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Further Studies on TLP (Tumor Liberated Protein)

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Isolation of the TLP Encoding Cdna, Gene Sequence and Analysis of its Expression Control

Radiation is the propagation of radiant energy in the form of waves or particles. It may also include beams of particles of which electrons, positrons, neutrons, protons, deuterons, and are the alpha particles are the best known. When, radioactivity is defined as the property, possessed by some materials, of spontaneously emitting alpha or beta particles or gamma rays as the unstable (or radioactive) nuclei of their gamma rays as the unstable (or radioactive) nuclei of their atoms disintegrate and the Radioisotopes are isotopes that are unstable, or radioactive, and give off radiation spontaneously and may be used either as a source of radiation energy or as a tracer.

Radioisotopes offer two advantages. First, they can be used in extremely small amount. Secondly, they can, be directed to various definitely known parts of the body. For example, radioactive sodium iodide found in the iodized salt used in many homes and their iodine concentrates in the thyroid where it is converted to the hormone thyroxin.

Other radioactive or "tagged" atoms can be routed to bone marrow, red blood cells, the liver, the kidneys, or made to remain in the blood stream, where they are measured using suitable instruments. When radiation is used for treatment, the energy absorbed by the body is used either to destroy tissue, particularly cancer, or to suppress some function of the body. Properly calculated and applied doses of radiation can be used to produce the desired effect with minimum side effects. More than 100 different radioisotopes that have been used by doctors during the past so many years, five have received by far the greatest attention. These are iodine-131, phosphorous-32, gold-198, chromium-51 and iron-59 whereas Radioactive sulphur (S^{35}) helps to study advantages and disadvantages offungicides.

Arsenic-74 and copper-64 are isotopes emitting positrons. Chromium-51 in the molecule sodium chromate, attaches itself to red blood cells, it is useful in several kind of tests. When chromium trichloride $CrCl_3$ is used as the tagging agent, the chromium is bound almost exclusively to plasma proteins, rather than the red cells.Chrmium-51 may thus be used for estimating the volume of plasma circulating in the heart and blood vessels. This ^{51}Cr procedure was used during the Korean War to determine how much blood had been lost by wounded patients, and helped to save many many lives. Iodine-131has been used as a tracer in determining cardiac output, which is the rate of blood flow from the heart. It has appeared recently that red blood cells tagged with ^{51}Cr are more satisfactory for this measurement than iodine-labeled albumin in the blood serum.

Cobalt-60, Vitamin B^{12} is a cobalt compound the few milligram of B^{12} in the body are stored in the liver and present in the blood stream.Iodine-1According to Tarro et al. [1] the putative TLP cDNA sequence synthesized by means of a degenerate oligonucleotide corresponds to a X chromosomal DNA sequence which is present in the NCI data bank (unpublished data produced most recently by bioinformatics analysis). This sequence lacks a conventional translation start codon and in addition it also does not match perfectly with the oligonucleotide used for RT-PCR. In addition, the translated amino acid sequence corresponding to this DNA sequence contains only the last three c-terminal amino acids of TLP-peptide: ASI. This argues against the coding of the TLP protein by the DNA fragment isolated in 2002.

Therefore, if our planned IP-Mass-Spec analysis proposed above results in TLP amino acid sequences which do not correspond to proteins available in the NCBI data bank, we will design new oligonucleotides based on the amino acid sequences of TLP we expect to obtain by mass spec. By means of these oligonucleotides we will synthesize a TLP-specific cDNA, then sequence it, and search by bioinformatics tools for its chromosomal location and gene organization with the help of NCBI DNA data bank. This will furthermore also allow us to characterize the promoter, enhancer and other regulatory regions of the TLP gene which might control its expression in normal and cancer cells. In addition, transcription factor binding site in the TLP promoter region identified by this approach will allow us to analyse by use of antibodies directed against corresponding transcription factors and chromatin immunoprecipitation (CHIP) assay whether or not they are involved in TLP expression in lung cancer cell lines and cancer tissues.

Evaluation of Tumor Specific TLP Expression and Establishment of Sensitive Assays

After having identified the TLP amino acid sequence and gene location, we aim to verify and characterize its expression pattern on the protein level by enzyme immunoassay and immunoblotting as well as on the RNA level by RT-PCR in a large number of lung cancer tissues and blood specimens in comparison with normal tissues. Thus, we should be able to confirm and extend the previous data shown by Tarro et al. [2] regarding TLP specificity and sensitivity and validate the use of this protein as a potential serological tumor marker. In order to do that, we will estimate the specificity and sensitivity values of TLP with reference to the state of the art, i.e. comparing the TLP values with those of the conventional markers currently used for lung cancer diagnosis.

In vitro Assessment of Tumorigenic Function of TLP in cell lines

To assess the tumorigenic properties of TLP, we will transfect the TLP gene *in vitro* in normal lung cell lines and evaluate its effects on cell proliferation and cell cycle by comparing transfected and not transfected lung cells. In case the *in vitro* results show alterations of the cell cycle, we propose to analyze the cell-cycle-related genes.

In vivo Assessment of Tumorigenic Function in Mice

To assess the tumorigenic properties of TLP *in vivo*, we will transfect an expression vector with the TLP gene into normal lung epithelium in mice and evaluate whether it is able to induce tumor formation. In case we observe tumor growth, we will isolate cells from the tumor and normal epithelium tissues to perform FACS analysis. In parallel, we will extract mRNA and evaluate genes regulating cell cycle, angiogenesis and metastasis through microarray analysis.

Functional Analysis of the TLP Protein as a Potential Therapeutic Target

In case we observe the expected tumorigenic effects of TLP *in vivo* in the mice, we intend to elucidate the physiological role of TLP in lung cancer cell by using a xenograft mouse model of lung cancer and test its potential as a therapeutic target by TLP-knockdown experiments. To this aim, we will inject lung cancer cells positive for TLP subcutaneously in mice and verified tumor formation. We then plan to perform the same experiment with the cell line after having silenced TLP gene expression with the help of TLP-shRNA carrying lentiviruses. Comparative analysis of tumor growth, FACS analysis of the cells of tumors from both experiments and eventually RNA-Seq analysis should provide unambiguous evidence whether or not TLP expression not only affects tumor growth, the cell cycle regulation, angiogenesis and metastasis but also whether TLP is a potential target for anticancer therapy [3].

References

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