TRYPTOPHANYL-tRNA SYNTHETASE AS A TARGET OF PHOSPHORYLATION BY PROTEIN KINASE OF CANCER SERUM

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**Introduction.** Tryptophanyl-tRNA synthetase (TrpRS) is an enzyme of protein biosynthesis catalyzing attachment of tryptophan to tRNA\(^{trp}\). TrpRS belongs to an ancient family of aminoacyl-tRNA synthetase. In contrast to other aminoacyl-tRNA synthetases TrpRS is dramatically induced by interferon in human cells. Earlier we demonstrated that mammalian TrpRS is a phosphoprotein phosphorylated *in vivo* and *in vitro* (1, 2). However little is known about protein kinases, which phosphorylate TrpRS and a role of phosphorylation in TrpRS activity.

**Methods.** Purified mammalian TrpRS was phosphorylated *in vitro* in the presence of \([\gamma^{32}\text{P}]\text{ATP}\), MgCl\(_2\) or MnCl\(_2\) and protein kinases of donor and cancer human sera. The routine synthesis of peptides containing serine, threonine and tyrosine residues of human TrpRS was performed. The N-terminal sequence analysis of the isolated phosphorylated TrpRS peptides was conducted. Phosphorylation of human TrpRS was characterized with phosphopeptide and phosphoamino acid mapping. ELISA and cytochemistry were performed using monoclonal anti-TrpRS antibodies blocked with excess of different TrpRS fragments.

**Results and Discussion.** We found that TrpRS and its fragments are phosphorylated by protein kinase that present in cancer sera whereas donor sera did not express a protein kinase activity towards TrpRS. To examine sites of phosphorylation in more details, we have constructed recombinant vectors containing different fragments of human TrpRS. The phosphorylated fragments of biochemically purified and recombinant TrpRS were subjected to N-terminal sequence analysis. In addition, the library of synthetic peptides containing potential phosphorylation sites of human TrpRS was created for screening sera. Effect of monoclonal antibodies against TrpRS on phosphorylation of TrpRS has been examined. The monoclonal antibodies to TrpRS have been mapped with phosphorylated and non-phosphorylated TrpRS fragments using cytochemistry and ELISA. Identification and characterization of serum protein
kinase that abnormally phosphorylate TrpRS is under progress. We study a link of the abnormal TrpRS phosphorylation with tumors of specific localization and morphology.

**Conclusion.** Fragments of human TrpRS used in this study as the targets for phosphorylation by serum protein kinases may be a useful tool for cancer diagnosis.

**References**