Abstract

Natural killer cells play a critical role in immune defence against tumours, virus-infected cells, and pathogens. They recognize target cells without prior sensitization and eliminate them utilizing cytotoxic activity, secretion of cytokines and chemokines. NK cells express activating and inhibitory receptors on their surface, which mediate external signals. The main role among activating receptors is played by a member of the natural cytotoxicity receptor family – the receptor NKp30. NKp30 recognises a wide range of ligands, including those involved in malignant processes such as B7-H6, BAG-6, and Galectin-3. Galectin-3 (Gal-3) is a cellular protein that plays multiple roles, from gene expression to tumour growth and defence from pathogens. It can suppress NK cell functions by binding to the NKp30 receptor and is a target for new antitumour therapy. However, little is known about the precise mechanism of NKp30:Gal-3 interaction, which is challenging to study due to several factors. Firstly, wild-type Gal-3 can create oligomeric structures through its N- and C-terminal domains, and form dimers through an odd cysteine in its carbohydrate recognition domain (CRD), which can interfere with measurements. Secondly, the ligand binding domain (LBD) of NKp30 carries three *N*-glycosylation sites (Asn42, Asn68, Asn121) involved in ligand binding, each with different contributions to it.

This master's thesis aims to gain a better understanding of the interaction between NKp30 and Galectin-3 using methods and approaches of structural biochemistry. To achieve this, NKp30 LBD *N*-glycosylation mutants were expressed in HEK293T cell line and a cysteine-less form of the carbohydrate recognition domain of Gal-3 was produced in *E. coli*. The binding affinity of this interaction was determined using two complementary methods: microscale thermophoresis and analytical ultracentrifugation. Another aim was to crystallize the Gal-3:NKp30 complex and solve its structure using X-ray crystallography, which has led so far only to crystallizing the cysteine-less Gal-3 CRD and successful solving of its structure.

Key words

Galectin-3, NKp30, NK cells, HEK293T, *N*-glycosylation