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Review

Natural killer cell-mediated immunosurveillance of human cancer

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ABSTRACT

The contribution of natural killer (NK) cells to immunosurveillance of human cancer remains debatable. Here, we discuss advances in several areas of human NK cell research, many of which support the ability of NK cells to prevent cancer development and avoid relapse following adoptive immunotherapy. We describe the molecular basis for NK cell recognition of human tumor cells and provide evidence for NK cell-mediated killing of human primary tumor cells *ex vivo*. Subsequently, we highlight studies demonstrating the ability of NK cells to migrate to, and reside in, the human tumor microenvironment where selection of tumor escape variants from NK cells can occur. Indirect evidence for NK cell immunosurveillance against human malignancies is provided by the reduced incidence of cancer in individuals with high levels of NK cell cytotoxicity, and the significant clinical responses observed following infusion of human NK cells into cancer patients. Finally, we describe studies showing enhanced tumor progression, or increased cancer incidence, in patients with inherited and acquired defects in cellular cytotoxicity. All these observations have in common that they, either indirectly or directly, suggest a role for NK cells in mediating immunosurveillance against human cancer. This opens up for exciting possibilities with respect to further exploring NK cells in settings of adoptive immunotherapy in human cancer.

1. Introduction

The concept of immunosurveillance of cancer was outlined half a century ago [1]. The general view was that tumor cell transformation is a frequent event and is under constant control by the immune system. A prediction of the concept was that genetically immunodeficient individuals, or those being treated with immunosuppressive drugs, would have a markedly increased incidence of cancer. At first, clinical observations provided only marginal support for this concept. The rate of spontaneous malignant transformation was likely overrated. However, based on data from experimental models in mice and epidemiological studies in humans [2], the concept has now gained acceptance in a wider research community. Indeed, patients with inherited or acquired immunodeficiencies, and patients on immunosuppressive drugs, have higher incidences of cancer [2]. Defects affecting T cells or other parts of the adaptive immune system have been particularly implicated in this context. However, innate constituents of the immune system, including natural killer (NK) cells, may also play a significantly important role.

NK cells were initially identified due to their ability to kill tumor cell lines *in vitro* [3,4]. Since that discovery, a large number of studies has demonstrated NK cell-mediated killing of many types of tumor cell lines *in vitro,* and in experimental animal models [5–7]. Studies have also shown that NK cells are involved in rejection responses against experimentally induced and spontaneously developing tumors in mice [8–10]. Indirect evidence for NK cell targeting of human tumors has come from studies of allogeneic hematopoietic stem cell transplantation (HSCT), in particular haploidentical HSCT against acute myeloid leukemia (AML) [11,12].

NK cell recognition of tumor cells is a tightly regulated process involving the interaction of specific ligands on the tumor cells with NK cell receptors and subsequent integration of signals derived from such receptors in the responding NK cells [13,14]. The earliest insights into the molecular specificity of NK cells were based on the observation that

Abbreviations: ADCC, antibody dependent cellular cytotoxicity; AML, acute myeloid leukemia; CMV, cytomegalovirus; CR, complete remission; HL, Hodgkin lymphoma; HLA, human leukocyte antigen; HLH, hemophagocytic lymphohistiocytosis; KIR, killer cell inhibitory immunoglobulin-like receptor; MDS, myelodysplastic syndrome; MHC, major histocompatibility complex; MIC, MHC class I-related chain; MM, multiple myeloma; NCR, natural cytotoxicity receptors; NK, natural killer; PR, partial response; SCT, stem cell transplantation

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NK cell cytotoxicity was triggered by tumor cells lacking expression of all (or certain) self-major histocompatibility complex (MHC) class I molecules, a phenomenon referred to as "missing-self" recognition [6,15]. These observations led to the identification of specific NK cell-inhibitory receptors that recognize MHC class I molecules [16,17] and were later followed by identification of NK cell-activating receptors, binding specific ligands expressed by tumor cells [18–23].

Furthermore, in contrast to what was initially thought, it is now clear that NK cells are not a homogeneous set of cytotoxic lymphocytes. Rather, during the past two decades we have gained a deep understanding of this population of lymphocytes revealing significant insights into their differentiation and functional diversification [24–32]. The extensive diversity in the human NK cell repertoire, both within and between individuals, is known to be driven by a combination of genetic variations in receptor expression, homeostatic turnover and epigenetic reprogramming following the response to pathogenic challenges, in particular by human cytomegalovirus (CMV) [28,31–35]. Much knowledge in this respect evolves from studies of NK cells in human peripheral blood. NK cells that reside in corresponding peripheral tissues including solid tumors are, however, still relatively less well-characterized [36]. In this respect, furthering our understanding of the biology of tumor-resident NK cells is essential to decipher their direct potential contribution to tumor control.

Herein, we review a series of findings relating to the complex interactions between NK cells and tumor cells. We discuss evidence for a direct role of NK cells in controlling human cancer development and/or progression, as well as studies showing enhanced cancer susceptibility and/or progression in patients with abnormal NK cell function. Together, these studies provide compelling evidence for an important role of NK cells in immunosurveillance against development and progression of cancers in humans. This concept encourages further efforts to develop new treatment options aimed at strengthening endogenous NK cell responses to tumor cells as well as designing protocols that utilize adoptive transfer of autologous and allogeneic NK cells to target human solid and hematological malignancies.

