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COMMENTARY

Targeting the tumor microenvironment to improve natural killer cell-based immunotherapies: On being in the right place at the right time, with resilience

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ABSTRACT

Natural killer (NK) cell immunotherapies that target solid tumors require NK cells in the proper place, at the right time, with optimal function and a susceptible target cell. Basic research and clinical correlative studies have provided evidence, for certain malignancies, that intratumoral NK cells delay tumor progression. Whether NK cells exert anti-tumor effects for solid tumors is determined by a number of factors including homing and activating receptor expression by NK cells themselves and the sensitivity of tumor cells to be targets of NK cell cytolysis, which depends on the chemokine and NK cell-inhibitory and activating receptor ligand expression by tumor cells. Chemotherapeutic agents that increase NK cell-activating receptor ligands on tumor cells have been clinically promising as well as ectopic gene expression in NK cells with factors that overcome the suppressive mechanisms of the tumor microenvironment (TME). Identifying agents that decrease myeloid-derived suppressor cells (MDSC) or T regulatory (Treg) cell frequencies or function would be important to co-administer with adoptively transferred NK cells to ameliorate immunosuppressive TMEs. Thus, studies indicate that critical factors for NK cell immunotherapies targeting the TMEs are: being in the right place at the right time, with resilience.

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Introduction

The immune system plays a crucial role to prevent local growth and dissemination of cancer. Immunotherapeutic approaches based on activating T cells, i.e. tumor-infiltrating lymphocyte (TIL) therapy, or blockade of immune checkpoints such as by use of anti-PD-1 or PDL1 antibodies have been shown to result in beneficial clinical responses in patients with advanced cancer (reviewed in [1,2]). Although these therapies are promising, many patients fail to achieve long-lasting clinical responses. Analysis of progressing tumor lesions in patients that fail to respond to T cell therapy often reveal reduced expression of MHC class I,[3-7] and such tumors are no longer susceptible to lysis by cytotoxic T cells. Instead, such tumor cells become sensitive to killing by Natural Killer (NK) cells.

NK cells were first identified due to their potent cytolysis of tumor cell targets.[8,9] They recognize their cellular targets by a combination of signals from inhibitory and activating receptors (reviewed in [10]). Thus, it is perturbations in the ligands for these NK cell receptors on target cells can result in cytolysis by NK cells. For example, loss of MHC class I, a canonical NK cell inhibitory receptor (KIR) ligand, from a cell's surface,[11] or an increase in expression of stress-induced molecules such as MICA/B,[12] a canonical killer cell activating receptor (KAR) ligand, can result in NK cell-mediated lysis of a cellular target. Later work established that NK cells are innate lymphocytes with some qualities of adaptive cells in that they have long-lived memory and are 'educated' and 'licensed' (i.e., tolerized) during development by the presence of NK cell receptor ligands in the natural host ([13]; reviewed in [14]). As NK cells do not depend on the recognition of specific antigens in the context of MHC class I as do cytotoxic T cells, one benefit of NK cell immunotherapy is that tumor antigens do not require specific *de novo* identification, however, specific cancers types need to be evaluated for alterations in NK cell ligands on tumor cells.

While NK cell immunotherapies have been used successfully for certain hematologic malignancies such as acute myeloid leukemia (AML), this modality of immunotherapy has not been used extensively for solid tumors. In many cases, for hematologic malignancies, allogeneic NK cells, either directly infused, or expanded *ex vivo* with IL-2 or IL-15 prior to infusion, have been used (reviewed in [15]). Providing one of the first clinical studies, in 2005, Miller et al. reported that infusion of haploidentical NK cells resulted in complete hematologic remission in 26% of poor prognosis acute myeloid leukemia (AML) patients.[16] However, the few clinical trials that have been performed with infusion of autologous NK cells in patients with solid tumors have reported varying clinical responses. After transfer of autologous *ex vivo* expanded NK cells, we found that 50% (7/14) of patients had stable disease including 2 patients who had more than a 30% decline in serum tumor markers and one patient with metastatic kidney cancer had a minor response. [17] Although the autologous NK cell therapy was well-tolerated and provided clinical evidence for NK cell mediated anti-tumor immunity in patients with solid tumors and advanced cancer, further improvement of this therapy is needed.

