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Research paper

Human cancer cells with stem cell-like phenotype exhibit enhanced sensitivity to the cytotoxicity of IL-2 and IL-15 activated natural killer cells

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ABSTRACT

Tumors harbor a population of cancer stem cells (CSCs) which can drive tumor progression and therapeutical resistance. Nature killer (NK) cells are best known for their ability to directly recognize and kill malignant cells. However, the susceptibility of cancer stem cells to NK cells is not fully understood. Here we demonstrated that human CD44+CD24− breast CSCs were shown enhanced sensitivity to IL-2 and IL-15 activated NK cells. CD44+CD24− CSCs expressed higher levels of NKG2D ligands ULBP1, ULBP2 and MICA. Blockade assay showed that the sensitivity of CSCs to NK cells-mediated lysis was mainly dependent on NKG2D. Furthermore, redox oxygen species (ROS)-low tumor cells were more sensitive to NK cells. The presence of antioxidant enzymes inhibitor L-S,R-buthionine sulfoximine or H_2O_2 retarded the cytotoxicity of NK cells to CD44+CD24− CSCs. In addition, NK cells could readily target CD133+ colonal CSCs. Our findings provide novel targets for NK cells-based immunotherapy and are of great importance for translational medicine.

1. Introduction

Cancer stem cells (CSCs), also termed tumor-initiating cells, are a small subset of cells with self-renewal properties and the ability to produce differentiated progeny [1]. CSCs have high tumorigenic and proliferative potential and can establish tumors which closely resemble histologic, cytologic, and architectural features of the primary human cancer [2]. Furthermore, CSCs are also associated tumor metastasis. CD133+CXCR4+ cancer stem cells are essential for pancreatic cancer metastasis [3,4]. The cancer stem cell hypothesis has been investigated in a variety of human cancers. The existence of CSCs was first evidenced by the identification of CD34+CD38− leukemia-initiating cells in acute myeloid leukemia [5], and then followed by CD44+CD24−epithelial-specific antigen (ESA)+ breast CSCs [6], CD133+ brain CSCs [7], CD44+α2β1hi CD133+ prostate CSCs [8], CD20+ melanoma CSCs [9], CD133+ colon CSCs [10], CD44+ CD24+ ESA+ pancreatic CSCs [11], CD133+, CD90+ or CD24+ liver CSCs [12–14]. Then, targeting CSCs is a promising strategy for cancer therapy [15,16]. However, owing to their unique survival mechanism, CSCs are resistant to the current chemotherapies and chemotherapy [12]. Whether CSCs are sensitive to cytotoxic cells in the host are not clearly clarified.

Innate and adaptive immune systems are involved in tumor progression and protection [17]. Natural killer cells are a subset of innate immune cells with specified functions in host defense [18]. NK cells are considered as professional cytotoxic cells during antiviral, antimicrobial and antiparasitic responses [19,20]. NK cells are also cytotoxic to transformed cells and tumor cells via direct killing or induction of apoptosis [21]. The immune system–tumor interactions are important for cancer protection. NK cells eliminate cancer cells by direct recognization and killing, irrespective of antigen-presenting cells [22]. Blockade of the interaction between NK cells and tumor cells results in inhibition of NK cell cytotoxicity [23]. Though NK cells could directly kill malignant cells, tumor cells have the ability to evade those attacks. The main mechanisms involve the expression of MHC-I molecule and secretion of soluble NKG2D ligands by tumor cells [24,25].

Thus, the goal of the present study was to evaluate the relationship between cancer stem cells and NK cells. Here we found that human CD44+CD24− breast cancer stem cells are sensitive to NK cells cytotoxicity. CD44+CD24− CSCs expressed NKG2D ligands ULBP1, ULBP2 and MICA. Blockade of NKG2D reduced NK cell-mediated lysis of CSCs. In addition, we found that ROS was

Abbreviations: NK, nature killer cells; CSCs, cancer stem cells; ROS, redox oxygen species; ULBP, UL16-binding proteins; MIC, MHC class I chain–related molecules.

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associated with CSCs' sensitivity to NK cells. We also found that NK cells could readily target CD133+ colonal cancer stem cells.

2. Materials and methods

2.1. Tumor cells cultures and agents

Human breast cell line MCF-7 and human colonal cancer cell line HCT116 were obtained from American Type Culture Collection (ATCC; Manassas, VA), and maintained in DMEM and RPMI 1640 media (Hyclone) supplemented with 10% FBS (GIBCO), respectively. To pharmaceutically raise ROS levels, MCF-7 cells were treated with 1 mM L-S,R-buthionine sulfoximine (Sigma-Aldrich) for eighteen hours. PE mouse anti-human CD44 and PerCP-Cy5.5 mouse anti-human CD24 antibodies were purchased from BD Pharmingen. Monoclonal mouse antibodies against human NKG2D, NKp44, NKp46, ULBP1, ULBP2 and MICA were obtained from R&D system. FITC-conjugated anti-mouse IgG was from eBioscience. 2,7-dichlorodihydrofluorescein diacetate (CM-H2DCFDA) was purchased from Invitrogen Molecular Probes.

