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ERDR1 enhances human NK cell cytotoxicity through an actin-regulated degranulation-dependent pathway

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ABSTRACT

Erythroid differentiation regulator 1 (ERDR1), which is a stress-related survival factor, exhibits anti-cancer effects against melanoma. However, the function of ERDR1 on immune cells has not been examined. We investigated whether ERDR1 regulates the cytotoxic ability of human natural killer (NK) cells, which are known as innate effector lymphocytes. In this study, treatment with recombinant ERDR1 resulted in enhanced NK cell cytotoxicity through the secretion of lytic granules. Furthermore, actin modulation was involved in the ERDR1-enhanced NK cell cytotoxicity. ERDR1 stimulated actin accumulation at the immunological synapse, which was induced by the activation of Vav-1 in NK cells. These findings suggest new insight into the function of ERDR1 function in the human immune system.

1. Introduction

Natural killer (NK) cells play an important role in the innate immune response and are crucial for defense against tumor cells and virus-infected cells [1–3]. NK cell activation is tightly regulated by the balance between stimulation of inhibitory and activating receptors [4,5]. The membrane-activating receptors recognize their ligands on the surface of tumor cells and activate NK cell functions such as tumor cell killing, cell survival, and cytokine production [6]. The activating receptors include the NK group 2 member D and natural cytotoxicity receptors (NKp30, NKp44, NKp46) that trigger NK cell lysis of target cells and are important mediators of NK cell cytotoxicity [7–9]. The other mechanisms of NK cell cytotoxicity include the death receptor-dependent pathway, involving Fas/Fas Ligand and the cytolytic granule-dependent pathway, which results in degranulation of cytolytic granules. When the Fas Ligand on NK cells recognizes the Fas receptor containing the conserved intracytoplasmic death domain, the NK cells activate the caspase enzymatic cascade and induce apoptosis of target cells [10,11].

Cytolytic granules are filled with perforin and granzymes and are important defense structures of the NK cells [12]. Once the NK cell recognizes and binds to its target cell, an immunological synapse is formed between the NK cell and its target cell [13]. The cytotoxic granules migrate and polarize toward the target cells and are secreted into the actin-rich immunological synapse in a calcium-dependent manner [14–16]. Perforin creates pores in the membrane of target cells, and then serine proteases and granzymes move into the target cells and induce apoptosis. The formation of the immunological synapse is especially dependent on actin rearrangement. Actin polymerization is critical for conjugating, docking, and fusing the immunological synapse, and the microtubule-organizing center regulates granule migration toward the immunological synapse. Therefore, the actin regulation pathway is crucially involved in NK cell cytotoxicity.

Erythroid differentiation regulator 1 (ERDR1) acts as a cytokine and stress-related survival factor and induces hemoglobin

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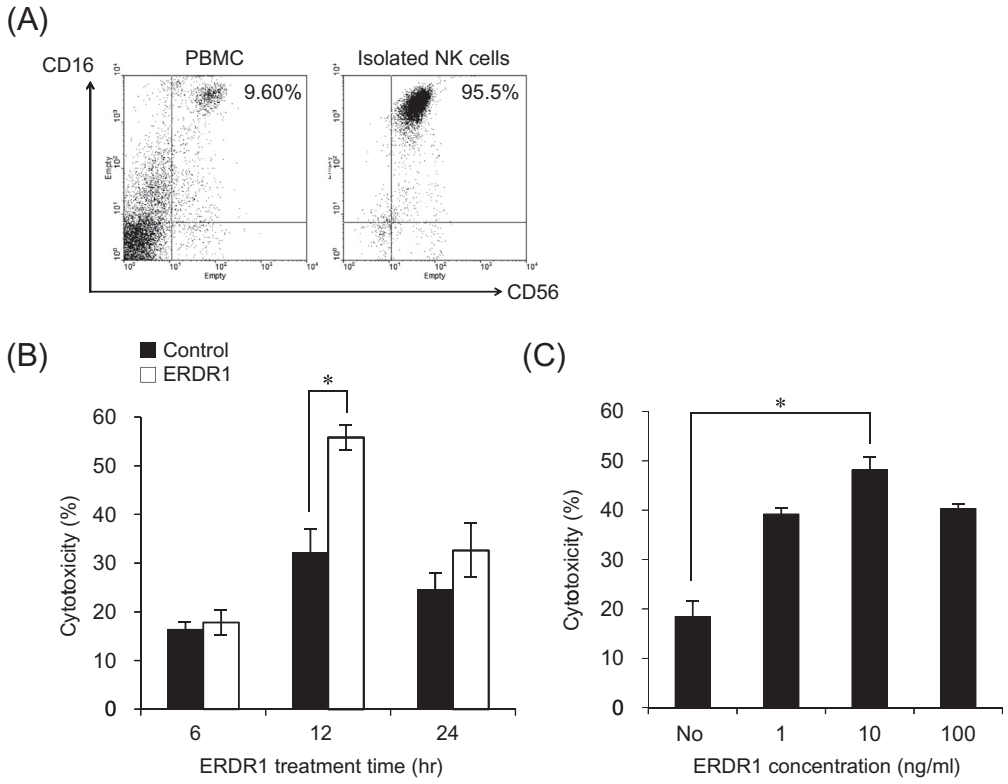