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In addition to infusion of autologous NK cells for immunotherapy, other approaches have augmented NK cells before infusion by genetic modification of cellular receptors or expression of homing receptors that are known to be important for specific types of malignancies. There has been much attention drawn to identifying methods for ectopic gene expression in NK cells without using viral vectors in order to eliminate potential safety concerns. One such example is the development of an NK expansion protocol that includes feeder cells transduced to express the CCR7 lymphoid-homing chemokine receptor.[18] It was found that the co-cultured NK cells expressed CCR7 via a process known as trogocytosis (transference of surface membrane molecules from target cells to effector cells such as NK cells or T cells). After adoptive transfer, these CCR7-expressing NK cells homed more efficiently to tumors.[18]

Genetic modifications of NK cells have included the expression of chimeric antigen receptors (CAR) that express a single chain antibody fragment recognizing a tumor-associated molecule with an intracellular activating domain, which, when engaged with the ligand on a target cell, leads to targeted NK-cell cytolytic activity. The most notable example of this has been the use of an anti-CD19 antibody fragment used for multiple myeloma or B cell lymphomas,[19] and in fact, mRNA electroporation was used for CAR ectopic gene expression in NK cells. Trogocytosis has also been used to mediate CAR expression in expanded NK cells.[20] Other therapeutics to augment NK anti-tumor activity are bi-specific antibodies with one chain specific to the NK cell CD16 Fc receptor and another arm of the antibody, directed against a tumor molecule.[21] In this way, the antibody-dependent cytotoxicity (ADCC) function of NK cells is harnessed and directed to tumor cells. Taken together, a variety of methods are being used to both increase the cytoxicity and effector function, as well as homing potential, of NK cells for immunotherapies, with special attention to innovative means of ectopic antigen expression for clinical efficacy and safety. Another approach being taken is to increase NK cell-mediated killing by sensitizing tumors using chemotherapeutic agents.

Rate-limiting factors for successful NK cell-based immunotherapy in patients with solid tumors

Of course, a rate-limiting factor for successful clinical responses is that the infused NK cells must display optimal tumor killing potential. We as well as others have shown have shown in several studies that various chemotherapy agents are able to sensitize tumors to NK cell-mediated killing *in vitro* and *in vivo*. Of note, *in vitro* exposure of tumor cells to chemotherapy agents such as proteasome inhibitors or doxorubicin increases expression of the death receptor, tumor necrosis factor–related apoptosis-inducing ligand receptor 2 (TRAIL-R2), sensitizing tumor cells to NK cell-mediated apoptosis ([22-24]; unpublished observations). Numerous studies have indicated that TRAIL signaling represents an important pathway that improves the ability of tumors to selectively serve as NK cell targets.

Novel chemotherapeutic agents are being examined for tumor sensitization to NK cell effector functions. For example, we have observed enhanced NK cell lysis of tumors using zoledronic acid (ZA), an osteoclast inhibitor that is used to prevent bone complications in patients with bone metastases. Treatment with ZA results in upregulation of TRAIL expression on NK cells resulting in significant increased anti-tumor activity by NK cells *in vitro* and in tumor-bearing mice.[25] Thus, combining standard chemotherapy with NK cell therapy represents a viable strategy in patients with solid tumors.

Homing receptor expression to increase NK cell intratumoral infiltration

NK cell infiltration within tumors of multiple cancers types is associated with improved prognosis.[26-28] For example, multivariate analyses have shown that a low number of tumor-infiltrating NK cells is an independent factor of poor prognosis whereas high levels of T cell infiltration is associated with a poor prognosis in patients with renal cell carcinoma (RCC), the most common form of kidney cancer.[28,29] Thus, RCC could be a prime candidate for NK cell immunotherapy. However, in order to fully harness the potential of adoptive infusion of NK cells in patients with solid tumors, infused cells need to first migrate toward the solid tumor. The ability of NK cells to infiltrate tumor microenvironments depends on the chemokine receptors they express as well as the chemokines that are secreted by the tumor cells themselves.

