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Next generation natural killer cells for cancer immunotherapy: the promise of genetic engineering

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Recent advances in the field of cellular therapy have focused on autologous T cells engineered to express a chimeric antigen receptor (CAR) against tumor antigens. Remarkable responses have been observed in patients receiving autologous CD19-redirected T cells for the treatment of B-lymphoid malignancies. However, the generation of autologous products for each patient is logistically challenging and expensive. Extensive research efforts are ongoing to generate an off-the-shelf cellular product for the treatment of cancer patients. Natural killer (NK) cells are attractive contenders since they have potent anti-tumor activity, and their safety in the allogeneic setting expands the cell sources for NK cell therapy beyond an autologous one. In this review, we discuss advantages and limitations of NK cellular therapy, and novel genetic engineering strategies that may be applied to overcome some of the limitations. Next-generation engineered NK cells are showing great promise in the preclinical setting and it is likely that in the next few years CAR-engineered NK cells will be incorporated into the current armamentarium of cell-based cancer therapeutics.

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Introduction

In the past decade, the field of cellular therapy has emerged as a powerful treatment modality for advanced cancers refractory to conventional therapy. Genetic engineering of immune cells has evolved from a promising concept to a practical solution for the treatment of a number of previously refractory types of cancer. Indeed, in the past few months, the Food and Drug Administration (FDA) approved the first gene-modified chimeric antigen receptor (CAR) T-cell therapy (tisagenlecleucel) for relapsed B-cell acute lymphoblastic leukemia (ALL) in children and young adults [1]. This was quickly followed by the approval of the second gene-modified CAR T-cell therapy (axicabtagene ciloleucel) for patients with certain types of relapsed large B-cell non Hodgkin lymphoma (NHL) [2,3]. These exciting new therapies mark a paradigm shift in the treatment of certain hematologic malignancies and are expected to cause a ripple effect in the field of cellular therapy and gene editing to target other cancers.

Despite its success, CAR modified autologous T-cell therapy has some undeniable limitations [4]. From a clinical standpoint, some of the patients are heavily pretreated and therefore lymphopenic, which makes it difficult to collect sufficient numbers of autologous lymphocytes to generate a clinically relevant dose of CAR-T cells for therapy. Furthermore, despite the high response rates, a significant number of patients still experience relapse as the cancer cells develop mechanisms to escape recognition by CAR-T cells [5•]. From a logistic standpoint, the generation of autologous CAR-T cells is cumbersome and therefore restrictive for large-scale clinical application. The process is also lengthy and time consuming and therefore not applicable for patients with rapidly progressing malignancies. An off-the-shelf, ready to use, allogeneic source of CAR-T cells is therefore attractive, however allogeneic T cells are notorious for causing graft-versus-host (GVHD), even after HLA matching [6].

Natural killer (NK) cells are very effective at mediating cytotoxicity against tumor cells and unlike T-cells, kill their targets in a non-antigen specific manner and without the need for prior sensitization [7]. Allogeneic NK cells lack the potential to cause GVHD [8,9], and could be made available as an off-the-shelf allogeneic product for immediate clinical use. Therefore, genetic engineering of NK cells by introducing a CAR to redirect their specificity is an active field of investigation. Notably, engineered CAR-NK cells retain their diverse arrays of activating and inhibitory receptors [10••], which in principle should make relapse due to downregulation of the CAR target antigen less likely than it is with CAR-T cells [11]. Therefore, the inherent qualities of NK cells make them attractive candidates for genetic engineering for the therapy of cancer.

In this review, we detail recent advances in the field of NK cell engineering for cancer immunotherapy and discuss advantages and limitations of these strategies.

