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DISSERTATION SUMMARY

Study of structure-function relationship of human galectin-1

Dmytro Demyndko

Institute of Genetics, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary

Lectins are proteins which specifically bind (or crosslink) carbohydrates. Galectins are defined as lectins having both galactose-binding ability and characteristic conserved amino-acid sequence. Galectin-1 is a homodimeric lectin with specificity for beta-galactosides. It is found in many species from fungi to mammals. In mammals its expression is developmentally regulated in a variety of organs from brain to thymus. Galectin-1 has been shown two participate in cell adhesion and growth, immunomodulation, apoptosis, metastasis, inflammation, and premRNA splicing. It has been proposed to play important roles by recognizing carbohydrate moieties on intracellular ligands, cell signaling receptors and extracellular glycoproteins, although precise knowledge of the mechanism of galectin-1 action is lacking. To reveal this mechanism we are conducting a structure-function study of the lectin.

We applied two approaches in our research. The first one is to study the extracellular function of lectin. cDNAs of wild type and carbohydrate non-binding mutant of galectin-1 were cloned into pET-His bacterial expression vector. Obtained recombinant proteins were successfully purified by affinity chromatography on Ni-NTA agarose utilizing 6xHis tag. Wilde type and mutant his-tagged proteins showed the similar properties in polyacrylamide gel electrophoresis and western blot analysis to the normal non-his tagged recombinant galectin-1. These proteins were tested for ability to induce apoptosis in human lymphoid T-cell line Jurkat. Galectin-1 and his-galectin-1 induced a similar degree of apoptosis in tested cell line. His-tagged carbohydrate non-binding mutant of galectin-1 did not induce apoptosis. This result can lead to a conclusion that apoptosis-inducing function of galectin-1 is dependent on its beta-galactoside binding ability. Other mutant forms of galectin-1 will be produced as recombinant proteins and similarly tested.

Second approach is to study intracellular function of the lectin. With this goal cDNA of wild type and mutant galectin-1 were cloned in mammalian retroviral expression vector pLXSN. These constructs in parallel with empty vector for control were introduced into Jurkat, BL-41, and Daudi cell lines by retroviral transfection. Successful transfection was confirmed by western blotting. Jurkat cell lines were analyzed further. Cell surface markers analysis by fluocytometry revealed no differences between cell lines expressing and non-expressing galectin-1. Existing data on participation of galectin-1 in processes of cell growth prompted us to conduct analysis of proliferation of transfected cells. In MTT proliferation assay cell line expressing wild type galectin-1 showed significantly lower proliferation rate than cell lines expressing mutant or no galectin-1. Again, as in case of apoptotic function of recombinant protein, this function of galectin-1 seems to be dependent on its beta-galactoside binding activity. Further experiments are needed to confirm this result. Participation of intracellular galectin-1 in regulation of apoptosis is of particular interest. Future experiments will test sensitivity of transfected cell lines to apoptosis by different agents including galectin-1 itself, anti-CD95 antibody, staurosporine and others. Galectin-1 has been shown to participate in T-cell receptor (TCR) signal transduction. Response of transfected cells to the activation through TCR will be studied in different aspects (calcium mobilization, tyrosine phosphorylation, phosphatase activity).

Althoug more experiments have to be done, already completed studies of recombinant proteins and transfected cell lines prove the dependence of galactin-1 function on its beta-galactoside binding ability. Its intracellular presence in many cell types suggests the importance of cytoplasmatic and nuclear protein glycosilation. Recent studies show that, indeed, some of the intracellular proteins are glycosilated *in vivo.*

Supervisor: Éva Monostori
E-mail: dima@nucleus.szbk.u-szeged.hu