Chemokine (C-X-C Motif) Receptor 3 (CXCR3) mediates leukocyte adhesion and trafficking, and its expression by NK cells has been implicated in efficient NK cell homing to tumor sites. Its ligands include chemokine C-X-C motif chemokine (CXCL)-9, -10 and -11. We recently showed that production of the tumor-derived inflammatory CXCL10 was rate-limiting in attracting infused activated CXCR3-positive NK cells.[30] Similarly, Wendel et al. showed that local IFN-γ treatment induces intratumoral infiltration of CXCR3-positive murine NK cells, resulting in delayed tumor progression in immunocompetent mice.[31] Others showed that tumors producing CX3CL1, or fractalkine, had increased NK cell infiltration and decreased tumor growth that depended on cognate receptor expression (CX3CR1) of the NK cells.[32]

Human anaplastic thyroid cancer (ATC) is a solid malignancy highly resistant to chemotherapeutic agents and no curative therapies exist. Furthermore, no immunogenic determinants for T or B cell responses have been identified, thus it is a potential candidate for NK cell immunotherapy. When we investigated chemokine and chemokine receptor expression, we discovered that CXCR3-positive NK cells were enriched in ATC tumors compared to peripheral blood.[33] When we examined the tumors, we also found a secretion of CXCL10. Thus, testing for the secretion of chemokines by specific tumor types as well as the chemokine receptors expression by NK cells for use in immunotherapies is important, in conjunction with *in vitro* cell migration studies to test for the ability of lymphocytes to home to tumors.

We showed that the ATC tumors infiltrated by primarily CXCR3-positive NK cells are very sensitive to killing by activated NKG2D-positive NK cells (NKG2D being a killer-activating receptor of NK cells for stress-induced ligands on target cells). However, despite displaying these features, ATC xenografts are not targeted by infused NK cells.[33] The lack of anti-tumor responses was due to the fact that ATC cells produce

high levels of prostaglandin E2 (PGE2) and suppressed the activity of the infused NK cells. Similar to patients with ATC, we have observed that CXCR3-positive NK cells preferentially accumulate in tumor tissue rather than in peripheral blood in patients with RCC, but are functionally suppressed (unpublished data). In a number of cancers it has been shown that NK cells isolated from tumors compared to the blood are suppressed and cannot recognize and kill tumor targets.[34,35] Thus, a major challenge to designing immunotherapies based on NK cell infusion for solid tumors is not only tumor-homing capabilities, but that one must overcome the tumor microenvironment, which exhibits, in many cancers, immunosuppressive properties.

Targeting the immunosuppressive tumor microenvironment

The tumor microenvironment exhibits accumulation of immunosuppressive cell types including myeloid-derived suppressor cells (MDSC), and T regulatory (Treg) cells and tumor-associated macrophages (TAM) as well as others (reviewed in[36]). These cell types suppress anti-tumor NK cell responses and limit their ability to kill tumor targets. A high tumor infiltration of immunosuppressive cell populations is known to correlate with poor prognosis in several cancers.[37-40] These cells as well as tumor cells themselves can produce an abundance of immunosuppressive factors including TGF-β (TGFβ), IL-10, arginase-1, nitric oxide, indoleamine 2,3-dioxygenase (IDO), PGE2, and reactive-oxygen species (ROS) that directly inhibit NK cell responses.

MDSCs are cellular immune modulators that tumors employ to suppress anti-tumor immune responses.[41] We as well as others have shown that MDSCs appear in higher frequencies in patients with cancer compared with healthy individuals, and inhibit the effect of immunotherapy.[42-44] When we examined melanoma patients we found a preponderance of MDSC, which could be recapitulated using an *ex vivo* model using patient-derived melanoma cell lines.[42,45] Co-culture with the tumor cells resulted in the conversion of monocytes into MDSC. These MDSC-like cells generated *in vitro* resemble patient-derived MDSCs in their ability to suppress NK cell responses through the production of PGE2, TGF-β, and ROS.[42,45] It has also been shown that treatment with Ipilimumab (anti-CTLA4) decreases MDSC frequencies in melanoma-affected individuals.[46] Therefore, treatments such as these may provide useful in conjunction with NK cell immunotherapies.