NK cell biology and role in cancer immune surveillance

As their name indicates, NK cells are a subset of effector lymphocytes involved in innate 'natural' immunity and being potent 'killers', they represent the first line of defense against pathogens and malignant cells [12]. They are characterized by CD56 and CD16 expression and lack of T cell receptor (TCR) and CD3 expression on their surface. NK cells can be further subdivided into two distinct subsets depending on their level of CD56 expression. The most common subtype present in the peripheral blood is $CD16^{+}CD56^{dim}$ and represents the more mature and highly cytotoxic phenotype; the second subtype is $CD16^{-}CD56^{bright}$, which characterizes a less mature immunoregulatory population mainly found in lymphoid tissues [13]. Unlike T cells, NK cells do not need prior antigen sensitization to kill their target cells. NK cells can mediate cytotoxicity through a number of mechanisms, the most important of which are degranulation and antibody dependent cellular cytotoxicity (ADCC), mediated by CD16 binding to the Fc portion of IgG1 opsonized on the surface of target cells [14]. Unlike other immune cells, which require time to acquire cytolytic activity, NK cells are 'ready to kill'. In fact, when NK cells form an immunologic synapse with their target cells, they release preformed cytolytic granules containing perforin and granzyme, leading to target cell lysis. In addition, engaged NK cells release molecules of the tumor necrosis factor (TNF) family, which induce death ligands such as FAS (first apoptosis signal) ligand and TRAIL (TNF-related apoptosis inducing ligand) on their surface. In turn, these ligands bind to death receptors on target cells, initiating an enzymatic cascade through caspases leading to apoptosis [14,15]. Activated NK cells also produce interferon (IFN)-γ, which activates dendritic cells and macrophages and has pleiotropic effects on the adaptive immune response [15]. In fact, the activity of NK cells is intricately complex and depends on the delicate integration of signals from multiple activating and inhibitory receptors, cytokines and chemokines [16].

NK cells can distinguish between normal and tumor cells through several mechanisms. To prevent the killing of healthy cells, NK cells primarily use inhibitory receptors, such as killer cell immunoglobulin-like receptors (KIRs) and CD94-NKG2A, that bind to major histocompatibility complex (MHC) class I molecules which are constitutively present on normal cells. Malignant cells can be recognized and killed by NK cells as they often downregulate or lose the expression of MHC-class I molecules [17] and/or by activating signals provided by multiple activating receptors on the surface of NK cells, which recognize stress ligands on the tumor cells. The vast array of cytokines and chemokines in the tumor microenvironment also play a role in the final disposition of NK cells to kill the transformed cells [18].

In the past few decades, numerous studies have implicated an important role for NK cells in tumor surveillance. In fact, quantitative and qualitative deficiencies of NK cells have been shown to contribute to cancer risk [19,20,21•]. In addition, in experimental animal models, specific depletion of NK cells has been shown to cause more aggressive tumor progression and metastasis [22]. More recently, cancer stem cells (CSC's) which are recognized to be particularly resistant to chemotherapy and radiotherapy, have been shown to be highly susceptible to NK cell killing, partly due to downregulation of MHC class I on their surface (reviewed in [23]). These studies point to a crucial role for NK cells in the immune surveillance of cancer.

Limitations of adoptive NK cellular therapy

Despite the lure of NK cells for adoptive therapy of cancer and their favorable safety profile, their efficacy in human trials has been modest at best. A number of factors limit the application of NK cell immunotherapy for the treatment of cancer. First, adoptively transferred NK cells have limited persistence *in vivo*, which while desirable from a safety standpoint, may hinder their efficacy [24]. Also, NK cell migration and their ability to penetrate tumor tissues have been reported to be inferior to that of T cells, which raises concerns regarding their usefulness in the setting of solid tumors [25]. Thus, strategies to increase infiltration of NK cells into tumors would be plausible to enhance antitumor efficacy. In addition, tumors develop mechanisms to evade NK cell surveillance such as upregulation of HLA molecules to disguise as normal cells, or downregulation of ligands for activating NK cell receptors [26]. The tumor microenvironment is also a major barrier to the effectiveness of NK cells. For instance, regulatory T cells (Treg cells) and myeloid-derived suppressor cells (MDSCs) frequently found at the tumor site can inhibit the function of NK cells [27]. Activated platelets in the malignant milieu have also been shown to suppress NK cell cytotoxicity through a range of mechanisms [7]. Furthermore, the tumor microenvironment is rich in immunosuppressive cytokines and metabolites such as TGF-β, adenosine, prostaglandin E2 (PGE2) and indoleamine 2,3-dioxygenase (IDO), which have been linked to NK cell dysfunction [7]. Finally, until relatively recently, the genetic manipulation of NK cells was considered to be challenging, yielding low efficiency and cell viability. However, recent optimizations in viral transduction, gene editing and electroporation technologies have renewed interest in strategies to enhance NK cell activity through genetic engineering [28]. These include approaches to make these cells persist longer, home to tumor sites, have enhanced cytotoxicity against tumors and be more adept at circumventing the immunosuppressive microenvironment (Figure 1).