EDITORIAL

iNK-CD64/16A cells: a promising approach for ADCC?

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ABSTRACT

**Introduction:** ADCC by natural killer (NK) cells is a key mechanism for several clinically successful tumor-targeting therapeutic antibodies. Most patients, however, have limited responses to this therapy and/or develop resistance. NK cells exclusively recognize IgG by their low-affinity FcR CD16A.

**Areas covered:** We describe in this editorial a novel recombinant FcR that consists of CD64, the only high affinity IgG receptor, and the transmembrane and cytoplasmic regions of CD16A, a potent activating receptor. CD64/16A was expressed in engineered iPSCs that were differentiated into NK cells (referred to as iNK cells).

**Expert opinion:** iNK-CD64/16A cells in combination with therapeutic antibodies provide a universal tumor antigen targeting approach and potential off-the-shelf cell therapy to treat various malignancies.

1. NK cells

NK cells are lymphocytes of the innate immune system that target stressed, infected, and neoplastic cells. In human peripheral blood, NK cells comprise approximately 10% of lymphocytes and the mature NK cells are identified as CD56dim CD16A+. Although NK cells share many functional characteristics with cytotoxic T cells, they do not rearrange or express T cell antigen receptor genes. Instead, their activation is determined by the integration of signals from an array of germline encoded inhibitory and activating receptors that recognize markers on target cells that signify altered-self or missing-self [1]. The latter process of direct cytotoxicity provides a mechanism for killing tumor cells that have escaped T cell immunosurveillance by downregulation of MHC class I.

Human NK cells also mediate indirect cytotoxicity by the recognition of IgG antibodies attached to target cells, referred to as antibody-dependent cell-mediated cytotoxicity (ADCC). This process is a key mechanism of action for various clinically successful anti-tumor therapeutic monoclonal antibodies (mAbs) [2], which are antibodies of the same isotype (e.g., IgG1) with specificity to the same epitope within an antigen. However, despite having a significant impact on some malignancies, most cancer patients respond poorly or develop resistance to anti-tumor mAb therapy. For instance, although the importance of EGFRs in the malignant progression of ovarian cancer is well established and over expression of EGFRs is a biomarker of advanced disease, antibody therapies such as cetuximab (anti-EGFR mAb) and trastuzumab (anti-HER2 mAb) have shown limited clinical benefit [3].

2. CD16A

Human NK cells mediate ADCC exclusively by the Fc gamma receptor CD16A (FcyRIIA), which binds to IgG1 (the isotype of most tumor-targeting therapeutic mAbs) and IgG3 [4]. CD16A associates with FcRγ and/or CD3ζ chains and is a potent activating receptor. CD16A engagement alone can trigger NK cell degranulation, whereas other NK cell activating receptors typically require a combination of signaling events to induce degranulation. Consistent with this role, CD16A is tightly regulated and this includes its rapid downregulation by a proteolytic process upon NK cell activation [4]. This process is referred to as ectodomain shedding and is primarily mediated by the membrane-associated protease ADAM17 (a disintegrin and metalloproteinase-17) [4]. CD16A shedding occurs in a cis manner at a specific extracellular location proximal to the cell membrane [4]. Site-directed mutagenesis proximal this cleavage site in CD16A has been shown to completely disrupt its shedding by engineered NK cells activated in various manners [5,6]. The therapeutic benefits of this cleavage-resistant version of CD16A in enhancing ADCC efficacy has been described in recent review articles [4,7].

Humans express three classes of FcγRs: FcγRI (CD64), FcγRII (CD32), and FcγRII (CD16). CD16A is intrinsically a low affinity FcγR [8], though two allelic variants of the receptor vary in their binding affinity for IgG [4]. CD16A with a valine (V) at position 158 binds to IgG1 with at least 2-fold higher affinity than CD16A with a phenylaniline (F) at this position [9], with the lower affinity variant being the dominant allele [10]. Cancer patients that are homozygous for the higher affinity allele have been reported to respond significantly better to therapeutic mAbs for various malignancies [2]. In addition, it has been recently reported that that the higher affinity version of CD16A can also enhance certain checkpoint mAb therapies by facilitating ADCC of regulatory T cells [11]. These studies demonstrate that the anti-tumor activity of therapeutic mAbs corresponds with higher affinity FcγR binding. Thus far, the main approach to enhance FcγR binding has been to modify...
the protein backbone or glycosylation of antibodies [2]. However, limitations of this approach are that the therapeutic mAbs need to be individually modified and optimized and, of course, if CD16A is cleaved from NK cells upon their activation, this strategy will have limited efficacy.

3. Recombinant FCyR

Our focus has been on generating a high affinity and non-cleavable recombinant FCyR for expression in engineered NK cells for use as an adoptive cell therapy in combination with anti-tumor therapeutic mAbs. Human CD64, which is expressed by certain myeloid leukocyte populations, is the only high affinity FCyR. It binds to monomeric IgG1 with at least 30-fold higher affinity than CD16A [8]. We generated a novel recombinant FCyR that consists of the extracellular region of human CD64, for high affinity IgG binding, and the transmembrane and intracellular regions of human CD16A to induce NK cell activation (Figure 1). This recombinant FCyR, referred to as CD64/16A, was initially functionally evaluated in the human NK cell line NK92. An advantage of these cells is that they lack endogenous FCyRs but are able to mediate ADCC upon their expression of CD16A [12]. CD64/16A does not contain the ADAM17 cleavage region found in CD16A (Figure 1), and in contrast to CD16A, it did not undergo a rapid downregulation in expression upon NK cell activation [12]. NK92 cells expressing CD64/16A efficiently attached to antibody-opsonized tumor cells, mediated ADCC, and produced cytokines during this process [12]. These findings demonstrated that the CD64 and CD16A components of the recombinant FCyR retained their respective functions.