2.2. Cancer stem cells isolation

CD44+CD24− breast cancer stem cells were isolated from MCF-7 cells by flow cytometry activated sorting (FACS). CD133+ colonal cancer stem cells were isolated from HCT116 cells by magnetic activation cell sorting (MACS) (Miltenyi Biotech). MCF-7 cells were stained with 10 μM a chloromethyl derivative of fluorescence probe CM-H2DCFDA to detect intracellular ROS levels. ROS-low and ROS-high subsets were isolated from MCF-7 cells by FACS. Immediately after cell sorting, cancer cells were used to carry out ^{51}Cr release assay.

2.3. Isolation and culture of NK cells

All experiments were performed in accordance with the Sichuan University's Ethics Committees. Human periphery blood was obtained from different health donors. NK cells were sorted by magnetic bead isolation using NK isolation kit from R&D systems. The procedure was according to the manufacturer's instructions. The purified NK cells were maintained in RPMI 1640 media supplemented with 10% FBS and 200 U/ml human IL-2 as well as 10 ng/ml IL-15 human.

2.4. ^{51}Cr release assay

Target tumor cells or cancer stem cells were labeled at 37 °C with 1 mCi of $Na_2{}^{51}CrO_4$ for 1 h. Labeled cells were then incubated in triplicate with IL-2 and IL-15 activated NK cells at different effector/target (E:T) ratios. After incubation for 4 h at 37 °C, plates were centrifuged (200 g, 5 min), and cell free supernatants were collected. Samples were counted on scintillation counter. Percentage of cytolysis by NK cells was calculated as (sample release – spontaneous release)/(maximum release - spontaneous release). To assess the role of ROS on the NK cells cytotoxicity to CD44+CD24− CSCs, 1 mM L-S,R-buthionine sulfoximine or 1 mM H_2O_2 were added into ^{51}Cr release assay system. Each experiment was done in triplicate.

2.5. Flow cytometry

MCF-7 cells were stained with CD44-PE, and CD24-PerCP-Cy5.5. To assess the expression of NKG2D ligands on cancer cell surface. MCF-7 Cells were also stained with antibodies against ULBP1, ULBP2 or MICA, followed by FITC-conjugated anti-mouse IgG. Stained cells were acquired with a FACSCalibur flow cytometer (BD Biosciences) and analyzed with CellQuest software (BD Biosciences). Data was determined in triplicate.

2.6. Blocking studies

To block the cytotoxicity of NK cells, 20 μg/mL antibodies against NKG2D, NKp44 and NKp46 were added into the ^{51}Cr release assay system, respectively. 4-h ^{51}Cr release assay was performed to evaluated the NK cells cytotoxicity.

2.7. Statistical analysis

Statistical significance was analyzed by Student's t test when compared between two groups and one-way ANOVA when compared among more than three groups. The p values less than 0.05 were considered as statistically significant.

3. Results

3.1. Human breast cancer stem cells are sensitive to NK cell cytotoxicity

To investigate the sensitivity of cancer stem cells to NK cells, ^{51}Cr releasing assay was performed. We first isolated NK cells by MACS from periphery blood of health donors, and activated with IL-2 and IL-15. CD44+CD24− population, well-known cancer stem cells in human breast cancer [6], was sorted by FACS from MCF-7 cell line. Then, a 4-h ^{51}Cr release assay was performed. We observed that CD44+CD24− CSCs showed enhanced susceptibility to NK cells killing (Fig. 1), suggesting that NK cells might preferentially kill cancer stem cells.

3.2. Human breast cancer stem cells express NK cell activation ligands

NK cells are able to recognize and kill infected and transformed cells through direct receptor–ligand interactions [26]. The interaction of NKG2D and its ligands is important to the recognition and killing activity of NK cells [27]. To investigate the potential mechanism of enhanced sensitivity of cancer stem cells to NK cells, we further assessed the expression of NK cell activation ligands on cancer stem cells. The levels of NKG2D ligands, including ULBP1, ULBP2 and MICA, were higher on CD44+CD24− cancer stem cells than non-CSCs (Fig. 2A and B). Those results suggested that NK

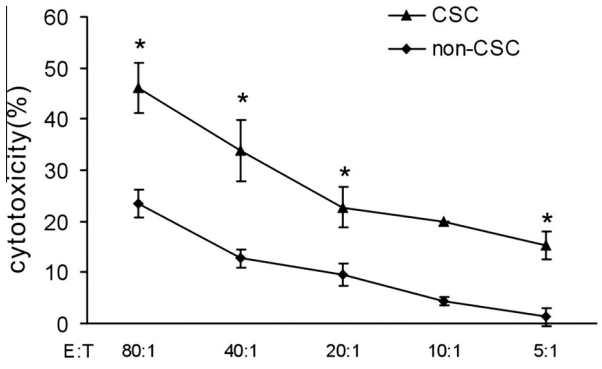

Fig. 1. Human breast cancer stem cells are sensitive to NK cell cytotoxicity. NK cells were isolated by MACS from periphery blood. CD44+CD24− breast cancer stem cells were sorted by FACS. CSCs were cultured with NK cells at different E:T ratios from 80:1 to 5:1. Cytotoxicity was assessed using the ^{51}Cr release assay. Two independent experiments were performed. Data was analyzed in triplicate. CSC, cancer stem cells. $^{*}P < 0.05$.