2. NK cell interactions with human tumor cells and the tumor microenvironment

NK cells can control cancers directly by interacting with tumor cells and indirectly by influencing the activities of other immune cells in the tumor microenvironment. Direct tumor cell lysis by NK cells is thought to be principally perforin-dependent, as extensively demonstrated in many experimental model systems [37]. However, NK cells can also induce tumor cell elimination through death receptor-mediated pathways such as TRAIL and FasL [38]. Further, activated NK cells produce numerous cytokines and chemokines [39], many of which have potent antitumor activities in addition to promoting other innate and adaptive immune responses. In the Sections 2.1–2.4, we cover current insights into the molecular basis for NK cell-mediated recognition of human tumor cells. In this context, we also describe the new insights into human NK cell differentiation and functional diversification that have emerged in recent years. We then present evidence for *ex vivo* killing of primary human tumor cells by NK cells. Finally, we briefly discuss some of the known characteristics of tumor tissue-resident NK cells. Evidence that NK cell recognition of tumor cells imposes a selection pressure on tumors, a process referred to as "immunoediting" [40,41], is also provided.

2.1. NK cell recognition of human tumor cells

Although evolution has likely selected NK cells on the basis of infectious pressure, the effector mechanisms used by NK cells also enable them to discriminate between normal non-transformed cells and transformed cancer cells. However, in contrast to T cells, NK cells do not recognize MHC-presented neoantigens. With the exception of the low affinity Fcγ receptor IIIA (CD16) that operates via antibodies, NK cells are equipped with germline-encoded receptors that recognize ligands associated with cellular stress, viral infection or transformation [13,14]. Below, we describe the principle mechanisms that govern NK cell recognition of cancer cells.

NK cells have long been known to express CD16 that mediates antibody-dependent cellular cytotoxicity (ADCC). ADCC is likely a key mechanism responsible for the action of several monoclonal antibodies currently used in clinical cancer treatment [42]. Several other NK cell activation receptors have subsequently been discovered and well characterized, all of which contribute to "natural cytotoxicity" [18–23]. One important group of human NK cell activation receptors is the natural cytotoxicity receptors (NCRs). Two of these receptors, NKp30 and NKp46, are constitutively expressed on all peripheral blood NK cells, whereas NKp44 is induced upon IL-2 stimulation [19,20,22]. The role of these receptors in tumor cell killing has been demonstrated by, e.g., receptor masking with anti-NCR antibodies inhibiting NK cell-mediated lysis of many different tumor cell lines. Other important activation receptors are NKG2D and DNAM-1. Human NKG2D recognizes the stress inducible molecules MHC class I-related chain, (MIC)A and MICB, as well as the UL16-binding proteins, while DNAM-1 recognizes the poliovirus receptor (PVR) and Nectin-2 [43]. In addition to these receptors, many other activation receptors, including 2B4 (CD244), NTB-A, NKp80, CD2, CD11a/CD18 and CD59, have also been characterized [13,14]. Several of these may have important coactivating or costimulatory functions in NK cell activation and tumor cell recognition.

NK cell cytotoxicity is also controlled by signals from inhibitory receptors [13,14]. Most of these receptors bind classical and/or non-classical MHC class I molecules [44]. These molecules are normally expressed on most healthy cells in the body but are often lost upon malignant transformation or during tumor progression [45]. In humans, killer cell inhibitory immunoglobulin-like receptors (KIR) and CD94/NKG2A play major roles as HLA class I-binding inhibitory NK cell receptors. KIRs recognize selected HLA-A, -B and -C alleles, whereas CD94/NKG2A receptors recognize HLA-E molecules [44,46]. Individuals differ in the number and type of inherited *KIR* genes, and expression of specific KIR gene-products is stochastic in the NK cell repertoire [47–51]. Thus, many NK cells express only a few of many possible inhibitory KIRs. Although NK cells do not undergo a strict selection process, most functionally mature NK cells express at least one inhibitory receptor (*i.e.*, a KIR and/or CD94/NKG2A) that is specific for a self-MHC class I ligand [48,52,53]. The clonal distribution of KIRs results in a system allowing NK cells to detect cells lacking expression of single MHC class I alleles, *i.e.*, mediating "missing-self" reactivity against tumor cells that have lost MHC class I expression. Thus, every individual has a unique repertoire of NK cells with varying proportions of cells that are capable of sensing alterations in HLA class I levels upon tumor transformation or viral infection. This diversity is potentially beneficial in settings of SCT and may be harnessed in settings of adoptive NK cell immunotherapy when used over HLA barriers [44]. Normally, NK cell inhibition dominates over activation. In some situations, however, the activation signals may override the inhibitory signals mediated by MHC class I molecules, as has been demonstrated, *e.g.*, for NKG2D-mediated triggering of some MHC class I-expressing tumor cell lines [54,55].

Interactions between inhibitory receptors and their cognate HLA class I ligands are not the only contributors to maintaining immune tolerance. Paradoxically, inhibitory input during homeostasis also determines the intrinsic functional potential of NK cells, a process termed "education" [56–58]. This further emphasizes the critical role of KIRs in generating functional diversity within and between individuals. Although a large number of studies have explored how variation in the *KIR* and *HLA* genetic loci influences disease risk and progression as well as outcomes during different therapies, including SCT [44], few investigators have interrogated the impact of functional diversity among NK cell repertoires.