MOLECULAR ONCOLOGY 9 (2015) 1904–1917

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Review

Harnessing adaptive natural killer cells in cancer immunotherapy☆

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ARTICLE INFO

Article history:
Received 3 September 2015
Received in revised form
28 September 2015
Accepted 4 October 2015
Available online 20 October 2015

Keywords:
NK cells
Immunotherapy
Killer cell immunoglobulin-like receptors
NKG2C
Adaptive

ABSTRACT

Natural killer (NK) cells are innate lymphocytes with a refined ability to recognize transformed cells through a broad array of activating receptors in combination with stochastically expressed inhibitory receptors that recognize MHC-class I. Recent advances in NK cell biology have revealed a high degree of functional plasticity that can be attributed to dynamic cell-to-cell interactions in concert with transcriptional and epigenetic reprogramming. Here, we discuss how new insights into the adaptive behavior of NK cells pave the way for next generation cell therapy based on guided differentiation and selective expansion of particularly cytotoxic NK cell subsets.

1. Introduction

Immunotherapy has rapidly emerged to become a cornerstone in the treatment of a large variety of cancer types. Current approaches in immunotherapy include the relatively basic administration of cytokines, vaccines (Melero et al., 2014) and antibodies (Michaud et al., 2014) as well as more advanced manipulations involving *in vitro* expansion and infusion of specific T cell (Restifo et al., 2012) and natural killer (NK) cell subsets (Vivier et al., 2012). The natural cytotoxic potential of these lymphocytes may also be harnessed through the insertion of genetically engineered chimeric antigen receptors

☆ This is a contribution to the special issue edited by Johanna Olweus, Cancer Immunotherapy.

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http://dx.doi.org/10.1016/j.molonc.2015.10.001
1574-7891/

(CAR) or T cell receptors (TCR), specific for tumor associated and patient specific neo-antigens, respectively (Gill and June, 2015; Schumacher and Schreiber, 2015). Currently, adoptive cell therapy platforms require enormous resources in order to facilitate tumor sequencing, bioinformatics and cell culture that fulfill the requirements for good manufacturing practice (GMP) conditions. It is likely, however, that refinement in these areas over the coming decade will enable the development of cost-effective, high-throughput platforms for large-scale implementation of advanced cellular therapies in the clinic. In this review we focus on the development of the next generation of NK cell immunotherapy, based on new insights into their functional plasticity (Vivier et al., 2011).

2. NK cell-based immunotherapy against cancer

NK cells were discovered in the mid 70's based on their intrinsic "natural" capacity to kill tumor cells (Herberman et al., 1975; Kiessling et al., 1975). Mice deficient in key activating NK cell receptors are more prone to develop carcinogen-induced tumors (Iguchi-Manaka et al., 2008), highlighting the biological relevance of NK cells in immune surveillance. In humans, a population-based functional screening of >3500 healthy individuals revealed an inverse correlation between NK cell cytotoxicity and the risk of developing cancer (Imai et al., 2000). Four decades of intense research have culminated in a rather detailed understanding of the biology of these potent cytotoxic lymphocytes, including their development and functional regulation by cytokines, and the broad array of activating and inhibitory receptors that they express (Cichocki et al., 2014). Insights into the molecular specificities of the missing self response, i.e. the ability of NK cells to sense the absence of self MHC class I molecules through stochastically expressed inhibitory receptors, suggest that NK cells may be particularly effective when transferred across HLA barriers (Karre, 2002; Ruggeri et al., 2002a; Valiante et al., 1997), in the context of allogeneic stem cell transplantation (Ruggeri et al., 2002a) or adoptive cell therapy (Miller et al., 2005). However, the research community has only recently begun to systematically address the potential role of NK cells in clinical settings. Currently, approximately 260 open studies are registered at ClinicalTrials.gov and the clinical translation of new insights in NK cell biology is an area of intense investigation.

Several recent reviews have covered historical landmarks of breakthroughs in NK cell biology (Cichocki et al., 2014), their functional regulation, mechanisms involved in maintenance of self tolerance (Goodridge et al., 2015; Kadri et al., 2015), as well as their role in the context of allogeneic stem cell transplantation (Cichocki et al., 2015). Other reviews have discussed strategies for *ex vivo* expansion (Pittari et al., 2015), de novo development of NK cells from induced pluripotent stem cells (iPSc) and human embryonic stem cells (hESC) (Eguizabal et al., 2014), genetic manipulation with CARs (Glienke et al., 2015), and prospects for using NK cells in both adult and pediatric hematological malignancies and solid tumors (Gras Navarro et al., 2015; Knorr et al., 2014; Leung, 2014; McDowell et al., 2015). In light of these, this review will focus entirely on the prospects for clinical translation of the most recent insights into the functional plasticity and adaptive behavior of NK cells. Several lines of evidence suggest that NK cells contribute to adaptive immunity both as mediators of memory responses (Min-Oo et al., 2013) and in their ability to regulate T cell homeostasis (Cook et al., 2014; Waggoner et al., 2012). Thus, in addition to overcoming regulatory and technical challenges pertaining to donor selection, generation of sufficient NK cell numbers and choice of the target specificity for therapy, we believe it will be of outmost importance to consider the fundamental mechanisms involved in creating the vast repertoire diversity of NK cells as well as the heritability and persistence of the effector potential during *in vivo* homeostasis. Before outlining the emerging clinical possibilities of harnessing adaptive NK cells, we will briefly review recent insights into their differentiation and functional reprogramming.

3. NK cell differentiation

At birth, the repertoire of human NK cells is usually naïve and devoid of cells bearing the features of full functional maturity and terminal differentiation (Bjorkstrom et al., 2010; Le Garff-Tavernier et al., 2010). Full development of mature phenotypic and functional NK cell profiles occurs only in response to environmental cues, from metabolism to infection, as observed with infection of mice raised under germ free conditions (Marcais et al., 2014). This development and accumulation of functional NK cells over time, likely occurs under persistent waves of environmental stimulation (Goodridge et al., 2015). The naïve state of NK cell differentiation is associated with high CD56 and CD62L expression, as well as expression of more broadly specific receptors, such as NKG2A and natural cytotoxicity receptors (NCRs) (Beziat et al., 2010; Bjorkstrom et al., 2010; Juelke et al., 2010). Moving along the spectrum of differentiation, cells are observed to progressively downregulate NKG2A and acquire CD16 as well as more specific inhibitory receptors, such as inhibitory killer cell immunoglobulin-like receptors (KIR). KIR acquisition results in a narrower HLA-I ligand specificity and determines the functional fate of the cell (Goodridge et al., 2015; Luetke-Eversloh et al., 2013). As the NK cell population veers toward terminal differentiation, receptor expression profiles become less random and emerging patterns of surface expression suggest an accumulation of differentiated cells enriched by specific receptor interactions. Terminally differentiated NK cells display diminished responsiveness to cytokines, which correlates with the reduction of cytokine receptors, manifested both at the protein and transcriptional level (Beziat et al., 2010; Bjorkstrom et al., 2010). Concurrently, there is a gain in cytolytic potential, as they express higher levels of granzyme B and perforin and become particularly efficient in mediating antibody-dependent cellular cytotoxicity (ADCC) (Lopez-Verges et al., 2010), as well as in the capacity for cytokine production in response to stimulation through activating receptors (Luetke-Eversloh et al., 2014b). It is during the approach towards terminal stages of maturation that the adaptive behavior of NK cells truly comes to light, and is most strikingly demonstrated during CMV infection in mice and humans alike (Cichocki et al., 2014).