ABSTRACT

The subject of this study is a receptor NKp80, also known as killer cell lectin-like subfamily F, member 1 (KLRF1). It is an activating receptor which forms homodimers on the surface of natural killer (NK) cells. Receptor NKp80 binds to a ligand, AICL, which is naturally expressed on all myeloid cells. Upon a substantial increase in AICL expression, for example in cancer cell, the cell then becomes a target for an NK cells expressing the receptor NKp80. Ultimately, the complex NKp80:AICL is therefore a potential target for the immunotherapeutic treatment of myeloid leukaemia.

The aim of the study was to the produce and purify a series of mutants of an extracellular domain of NKp80 by replacing cysteins by serines in a segment of extracellular domain called the stalk region. Here, by introducing the mutations, we studied their effect on homodimer formation. The proteins were prepared in HEK293S GnTI$^-$ cells using stable transfection. Altogether, we produced seven mutants with all possible combinations of mutations of the three cysteins in the stalk region. We then analysed the proteins using size exclusion chromatography and differential scanning fluorimetry. Lastly, we deglycosylated the proteins to verify that NKp80 is present in several glycoforms.

Our results show that none of the variants of NKp80 form covalent dimers. However, regarding the elution volume of the proteins we may suggest that they form non-covalent dimers. In addition, we ascertained that there is no significant correlation between the position of a mutation or their combinations and the level of expression or their thermal stability. Finally, we confirmed the presence of several glycoforms in all prepared proteins.

KEY WORDS

NK cell, NKp80, AICL, immunotherapy, HEK293S GnTI-