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NK Cell-Mediated Targeting of Human Ovarian Cancer and Possibilities for Immunotherapy

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Abstract

Silva RF, Alves PCM, Guimarães F. NK Cell-Mediated Targeting of Human Ovarian Cancer and Possibilities for Immunotherapy. ARBS Annu Rev Biomed Sci 2011;13:23-29. A better understanding of the NK cell receptor-ligand interaction opened possibilities for new therapeutic strategies. The well known mismatch KIR/ HLA interaction is of great importance for NK cell stimulation. Lately, the interaction of the activating receptor DNAM-1 with its ligand PVR, has been highlighted in many ovarian carcinoma studies. Autologous and allogeneic NK cells, along with monoclonal antibodies, have been tested in current immunotherapies. This review presents information on NK cell receptor-ligand interactions and its possibilities for NK-based immunotherapy against ovarian cancer.

Keywords: Natural killer cells, immunotherapy, ovarian neoplasm, NK cell receptors.

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1. Introduction

The poor prognosis of ovarian carcinoma has driven studies to develop more efficient therapeutic strategies. The possibility to use natural killer (NK) cells for the treatment of human cancer has increased recently (Ljunggren & Malmberg, 2007; Sutlu & Alici, 2009). This is a consequence of an increased comprehension of the molecular processes and receptor-ligand interactions which influences NK cell

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recognition and elimination of tumor cells. New cellular isolation and expansion methods ex vivo provide sufficient quantities of human NK cells for clinical trials (Barkholt *et al.*, 2009). Additionally, drugs, antibodies and genetic manipulation can be combined to the therapeutic strategy in order to exploit NK cells antitumor function (Malmberg *et al.*, 2008). The purpose of this review is to present information on NK cell specific molecular receptor-ligand interactions with ovarian cancer cells and its usefulness for the development of an NK-based immunotheraphy.

2. NK Cells

NK cells are lymphocytes initially indentified by its functionality, based on their capacity of eliminating a variety of tumor cells without previous stimulation (Kiessling *et al.*, 1975; Herbermen *et al.*, 1975). In humans, NK cells constitute 5-15% of lymphocytes present in the peripheral blood, identified by the absence of CD3 cell surface molecule and by the expression of CD56 (CD3-CD56+), an isoform of neural cell adhesion molecule (NCAM) also found on a minority of T cells (Robertson *et al.*, 1990; Arnon *et al.*, 2006). NK cells can be further grouped into two functional subtypes considering the expression of the CD56 marker. The majority (90%) of the NK cells that are found in the peripheral blood express low maker density, they are categorized as CD56dim and the minority of them (10%) express high marker density, they are categorized as CD56bright (Lanier *et al.*, 1986; Sutlu & Alici, 2009). CD56dim cells can be found in the bone marrow, peripheral blood and spleen. They express high levels of CD16 (FcγRIIIA) receptors, have great cytolytic potential and produce less variety of cytokines. CD56bright are mainly found in peripheral lymphoid tissues, express low levels of CD16, have low cytolytic capacity and produce the largest variety of immunoregulatory cytokines. It has been hypothesized that CD56bright cells are less mature than CD56dim, since there is an increase in the expression of specific receptors of the subtype "dim" (like activation receptors and CD16+) when the "bright" subtype is stimulated by IL-2 (Lanier *et al.*, 1986; Di Santo, 2006). Another evidence which supports this hypothesis is the existence of large quantities of CD56bright cells associated with lymphocytes CD34+CD45RA+, NK precursor cells in secondary lymphoid tissues. In these tissues there are still plenty of antigen presenting cells expressing interleukin 15 (IL-15) attached to its surface, this cytokine is known to be important in the maturation of NK cells. (Mattei *et al.*, 2001; Fehniger *et al.*, 2003; Freud *et al.*, 2005; Caligiuri, 2008). Therefore, the development of NK cells does not only occur in the bone marrow, but it also occurs in secondary lymphoid tissues.