We also expressed CD64/16A in primary NK cells derived from human induced pluripotent stem cells (iPSC). iPSCs are routinely generated from a variety of cell sources, they can be maintained as clonal cell lines, and are very stable and genetically-modifiable cells [7]. A defined method for deriving cytolytic NK cells from iPSCs in vitro was first established at the University of Minnesota [13]. iPSC-derived NK (iNK) cells overcome issues with donor and patient-obtained NK cells, such as heterogeneity and challenges with genetic modification, and can be produced in essentially unlimited numbers [7]. Undifferentiated iPSCs were transduced to express CD64/16A using a sleeping beauty transposon plasmid for nonrandom gene insertion and stable expression. The iPSCs were differentiated into hematopoietic cells and then into CD56+ CD3− iNK cells by a two-step process. The generated iNK cells were cytolytic effectors responsive to CD64/16A engagement of antibody-bound tumor cells [12].

Due to its high affinity property and resistance to shedding, CD64/16A is likely to alter NK cell attachment to antibody-opsonized target cells. For instance, an individual NK cell can kill multiple target cells by a process of serial killing. During ADCC, this process involves localized CD16A shedding resulting in transient attachments by NK cells for sequential engagements of target cells [6]. Serial killing by engineered NK cells expressing CD64/16A during ADCC has not yet been examined. It is anticipated, however, that the recombinant FCyR would mediate stable rather than transient tethers to antibody-opsonized target cells and lead to higher attachment levels of NK cells on tumor cells. Indeed, serial killing by adoptively transferred NK cells expressing endogenous CD16A could be readily impaired considering the various circumstances that lead to its downregulation. This includes during ex vivo NK cell expansion for adoptive transfer, by circulating NK cells in individuals receiving mAb therapy, and by infiltrating NK cells in the tumor microenvironment of patients [4].

Most importantly, high affinity CD64/16A could serve as a docking platform for therapeutic mAbs and receptor-IgG chimeric proteins on the surface of engineered NK cells [12]. Therapeutic mAbs have become one of the fastest growing classes of drugs, and tumor-targeting mAbs are the most widely used and characterized immunotherapy for hematologic and solid tumors. In addition to recognizing cell surface tumor antigens, mAbs can be used to recognize intracellular tumor antigens such as oncoogenic protein/HLA complexes [14]. Tumor-associated markers can also be detected by receptor-IgG chimeric proteins, such as NKG2D-IgG, which binds MICA and MICB that are upregulated by various tumor cells [15]. Hence, a broad and ever-expanding selection of tumor antigen-targeting elements could be docked to iNK cells expressing CD64/16A to treat assorted malignancies and reduce the likelihood of tumor escape. Moreover, this approach could greatly decrease the levels of tumor targeting mAbs administered to patients, reducing toxicities they can exert and their high cost for treatment.
Due to the high affinity state of CD64/16A, free IgG in the blood or tumor microenvironment could displace therapeutic mAbs pre-docked to CD64/16A on engineered NK cells. However, others have shown that monomeric IgG adsorbed to CD64 is not displaced by free IgG [16]. Instead, monomeric IgG bound to CD64 mainly detaches within an acidic lysosomal compartment following internalization of the receptor [16]. Thus, therapeutic mAbs pre-docked to CD64/16A may not be readily displaced by serum IgG. If therapeutic mAb and engineered NK cells were administered separately, it is anticipated that CD64/16A would be immediately occupied by serum IgG. Indeed, CD64 on circulating myeloid cells has been shown to be saturated by serum IgG; however, this does not prevent these cells from binding IgG-opsonized targets. This is because FcγRs undergo an increase in affinity and avidity mediated by inside-out signaling, increased clustering, and increased expression upon leukocyte stimulation, resulting in preferential binding to IgG-opsonized targets [17]. This process is similar to the increased binding activity by integrin adhesion molecules upon leukocyte activation [18]. However, if free IgG is determined to markedly impair ADCC by iNK cells expressing CD64/16A, the Fc region of therapeutic mAbs can be engineered to bind FcγRs with higher affinity than serum IgGs [2].

4. Expert opinion

Recombinant CD64/16A expressed on NK cells is designed to bind anti-tumor therapeutic mAbs with high affinity, not to undergo downregulation by shedding, and to induce robust NK cell activation in order to mediate ADCC of resilient cancer cells in an immunosuppressive microenvironment. CD64/16A provides a universal targeting approach when combined with anti-tumor mAbs, either administered to patients or docked on to the surface of engineered NK cells prior to patient infusion. The latter approach offers the advantage of arming NK cells with switchable and mixable combinations of tumor-targeting elements (Figure 2). Other universal targeting approaches have been described including a chimeric antigen receptor (CAR) containing avidin in its extracellular domain to bind biotinylated mAbs or containing a leucine zipper adaptor to bind an scFv with a leucine zipper adaptor [19]. Advantages of CD64/16A are that its components are native proteins and would not be expected to be immunogenic, and the anti-tumor mAbs that bind CD64/16A do not have to be further modified and optimized. iPSCs provide a state-of-the-art platform for CD64/16A expression, as well as other genetic modifications to enhance the in vivo persistence, resistance to immune suppression and recognition, and tumor homing of derived iNK cells. These cells offer an important resource for an off-the-shelf, standardized, and clonal cell therapy that can be efficiently and reliably produced in very high numbers [7].

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Declaration of interest

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