Fig. 1. Human primary NK cell cytotoxicity is enhanced by recombinant ERDR1 treatment. (A) Human primary NK cells were separated from whole human blood of healthy donors using Ficoll-Paque density gradient centrifugation followed by magnetic antibody cell sorting. PBMC or the separated cells were stained with FITC-conjugated mouse anti-human CD56 antibody and PE-conjugated mouse anti-human CD16 antibody to determine the purity of the isolated NK cells. (B) Human primary NK cells were incubated with or without 10 ng/ml recombinant ERDR1. After 6, 12, or 24 h, the cytotoxicity assay was performed. (C) Recombinant ERDR1 (0, 1, 10, or 100 ng/ml) was added for 12 h, and then the cytotoxicity assay was performed with human primary NK cells. Error bars indicate the standard deviation. The data represent one experiment performed in triplicate. The Student's *t*-test was performed to determine statistical significance.

synthesis in the myelomonocytic cell line, WEHI-3B [17,18]. In human keratinocytes, ERDR1 is involved in cell growth and survival and is regarded as a pro-apoptotic factor [19]. Recently, ERDR1 expression is negatively regulated by IL-18, causing anti-metastatic functions in melanoma [20]. Additionally, ERDR1 inhibits gastric cancer cell migration and invasion [21].

ERDR1 is expressed in human keratinocytes and is detected in the cultured supernatants of murine erythroleukemia cells [17,19]. Although ERDR1 is regarded as a metastasis suppressor in cancer cells, its role in immune cells is still unknown. In this study, we investigated the function of ERDR1 in NK cell cytotoxic activity and the related mechanism. Recombinant ERDR1 enhanced NK cell cytotoxicity through the degranulation pathway with release of lytic granules via an actin-related mechanism. These findings suggest that ERDR1 may have anti-cancer applications that involve the human immune system.

2. Materials and methods

2.1. Cell preparation and culture

After IRB approval (Inje IRB/1) at Inje University Busan Paik Hospital (Korea), primary NK cells were obtained from the whole blood of healthy human donors using Ficoll-Paque (Sigma–Aldrich) density gradient centrifugation followed by use of the MACS NK cell isolation kit (Miltenyi Biotec). The purity of the isolated primary NK cells (CD16+CD56+) was 90–99%. Cells were maintained in Roswell Park Memorial Institute culture medium (RPMI; Gibco) supplemented with 2 mM L-glutamate and 10% FBS (Gibco) at 37 °C in 5% CO_2.

2.2. Cytotoxicity assay

Recombinant ERDR1 (based on NCBI Reference Sequence: NP_579940.1) was purchased from IL-YANG Pharm. Co., Ltd. (Korea). Concanamycin A (Sigma–Aldrich) was used as a potent inhibitor of perforin-mediated cytotoxicity [22]. Latrunculin A (Molecular Probes Inc.) was used to block actin polymerization [23]. A human leukemia cell line, K-562 cells (American Type Culture Collection), was used as the target cell and was stained with carboxyfluorescein diacetate succinimidylester (CFDA-SE; Molecular Probes Inc.). The effector:target ratio in all cytotoxicity experiments was 2.5:1. NK cells were incubated with CFDA-SE-labeled target cells for 2 h at 37 °C in 5% CO_2. Thereafter, the specific lysis of target cells was analyzed after staining with 7-amino-actinomycin D (7AAD) staining solution (BD Biosciences). To properly analyze the data, the following control target cell groups were used for flow cytometry and compensation: target cells without CFDA-SE or 7AAD staining, target cells with CFDA-SE staining only, target cells with 7AAD staining only, and target cells with both CFDA-SE and 7AAD staining. Analysis was done with a FACSCalibur (BD Biosciences) flow cytometer and the CELLQuest program (BD Biosciences). Cytotoxicity was calculated using the following formula:

$$\%\ \text{cytotoxicity} = \frac{\text{Killed target cells}}{\text{Killed target cells} + \text{Live target cells}} \times 100$$

2.3. CD107a assay

The degranulation of NK cells was examined using a CD107a detection kit (MBL) [24]. Primary human NK cells were treated with FITC-labeled CD107a monoclonal antibody for 3 h at 37 °C.