Due to their essential roles during cancer progression and ability to reduce the effects of immunotherapy, MDSCs and Tregs are attractive therapeutic targets in patients with cancer.

Tregs suppress NK cell activity by producing high amounts of TGF-β,[47] or by acting as a sink for IL-2.[48] We also found that in both a murine RCC model *in vivo* and a human RCC system *in vitro*, Tregs suppressed NK cell activity via the production of TGF-β.[49] Therefore, another approach to overcome the immunosuppressive mechanisms of the TME has been to engineer NK cells to silence, or express dominant negative (DN), TGF-β receptors [50] in order to decrease NK cell responsiveness to TGF-β. Also, as the TME is hypoxic and known to affect lymphocyte activity, expressing anti-oxidants in adoptively transferred NK cells, is another potential strategy to overcome oxidative stress. Various drugs, including fluorouracil (5-FU), cyclophosphamide, doxorubicin, celecoxib, and sunitinib have shown effective targeting of MDSCs or Tregs. However, these agents act broadly and can also negatively impact the immune system. Thus, novel and more potent compounds that could specifically eliminate MDSCs and Tregs in the TME are needed.

Conclusions

It would be beneficial, to inform the design of NK cell immunotherapies that target solid tumors, to examine tumors of specific cancers for NK cell accumulation and tumor cells themselves for NK cell receptor ligand expression. This could be useful in determining the best type of modifications or chemotherapeutic agents to use to augment adoptive NK cell therapy. TME are highly immunosuppressive and affect NK cell function. Therapies that engineer NK cells to express factors that counter the immunosuppressive TME, or treatments with agents that decrease immunosuppressive cell types in the TME, are promising. Evidence indicate that NK cell homing to tumors is important for NK-cell mediated protection from certain cancers and thus, it is important to boost the homing potential of adoptively transferred NK cells. Using *in vitro* models that recapitulate tumor microenvironments such as in vitro NK cell – tumor migration assays and MDSC co-culture models provide useful research tools to examine NK cell – tumor interactions. Chemotherapeutic agents that sensitize tumors as targets of NK cell cytolytic function, and those that decrease immunosuppressive cell types in the TME, have been used clinically, although more specific, targeted drugs are needed. Taken together, there have been significant advances in NK cell immunotherapies targeting solid tumors that take into account NK cell homing, resilience in the TME, and clinical safety and efficacy, yet there still is a need for a deeper understanding of the NK cell parameters that (1) limit tumor progression for different malignancies, and (2) overcome the immunosuppressive aspects of the TME.

Disclosure of potential conflicts of interest

There are no conflicts of interest for S. Murray or A. Lundqvist.

References

[1] Rosenberg SA, Restifo NP, Yang JC, Morgan RA, Dudley ME. Adoptive cell transfer: a clinical path to effective cancer immunotherapy. Nat Rev Cancer 2008; 8:299-308; PMID:18354418; http://dx.doi.org/10.1038/nrc2355

[2] Callahan MK, Wolchok JD. At the bedside: CTLA-4- and PD-1-blocking antibodies in cancer immunotherapy. J Leukoc Biol 2013; 94:41-53; PMID:23667165; http://dx.doi.org/10.1189/jlb.1212631

[3] Leone P, Shin EC, Perosa F, Vacca A, Dammacco F, Racanelli V. MHC class I antigen processing and presenting machinery: organization, function, and defects in tumor cells. J Natl Cancer Inst 2013; 105:1172-87; PMID:23852952; http://dx.doi.org/10.1093/jnci/djt184