NK cells are mentioned as lymphocytes of the innate immune response, mediating effector functions against malignant or virus infected cells. However, NK cells production of pro-inflammatory cytokines such as interferon-γ (INF-γ), tumor necrosis factor-α (TNF-α) and granulocyte macrophage colony-stimulation factor (GM-CSF) regulates the innate immune response and contributes to the development of the adaptive immune response (Wiltrout, 2000). The cytotoxic activity of NK cells occurs after contact with target cells, followed by the secretion of perforin and granzyme-B or induction of apoptosis by ligands FasL and TRAIL (TNF-related apoptosis-inducing ligand) (Wiltrout, 2000; Cooper *et al.*, 2001; Wu & Lanier, 2003; French & Yokoyama, 2003; Hayakawa *et al.*, 2004).

3. NK Receptors

NK cells cytolytic function is determined by the balance of inhibitory and activating signals resulted from the interaction between NK cells receptors and target cells ligands (Cerwenka *et al.*, 2001; Lanier, 2005). Several activating and inhibitory receptors have been described in NK cells, some of them have been characterized focusing on the recognition process of ovarian cancer cells. Among the major inhibitory receptors are: KIR (killer immunoglubuline-like receptors) that recognize ligand molecules of the human leukocyte antigen (HLA) class I from the groups A, B and C; CD94-NKG2A receptor that recognizes the HLA class I from the group E; and ILT2 (immunoglobulin-like transcript 2) receptor that recognizes a relatively conserved region in the class I molecule, providing broad class I specificity (Farag *et al.*, 2002; Moretta *et al.*, 2004; Lanier, 2005; Malmberg *et al.*, 2008). HLA class I is expressed by virtually all nucleated cells of a person, playing a key role in the recognition of body cells as "self" by the immune system. Once committed to HLA class I "self", the inhibitory receptors KIR signal with dominance over the signal of activating receptors, therefore hindering the cytolytic function of NK cells (Bryceson *et al.*, 2006; Caligiuri, 2008). Thus, NK cells spare autosomal cells that express normal levels of HLA class I molecules while eliminating abnormal cells, such as tumor cells or cells infected by viruses, which frequently lose their expression of HLA class I (Ljunggren *et al.*, 1990; Garcia-Lora *et al.*, 2003; Schanoski *et al.*, 2004).

Among the activating receptors stand out the natural cytotoxic receptors (NCR) NKp46 and NKp30

that are expressed constitutively in NK cells, and the NKp44 receptor that is expressed after NK cells activation by interleukin 2 (IL-2) (Arnon *et al.*, 2006; Fuchs *et al.*, 2005). These receptors also assist in the recognition of tumor cells, cells infected by viruses and maturation regulation of dendritic cells. However, little is known about the identity of the ligands for NCR (Cooper & Caligiuri, 2004; Arnon *et al.*, 2005). The density of NCR expression varies among individuals and correlates directly with the ability of NK cells to eliminate abnormal cells (Sivori *et al.*, 1999; Pende *et al.*, 2001). Unlike the NCR, the NKG2D is an activating receptor whose expression is not restricted to NK cells, it is also expressed by cytotoxic T lymphocytes (γ/δ T e α/β T-CD8). The NKG2D recognizes as ligands molecules homologous to HLA class I, represented by transmembrane proteins such as MIC/A, MIC/B, ULBP4 (UL16-biding protein) and proteins anchored to the cell surface by glicofosfatidilinositol (GPI) as ULBP 1, 2 and 3 (Bauer *et al.*, 1999; Biassoni *et al.*, 2001; Cosman *et al.*, 2001; Raulet, 2003; Coudert & Held, 2006). In humans, increased expression of MIC and ULBP is related to different forms of cellular stress, such as viral infection and malignant transformation (Bauer *et al.*, 1999; Cosman *et al.*, 2001; Onda *et al.*, 2001). In fact, the expression of MICA/B has been observed in most human epithelial tumors, including breast, ovarian, colon, kidney and lung carcinomas (Biassoni *et al.*, 2001; Diefenbach & Raulet, 2002), contributing to the possible susceptibility of these tumors to the cytotoxic activity of NK cells. DNAX accessory molecule-1 (DNAM-1) is another important NK cells activating receptor. The ligands indentified for DNAM-1 receptor are the Poliovirus receptor (CD155) and Nectin-2 (CD112), with CD155 displaying to have a predominant role in the induction of DNAM-1 responses. Furthermore, CD155 is commonly expressed on normal cells and overexpressed on various tumor types, including ovarian carcinoma (Lanier, 2005; Carlsten *et al.*, 2009). DNAM-1 contributes to tumor immune surveillance and plays a crucial role in NK cell-mediated recognition of several types of human tumors.

