Abstract

Natural killer cells (NK cells) are part of the immune system in human and other mammals. The task of these cells, which belong to the non-specific immunity, is to induce apoptosis in other cells of the body that may represent a threat for the body (i.e., tumour or virally infected cells).

NK cells have a variety of surface receptors to recognize their target cells. A number of receptors are well-known today and they may be divided into groups based, e.g., on their structural similarities or on the type of signal which these receptors present to NK cells. Accordingly, we distinguish activation and inhibitory receptors. Inhibitory receptors inhibit NK cell response, while activation receptors elicit this response. During NK cell contact with another cell, the resulting NK cell behaviour is always the result of a certain balance of activation and inhibitory receptor responses.

The NKp44 receptor is an immunoglobulin-like receptor. This receptor is very unique among other receptors in many respects, for example because it is associated with both activation and inhibitory motif. The ligand of this receptor is a proliferating cell nuclear antigen (PCNA). PCNA is a clamp protein important, inter alia, during DNA replication, in which it anchors other replisome proteins.

This work is focused on recombinant preparation of NKp44 receptor (PCNA protein was already prepared earlier) and a study of interaction between both of these binding partners. NKp44 was prepared recombinantly in the HEK293S GnTI$^-$ expression system. The protein was prepared in several variants of the fusion constructs (some carrying mutation and differing in the orientation of the IgG Fc fragment).

The most suitable candidate was then selected from the prepared constructs and used to analyze the binding between the two proteins. Biophysical methods of analytical ultracentrifugation and microscale thermophoresis were used for this purpose. Some interaction between proteins was observed by microscale thermophoresis, although the accuracy of the determination of the dissociation constant of the NKp44:PCNA complex was really very poor. On the other hand, analytical ultracentrifugation did not show any interaction.

Key words

NK cell, NKp44, PCNA, HEK293, MST