[4] Seliger B, Wollscheid U, Momburg F, Blankenstein T, Huber C. Coordinate downregulation of multiple MHC class I antigen processing genes in chemical-induced murine tumor cell lines of distinct

origin. Tissue antigens 2000; 56:327-36; PMID:11098932; http://dx.doi.org/10.1034/j.1399-0039.2000.560404.x

[5] Atkins D, Ferrone S, Schmahl GE, Storkel S, Seliger B. Down-regulation of HLA class I antigen processing molecules: an immune escape mechanism of renal cell carcinoma? J Urol 2004; 171:885-9; PMID:14713847; http://dx.doi.org/10.1097/01.ju.0000094807.95420.fe

[6] Seliger B, Hohne A, Knuth A, Bernhard H, Ehring B, Tampe R, Huber C. Reduced membrane major histocompatibility complex class I density and stability in a subset of human renal cell carcinomas with low TAP and LMP expression. Clin Cancer Res 1996; 2:1427-33; PMID:9816317

[7] Hicklin DJ, Wang Z, Arienti F, Rivoltini L, Parmiani G, Ferrone S. beta2-Microglobulin mutations, HLA class I antigen loss, and tumor progression in melanoma. J Clin Investig 1998; 101:2720-9; PMID:9637706; http://dx.doi.org/10.1172/JCI498

[8] Kiessling R, Klein E, Wigzell H. "Natural" killer cells in the mouse. I. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Specificity and distribution according to genotype. Eur J Immunol 1975; 5:112-7; PMID:1234049; http://dx.doi.org/10.1002/eji.1830050208

[9] Kiessling R, Klein E, Pross H, Wigzell H. "Natural" killer cells in the mouse. II. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Characteristics of the killer cell. Eur J Immunol 1975; 5:117-21; PMID:1086218; http://dx.doi.org/10.1002/eji.1830050209

[10] Long EO, Kim HS, Liu D, Peterson ME, Rajagopalan S. Controlling natural killer cell responses: integration of signals for activation and inhibition. Ann Rev Immunol 2013; 31:227-58; PMID:23516982; http://dx.doi.org/10.1146/annurev-immunol-020711-075005

[11] Karre K. Natural killer cell recognition of missing self. Nat Immunol 2008; 9:477-80; PMID:18425103; http://dx.doi.org/10.1038/ni0508-477

[12] Bauer S, Groh V, Wu J, Steinle A, Phillips JH, Lanier LL, Spies T. Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. Science 1999; 285:727-9; PMID:10426993; http://dx.doi.org/10.1126/science.285.5428.727

[13] Kim S, Poursine-Laurent J, Truscott SM, Lybarger L, Song YJ, Yang L, French AR, Sunwoo JB, Lemieux S, Hansen TH, et al. Licensing of natural killer cells by host major histocompatibility complex class I molecules. Nature 2005; 436:709-13; PMID:16079848; http://dx.doi.org/10.1038/nature03847

[14] Elliott JM, Yokoyama WM. Unifying concepts of MHC-dependent natural killer cell education. Trends Immunol 2011; 32:364-72; PMID:21752715; http://dx.doi.org/10.1016/j.it.2011.06.001

[15] Childs RW, Carlsten M. Therapeutic approaches to enhance natural killer cell cytotoxicity against cancer: the force awakens. Nat Rev Drug Dis 2015; 14:487-98; PMID:26000725; http://dx.doi.org/10.1038/nrd4506

[16] Miller JS, Soignier Y, Panoskaltsis-Mortari A, McNearney SA, Yun GH, Fautsch SK, McKenna D, Le C, Defor TE, Burns LJ, et al. Successful adoptive transfer and in vivo expansion of human haploidentical NK cells in patients with cancer. Blood 2005; 105:3051-7; PMID:15632206; http://dx.doi.org/10.1182/blood-2004-07-2974

[17] Lundqvist A, Berg M, Smith A, Childs RW. Bortezomib Treatment to Potentiate the Anti-tumor Immunity of Ex-vivo Expanded Adoptively Infused Autologous Natural Killer Cells. J Cancer 2011; 2:383-5; PMID:21750690; http://dx.doi.org/10.7150/jca.2.383