4. NK Cell in Response Against Ovarian Cancer

Since NK cells were first described, more than 30 years ago, new cancer therapies based on their capacity to lysis tumor cells have been developed. Although, several studies have demonstrated the ability of NK cells to target tumor cells in vitro and in vivo (Smyth *et al.*, 2002; Wu & Lanier 2003; Malmberg *et al.* 2008), only in the last decade have been obtained direct evidences on how receptor-ligand interactions drive targeting of tumor cells by NK cells in humans. This information has prompted new insights on therapeutic uses for NK cells.

NK cells from ovarian carcinoma patients were initially reported to display none or poor cytolitic activity against ovarian cell lines, fresh ovarian tumors, and even against the prototype NK cell target K562 (Lotzova *et al.*, 1986; Lotzova *et al.*, 1988; Roszkowski *et al.*, 1993; Malberg, 2004). Additionally, NK cell cytolitic activity against tumor cells was significantly lower among patients with ovarian carcinoma than in patients with benign masses (Lutgendorf *et al.*, 2005). However, antitumor function of NK cells from patients with ovarian cancer can be reestablished as demonstrated by in vitro stimulation of effector cells with recombinant IL-2 (Lotzova *et al.*, 1986; Lotzova *et al.*, 1988) or by enriching the preparation of effector cells with large granular lymphocyte, corresponding to the NK cells (Lotzova *et al.*, 1988). These observations support the idea of overcoming the immunosuppression often seen in patients with cancer, by using strategies for ex vivo expansion and stimulation of cytotoxic cells.

Similarly to other malignancies, ovarian cancer exploits an array of immunological ways to create a suppressive environment to prevent being eliminated by the immune response (Yigit *et al.*, 2010). Two immunosupressive mechanisms capable of affecting NK cell functions have already been detected in ovarian carcinoma patients, one involving recruitment of regulatory T CD4+CD25+ (Treg) lymphocytes to the tumor site, and other involving selective down-regulation of NK cell-activating receptor DNAM-1 (Curiel *et al.*, 2004; Yigit *et al.*, 2010; Carlsten *et al.*, 2009). Specific recruitment of Treg lymphocytes to the tumor site and ascites was correlated to a reduced survival of patients with ovarian cancer (Curiel *et al.*, 2004). Additionally, Treg lymphocytes have been reported to affect NK cell proliferation, cytotoxicity and IFN-γ production (Ghiringhelli *et al.*, 2005; Smyth *et al.*, 2006). Ovarian carcinoma cells expressing the DNAM-1 ligand CD155 led to down-regulation of DNAM-1 activating receptor, explaining the hyporesponsiveness found in tumor-associated NK cells compared to the autologous peripheral blood NK cells (Carlsten *et al.*, 2009).

