ABSTRACT

Natural Killer (NK) cells can spontaneously destroy target cells, such as infected, damaged, or malignant cells. NK cell cytotoxicity is mediated by activating receptors on their surface, such as NKp80 (Natural Killer protein 80 kDa). The human receptor NKp80 stimulates cytotoxicity through its interaction with its ligand AICL (Activation Induced C-type Lectin), which is constitutively expressed by all myeloid cells. In pathological conditions, such as cancerous or damaged cells, AICL is often upregulated, resulting in the lysis of these cells by NK cells expressing NKp80. This interaction is thus a promising immunotherapeutic target for treating myeloid leukaemia.

However, the structures of both proteins have remained elusive. Therefore, we have focused on the successful production of the extracellular domain of these proteins. To improve the production yield of NKp80, we introduced a series of mutations in the stalk region to study their effect on production, stability, and homodimer formation. The introduced mutations significantly increased the production yield allowing for a large-scale production with subsequent crystallisation of the protein. The crystallisation resulted in the elucidation of the hitherto unknown structure of NKp80 homodimer at a resolution of 2.9 Å. However, the large-scale production revealed an unexpected partial proteolysis of the N-terminal stalk region during deglycosylation.

The production of AICL proved to be rather complicated due to intracellular retention of the protein and likely degradation of the protein due to misfolding issues. To answer this, various approaches were explored, including elongation of the stalk region based on similarity with homologous CD69, intracellular production, insect cell production and others. Finally, a fusion construct of AICL with the Fc fragment on the C-terminus was designed and produced with a yield of 30 mg per 400 ml media. The cleavage of the construct was briefly investigated and is to be fully optimised for the large-scale preparation of the pure AICL.

Our future aims are to produce the pure ligand AICL with a prospect to elucidate the structure of the ligand, study the receptor-ligand interaction, and thus fully investigate its therapeutic potential.

KEYWORDS

NK cell, NKp80, AICL, protein crystallization, X-ray crystallography