[18] Somanchi SS, Somanchi A, Cooper LJ, Lee DA. Engineering lymph node homing of ex vivo-expanded human natural killer cells via trogocytosis of the chemokine receptor CCR7. Blood 2012; 119:5164-72; PMID:22498742; http://dx.doi.org/10.1182/blood-2011-11-389924

[19] Shimasaki N, Fujisaki H, Cho D, Masselli M, Lockey T, Eldridge P, Leung W, Campana D. A clinically adaptable method to enhance the cytotoxicity of natural killer cells against B-cell malignancies. Cytotherapy 2012; 14:830-40; PMID:22458956; http://dx.doi.org/10.3109/14653249.2012.671519

[20] Cho FN, Chang TH, Shu CW, Ko MC, Liao SK, Wu KH, Yu MS, Lin SJ, Hong YC, Chen CH, et al. Enhanced cytotoxicity of natural killer cells following the acquisition of chimeric antigen receptors through trogocytosis. PloS one 2014; 9:e109352.

[21] Gleason MK, Verneris MR, Todhunter DA, Zhang B, McCullar V, Zhou SX, Panoskaltsis-Mortari A, Weiner LM, Vallera DA, Miller JS. Bispecific and trispecific killer cell engagers directly activate human NK cells through CD16 signaling and induce cytotoxicity and cytokine production. Mol Cancer ther 2012; 11:2674-84; PMID:23075808; http://dx.doi.org/10.1158/1535-7163.MCT-12-0692

[22] Lundqvist A, Abrams SI, Schrump DS, Alvarez G, Suffredini D, Berg M, Childs R. Bortezomib and depsipeptide sensitize tumors to tumor necrosis factor-related apoptosis-inducing ligand: a novel method to potentiate natural killer cell tumor cytotoxicity. Cancer Res 2006; 66:7317-25; PMID:16849582; http://dx.doi.org/10.1158/0008-5472.CAN-06-0680

[23] Sarhan D, Wennerberg E, D'Arcy P, Gurajada D, Linder S, Lundqvist A. A novel inhibitor of proteasome deubiquitinating activity renders tumor cells sensitive to TRAIL-mediated apoptosis by natural killer cells and T cells. Cancer Immunol Immunother 2013; 62:1359-68; PMID:23689729; http://dx.doi.org/10.1007/s00262-013-1439-1

[24] Wennerberg E, Sarhan D, Carlsten M, Kaminskyy VO, D'Arcy P, Zhivotovsky B, Childs R, Lundqvist A. Doxorubicin sensitizes human tumor cells to NK cell- and T-cell-mediated killing by augmented TRAIL receptor signaling. Int J Cancer J Int du Cancer 2013; 133:1643-52; PMID:23504627; http://dx.doi.org/10.1002/ijc.28163

[25] Sarhan D, D'Arcy P, Wennerberg E, Liden M, Hu J, Winqvist O, Rolny C, Lundqvist A. Activated monocytes augment TRAIL-mediated cytotoxicity by human NK cells through release of IFN-gamma. Eur J Immunol 2012; PMID:22996291

[26] Takanami I, Takeuchi K, Giga M. The prognostic value of natural killer cell infiltration in resected pulmonary adenocarcinoma. The J Thorac Cardiovasc Surg 2001; 121:1058-63; PMID:11385371; http://dx.doi.org/10.1067/mtc.2001.113026

[27] Ishigami S, Natsugoe S, Tokuda K, Nakajo A, Che X, Iwashige H, Aridome K, Hokita S, Aikou T. Prognostic value of intratumoral natural killer cells in gastric carcinoma. Cancer 2000; 88:577-83; PMID:10649250; http://dx.doi.org/10.1002/(SICI)1097-0142(20000201)88:3%3c577::AID-CNCR13%3e3.0.CO;2-V

[28] Donskov F, von der Maase H. Impact of immune parameters on long-term survival in metastatic renal cell carcinoma. J Clin Oncol 2006; 24:1997-2005; PMID:16648500; http://dx.doi.org/10.1200/JCO.2005.03.9594