Immunosuppression is not advantageous for immunotherapies, but by knowing the mechanisms of the suppression, new strategies can be developed in an attempt to overcome this therapeutic barrier. Recent studies have demonstrated the possibility of generating CD56+ NK and NKT-like lymphocytes by ex vivo expansion of PBMC (peripheral blood mononuclear cells) from patients with ovarian carcinoma (Alves *et*

al., 2011). Such effector cell preparations displayed antitumor function, showing the feasibility of overcoming the immune impairment often inferred to cancer patients. Additionally, the NK cells present in the ex vivo effector cells expansion were CD16+, indicating their activation status and their cytotoxic potential mediated by antibodies (Borghaei *et al.*, 2009).

Allogeneic human NK cells are also known to recognize and kill freshly isolated ovarian carcinoma cells. The degranulation of NK cells is dependent on signaling through DNAM-1 receptors with an additional contribution of NKG2D receptors by recognizing corresponding ligands expressed on the surface of ovarian carcinoma cells (Carlsten *et al.*, 2007). The relative high expression of CD155 in combination with reduced levels of HLA class 1 molecules on ovarian carcinoma cells, labels them as an ideal target for autologous NK cells as well (Carlsten et al 2009). Allogeneic NK cells from healthy donors can be a promising immunotherapy strategy since they don't exhibit impaired functions consequently derived from induced immune suppression. Besides, allogeneic NK cells can be specifically activated by the mismatch interaction of KIR/HLA and by the positive interaction between the activating receptor DNAM-1 and its ligand PVR (Carlsten *et al.*, 2007). Graft-versus-host disease (GVHD) is of major concern in the usage of allogeneic NK cells for immunotherapy. However, many recent clinical trials have demonstrated no GVHD in patients with malignancies treated with allogeneic NK cells (Passweg *et al.*, 2004; Miller *et al.*, 2005; Barkholt *et al.*, 2009).

Monoclonal antibodies (mAbs) usage in cancer therapy is based on targeting tumor cells that express tumor associated antigens. Many mAbs targeting antigens associated to cancer cells have been developed in the past few years and it is lately, one of the most important drugs approved for the treatment of cancer, including ovarian carcinoma (McCall *et al.*, 2001; Chan *et al.*, 2006; Seimetz *et al.*, 2010; Esser *et al.*, 2011). The efficacy of mAbs on hematological and some solid malignancies has been shown, but in the case of ovarian carcinoma, it has not been yet validated (Mabuchi *et al.*, 2010). Several mAbs have been investigated for a potential treatment against ovarian cancer, such as bevacizumab; a vascular endothelial growth factor-targeted mAb therapy, trastuzumab and cetuximab; an epidermal growth factor-targeted mAb therapy, oregovomab; a CA-125-targeted mAb therapy, the mAb human milk fat globule 1 (HMFG1); a MUC1-targeted therapy and catumaxomab; a trifunctional antibody with two different antigen-binding specificities, epithelial cell adhesion molecule (EpCAM) and CD3 antigen (Seimetz *et al.*, 2010; Mabuchi *et al.*, 2010). Their efficacy and side effects have been demonstrated in clinical trials but still, further research is needed to create more efficient, precise and less toxic immunotherapy strategies for ovarian carcinoma (Mabuchi *et al.*, 2010). Combined therapies using mAbs with NK cells are believed to benefit from antibody-dependent cell-mediated cytotoxicity (ADCC), since NK cells express CD16 (FcγRIIIA) receptor that recognizes the Fc portion of mAbs (McCall *et al.*, 2001; Borghaei *et al.*, 2009).

5. Conclusion

Due to an increased knowledge of the molecular processes and receptor-ligand interactions of NK cells, studies have been conducted using these lymphocytes for the development of cancer treatments. Currently, clinical trials with sufficient amount of NK cells can be conducted, due to improvements on the isolation and expansion methods. In overall, autologous and allogeneic NK cells, the KIR/HLA mismatch, DNAM-1/PVR interaction and monoclonal antibodies, currently are the most promising factors for NK-based immunotherapy against ovarian cancer.

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