Structure and function of C-type lectin NK cell receptors studied by recombinant expression and protein crystallography

Abstract of Ph.D. thesis

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Natural killer cells (NK cells) were found out for their ability to spontaneously kill certain allogeneic tumour cell lines, without any previous sensitization. NK cells are part of non-adaptive immune response with very short reaction time against pathogens such as viruses, intracellular bacteria, parasites, and they are responsible for elimination of certain tumour cells and thus they are able to fight against malignancy and formation of metastasis. Activity of NK cells is regulated by the balance between activation and inhibitory signals mediated by the NK cell surface receptors. From the structural point of view, the majority of NK cell surface receptors could be classified as the C-type lectin or immunoglobulin-like receptors. One of many C-type lectin subgroups are type II lymphocyte receptors that are expressed on the NK cell surface.

This study had two main aims. The first one was to find suitable expression and purification systems for selected C-type lectin receptors of NK cells and the other one was to perform their biochemical and structural characterization. The following specific aims were adopted: to develop a refolding and purification protocol providing sufficient amount of human CD69 and rat NKR-P1A and B for their structural and functional studies; to study the structure of CD69 and NKR-P1 receptors by protein crystallography; to develop eukaryotic expression system suitable for recombinant expression of native NK cell lectin receptors and to develop suitable affinity purification protocol for easy purification of secreted recombinant proteins from conditioned culture medium; and also to manage sedimentation analysis of recombinant proteins in analytical ultracentrifuge.

These aims were achieved and the results could be seen in attached publications and manuscripts. In case of CD69 and NKR-P1 receptors the main focus was an optimization of the expression constructs together with careful optimization of refolding and purification strategy. This enabled both their biochemical characterization (e.g. carbohydrate binding specificity, *in vivo* stability and binding cooperativity of CD69), their crystallization and three dimensional structure determination – a structure of CD69 with the highest resolution known was obtained, whilst for NKR-P1 the first structure solved within this NK receptor family was determined, containing also an unique structural motif not observed in NK cell receptors so far. Structural work was underlined by molecular modeling and Raman spectroscopy studies pointing out some possible mechanisms determining the receptor's binding specificity.

We were not able to prepare all investigated receptors in bacterial expression systems, therefore an eukaryotic expression system was established, based on transient transfection of suspension human HEK293 cell line adapted to serum-free medium cultivation. Further, this system might also allow us to study the effect of receptors dimerization and glycosylation on their binding properties. Firstly, it was necessary to optimize the transfection process to achieve maximum transfection efficiency and production yield, to this end we used two reporter proteins: secreted alkaline phosphatase and green fluorescent protein. When expressed by transient transfection in square shaped bottles and purified by IMAC and gel filtration, the final yield of pure recombinant rat NKR-P1B and Clrb, selected as a model receptors, varied between 1 and 5 mg per liter of production medium, with the production of native covalent homodimers being feasible and characterized.