[29] Jochems C, Schlom J. Tumor-infiltrating immune cells and prognosis: the potential link between conventional cancer therapy and immunity. Exp Biol Med 2011; 236:567-79; PMID:21486861; http://dx.doi.org/10.1258/ebm.2011.011007

[30] Wennerberg E, Kremer V, Childs R, Lundqvist A. CXCL10-induced migration of adoptively transferred human natural killer cells toward solid tumors causes regression of tumor growth in vivo. Cancer Immunol Immunother 2015; 64:225-35; PMID:25344904; http://dx.doi.org/10.1007/s00262-014-1629-5

[31] Wendel M, Galani IE, Suri-Payer E, Cerwenka A. Natural killer cell accumulation in tumors is dependent on IFN-gamma and CXCR3 ligands. Cancer Res 2008; 68:8437-45; PMID:18922917; http://dx.doi.org/10.1158/0008-5472.CAN-08-1440

[32] Lavergne E, Combadiere B, Bonduelle O, Iga M, Gao JL, Maho M, Boissonnas A, Murphy PM, Debre P, Combadiere C. Fractalkine mediates natural killer-dependent antitumor responses in vivo. Cancer Res 2003; 63:7468-74; PMID:14612547

[33] Wennerberg E, Pfefferle A, Ekblad L, Yoshimoto Y, Kremer V, Kaminskyy VO, Juhlin CC, Hoog A, Bodin I, Svjatoha V, et al. Human anaplastic thyroid carcinoma cells are sensitive to NK cell-mediated lysis via ULBP2/5/6 and chemoattract NK cells. Clin Cancer Res 2014; 20:5733-44; PMID:25212604; http://dx.doi.org/10.1158/1078-0432.CCR-14-0291

[34] Platonova S, Cherfils-Vicini J, Damotte D, Crozet L, Vieillard V, Validire P, Andre P, Dieu-Nosjean MC, Alifano M, Regnard JF, et al. Profound coordinated alterations of intratumoral NK cell phenotype and function in lung carcinoma. Cancer Res 2011; 71:5412-22; PMID:21708957; http://dx.doi.org/10.1158/0008-5472.CAN-10-4179

[35] Levy EM, Roberti MP, Mordoh J. Natural killer cells in human cancer: from biological functions to clinical applications. J Biomed Biotechnol 2011; 2011:676198; PMID:21541191; http://dx.doi.org/10.1155/2011/676198

[36] Gajewski TF, Schreiber H, Fu YX. Innate and adaptive immune cells in the tumor microenvironment. Nat Immunol 2013; 14:1014-22; PMID:24048123; http://dx.doi.org/10.1038/ni.2703

[37] Diaz-Montero CM, Salem ML, Nishimura MI, Garrett-Mayer E, Cole DJ, Montero AJ. Increased circulating myeloid-derived suppressor cells correlate with clinical cancer stage, metastatic tumor burden, and doxorubicin-cyclophosphamide chemotherapy. Cancer Immunol Immunother 2009; 58:49-59; PMID:18446337; http://dx.doi.org/10.1007/s00262-008-0523-4

[38] Kono K, Kawaida H, Takahashi A, Sugai H, Mimura K, Miyagawa N, Omata H, Fujii H. CD4(+)CD25high regulatory T cells increase with tumor stage in patients with gastric and esophageal cancers. Cancer Immunol Immunother 2006; 55:1064-71; PMID:16328385; http://dx.doi.org/10.1007/s00262-005-0092-8

[39] Ichihara F, Kono K, Takahashi A, Kawaida H, Sugai H, Fujii H. Increased populations of regulatory T cells in peripheral blood and tumor-infiltrating lymphocytes in patients with gastric and esophageal cancers. Clin Cancer Res 2003; 9:4404-8; PMID:14555512

[40] Porembka MR, Mitchem JB, Belt BA, Hsieh CS, Lee HM, Herndon J, Gillanders WE, Linehan DC, Goedegebuure P. Pancreatic adenocarcinoma induces bone marrow mobilization of myeloid-derived suppressor cells which promote primary tumor growth. Cancer Immunol Immunother 2012; 61:1373-85; PMID:22215137; http://dx.doi.org/10.1007/s00262-011-1178-0

