FUNCIONAL ANALYSIS OF THE HUMAN REGULATORY PROTEIN KI-1/57 AND ITS INTERACTING PROTEINS

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The protein Ki-1/57 was first identified by the cross reaction of the CD30 monoclonal antibody Ki-1 in Hodgkin lymphoma cell. The expression of Ki-1/57 in diverse cancer cells and its phosphorylation in blood leukocytes after mitogenic activation, suggested a role of this protein in the cellular signaling. To obtain clues for the functional context of Ki-1/57 protein, our group explored the yeast twohybrid system to screen a human fetal brain cDNA library for interacting proteins. Protein fragments Ki-1/57(1-150) and Ki-1/57(122-413) were used as baits. Our analyses resulted in the identification of several transcriptional regulating proteins, among others: Chromo-Helicase-DNA-binding domain protein-3 (CHD3), RACK-1 (Receptor of Activated Kinase 1), DAXX and Topors but also in proteins that contain RNA binding domains, including: CIRBP (Cold Induced RNA Binding Protein) and SFRS9 (Splice factor Arg/Ser rich 9). The latter two were expressed and purified as GST-CIRBP and 6xHis-SFRS9 fusion proteins in E. coli or insect cells, respectively. We used the recombinant proteins to performed pull down assays and were able to confirm the interaction of GST-CIRBP with Ki-1/57. At this stage we are performing new yeast two-hybrid assays with full-length Ki-1/57 protein and bone marrow and lymphocytes cDNA libraries to evaluate the interaction profile of Ki-1/57 in other tissues. Supported by: FAPESP, LNLS, CNPq