 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. In parallel several immunoprecipitation assays were carried out using cell extracts of A549 and H23 lines in order to obtain a precipitate containing only the TLP protein. 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. 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.

Biography

Giulio Tarro, graduated from Medicine School of Naples University. Research Associate at Division of Virology and Cancer Research, Children’s Hospital and Assistant Professor of Research Pediatrics, College of Medicine, Cincinnati, Ohio. Professor of Oncological Virology at University of Naples. He worked for National Research Council, Rome, and for National Cancer Institute, Frederick Center, Maryland. He became Division Chief of Virology, and then Department Chief of Diagnostic Laboratories, D. Colugno Hospital for Infectious Diseases, Naples; Emeritus, 2006-. Since 2007 he is Chairman Committee on Biotechnologies and Virus Sphere World Academy Biomedical Technologies, UNESCO, and Adjunct Professor Department Biology Temple University College of Science and Technology, Philadelphia. President Foundation de Beaumont Bonelli for Cancer Research.

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