[41] Poschke I, Kiessling R. On the armament and appearances of human myeloid-derived suppressor cells. Clin Immunol 2012; 144:250-68; PMID:22858650; http://dx.doi.org/10.1016/j.clim.2012.06.003

[42] Mao Y, Poschke I, Wennerberg E, Pico de Coana Y, Egyhazi Brage S, Schultz I, Hansson J, Masucci G, Lundqvist A, Kiessling R. Melanoma-Educated CD14+ Cells Acquire a Myeloid-Derived Suppressor Cell Phenotype through COX-2-Dependent Mechanisms. Cancer Res 2013; 73:3877-87; PMID:23633486; http://dx.doi.org/10.1158/0008-5472.CAN-12-4115

[43] Poschke I, Mougiakakos D, Hansson J, Masucci GV, Kiessling R. Immature immunosuppressive CD14+HLA-DR-/low cells in melanoma patients are Stat3hi and overexpress CD80, CD83, and DC-sign. Cancer Res 2010; 70:4335-45; PMID:20484028; http://dx.doi.org/10.1158/0008-5472.CAN-09-3767

[44] Kitano S, Postow MA, Ziegler CG, Kuk D, Panageas KS, Cortez C, Rasalan T, Adamow M, Yuan J, Wong P, et al. Computational algorithm-driven evaluation of monocytic myeloid-derived suppressor cell frequency for prediction of clinical outcomes. Cancer Immunol Res 2014; 2:812-21; PMID:24844912; http://dx.doi.org/10.1158/2326-6066.CIR-14-0013

[45] Mao Y, Sarhan D, Steven A, Seliger B, Kiessling R, Lundqvist A. Inhibition of tumor-derived prostaglandin-e2 blocks the induction of myeloid-derived suppressor cells and recovers natural killer cell activity. Clin Cancer Res 2014; 20:4096-106; PMID:24907113; http://dx.doi.org/10.1158/1078-0432.CCR-14-0635

[46] Pico de Coana Y, Poschke I, Gentilcore G, Mao Y, Nystrom M, Hansson J, Masucci GV, Kiessling R. Ipilimumab treatment results in an early decrease in the frequency of circulating granulocytic myeloid-derived suppressor cells as well as their Arginase1 production. Cancer Immunol Res 2013; 1:158-62; PMID:24777678; http://dx.doi.org/10.1158/2326-6066.CIR-13-0016

[47] Ghiringhelli F, Menard C, Terme M, Flament C, Taieb J, Chaput N, Puig PE, Novault S, Escudier B, Vivier E, et al. CD4+CD25+ regulatory T cells inhibit natural killer cell functions in a transforming growth factor-beta-dependent manner. J Exp Med 2005; 202:1075-85; PMID:16230475; http://dx.doi.org/10.1084/jem.20051511

[48] Gasteiger G, Hemmers S, Firth MA, Le Floc'h A, Huse M, Sun JC, Rudensky AY. IL-2-dependent tuning of NK cell sensitivity for target cells is controlled by regulatory T cells. J Exp Med 2013; 210:1167-78; PMID:23650441; http://dx.doi.org/10.1084/jem.20122462

[49] Lundqvist A, Yokoyama H, Smith A, Berg M, Childs R. Bortezomib treatment and regulatory T-cell depletion enhance the antitumor effects of adoptively infused NK cells. Blood 2009; 113:6120-7; PMID:19202127; http://dx.doi.org/10.1182/blood-2008-11-190421

[50] Yang B, Liu H, Shi W, Wang Z, Sun S, Zhang G, Hu Y, Liu T, Jiao S. Blocking transforming growth factor-beta signaling pathway augments antitumor effect of adoptive NK-92 cell therapy. Int Immunopharmacol 2013; 17:198-204; PMID:23806302; http://dx.doi.org/10.1016/j.intimp.2013.06.003