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REVIEW

The role of NKT cells in gastrointestinal cancers

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ABSTRACT

Gastrointestinal (GI) cancers represent a complex array of cancers that affect the digestive system. This includes liver, pancreatic, colon, rectal, anal, gastric, esophageal, intestinal and gallbladder cancer. Patients diagnosed with certain GI cancers typically have low survival rates, so new therapeutic approaches are needed. A potential approach is to harness the potent immunoregulatory properties of natural killer T (NKT) cells which are true T cells, not natural killer (NK) cells, that recognize lipid instead of peptide antigens presented by the non-classical major histocompatibility (MHC) molecule CD1d. The NKT cell subpopulation is known to play a vital role in tumor immunity by bridging innate and adaptive immune responses. In GI cancers, NKT cells can contribute to either antitumor or protumor immunity depending on the cytokine profile expressed and type of cancer. This review discusses the complexities of the role of NKT cells in liver, colon, pancreatic and gastric cancers with an emphasis on type I NKT cells.

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Introduction

Natural killer T (NKT) cells are a specialized small population of T cells that play a critical role in connecting the innate and adaptive immune systems.[1–3] They contain various pre-formed mRNAs for cytokines such as IFN-γ, IL-4, -10 and -13 that are rapidly translated upon antigen stimulation and subsequently provide immediate immune protection and activation of downstream adaptive immune responses.[4–6] The name natural killer T cell originated from the discovery of a subpopulation of $CD3^+$ T cells that express the NK cell marker, NK1.1.[7,8] Defining NKT cells based on T cells that express NK1.1 was problematic because not all NKT cells express NK1.1 and many activated T cells express NK1.1 as an activation marker.[9] NKT cells differ from conventional T cells in that their receptors recognize lipids presented by the non-classical MHC I-like molecule, CD1d.[3,9,10] Like class I HLA-A, -B and -C molecules, CD1d is expressed in most nucleated cells,[11–13] but it is often suppressed in tumors that have escaped killing by NKT cells.[14–16] Currently, NKT cells are defined as any TCR^+ cell that recognizes lipids presented by CD1d. An important discovery was that NKT cells (now called invariant NKT or iNKT or type I NKT cells; see below) possess a semi-invariant T cell receptor alpha (TCRα) chain, defined as Vα14-Jα18 in mice and Vα24-Jα18 in humans paired with a limited number of Vβ chains, Vβ8.2, 7, and 2 in mice and Vβ11 in humans.[17–20] Furthermore, α-galactosylceramide (α-GalCer), the prototypical glycolipid derived from a marine sponge or possibly a *Sphingomonas bacterium* symbiotic to the sponge, is presented by CD1d and recognized by NKT cells expressing the semi-invariant TCR.[3,6,9] Compared to a subset of conventional T cells specific for any single antigen, the population of NKT cells that recognize α-GalCer is quite large with representation being 1–2% of mouse spleen cells, 15–30% of liver cells, and up to 40% of $CD3^+$ T cells in the bone marrow.[1,21]

The discovery of NKT cells was further complicated by the studies that demonstrated the existence of a T cell population that does not possess a semi-invariant TCR and is able to recognize other lipids, but not the prototypical NKT cell agonist α-GalCer, presented by CD1d.[1,9] As a result, the NKT cells that utilize the semi-invariant TCR were named invariant NKT (iNKT) cells or type I NKT cells, and the others that use different TCRs were called non-invariant NKT cells or type II NKT cells. The heterogeneous TCR repertoire of type II NKT cells enables them to respond to a diverse range of lipid antigens in a similar fashion as conventional T cells' ability to recognize a large repertoire of peptide antigens. This is in contrast to type I NKT cells, which can recognize only a limited number of lipid antigens due to the utilization of a semi-invariant TCR. The discovery that type II NKT cells recognize sulfatide from the myelin sheaths of the central nervous system, and the subsequent development of sulfatide-loaded-CD1d tetramer, allowed the detection and characterization of this subset of type II NKT cells.[22] However, due to the diversity of type II NKT cell TCRs, it is highly unlikely that sulfatide-loaded-tetramer is capable of detecting all the subsets of type II NKT cells. The identification of additional type II NKT cell antigens is desperately needed to develop new tetramers to detect more type II NKT cell subsets. This would provide the capabilities to perform studies to further advance our knowledge about the type II NKT cell subpopulation and their function in the immune system. This review will focus on the role of NKT cells in GI cancers.

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Type I NKT cells in tumor immunity

The ability of NKT cells to function as a bridge between the innate and adaptive immune system plays a critical role in tumor immunity. The anti-tumor functions of type I NKT cells primarily depend on their ability to secrete the Th1 cytokine IFN-γ and TNF-α.[23,24] This is in contrast to the role of type I NKT cells in protection against autoimmunity, which depends on Th2 cytokines IL-4 and IL-13.[23–25] The role of type I NKT cells in tumor immunity was initially discovered when it was shown that α-GalCer has anti-tumor activity and could potently activate type I NKT cells and induce IFN-γ production.[26] NKT cells also have the ability to secrete lytic granules and directly lyse CD1d-positive tumors.[27] However, many tumors have suppressed CD1d expression by the time they are clinically detectable.[14–16] Perhaps as an escape mechanism from NKT cell-mediated tumor immunosurveillance. In addition, other studies have revealed a mechanism that involves NKT cell-mediated killing *in vivo* through Fas–FasL interaction.[28]

The studies using Jα18 KO mice, which lack type I NKT cells, further supported the importance of type I NKT cells in tumor immunity. In the absence of α-GalCer stimulation, the protection against murine tumors mediated by low-dose IL-12 treatment was dependent on type I NKT cells, as this protection was lost in Jα18 KO mice.[29–31] The immunosurveillance against spontaneous methylcholanthrene-induced sarcomas or in transgenic mice expressing the HER2 oncogene was deficient in Jα18 KO mice and demonstrates the importance of type I NKT cells in rejecting tumors.[32] This study also showed that the tumor immunosurveillance and protection elicited by exogenous IL-12 were dependent on type I NKT cells. This discovery was corroborated by the findings that NKT cells can also promote tumor immunity by inducing dendritic cells (DCs) to produce IL-12, which potently stimulates NK cells and IFN-γ production.[33] Furthermore, the adoptive transfer studies revealed that protection in Jα18 KO mice against methylcholanthrene-induced sarcomas and lung metastasis could be restored by the transfer of type I NKT cells from WT mice.[34]

Although NKT cells do express granzymes and perforin and can directly kill tumor cells, the primary mechanism of tumor protection is mediated through the production of IFN-γ and downstream activation of effector NK and $CD8^+$ T cells.[34] In fact, experiments using mice that lack NK, NKT, or T and B cells demonstrated that the sequential production of IFN-γ by NKT cells followed by NK cell production of IFN-γ was necessary for α-GalCer-mediated protection.[35,36]

The activation of type I NKT cells by α-GalCer also induces IL-12 production by DCs and plays a critical role in protection against tumors.[33] The mechanism of IL-12 production by DCs induced by α-GalCer was dependent on the direct interaction between NKT cells and DCs through CD40-CD40L binding.[37] In addition to stimulating IL-12 expression, NKT also has the ability to induce maturation of DCs, subsequently enhancing antigen-presenting capabilities and costimulatory molecule expression, and thus activation of $CD4^+$ and $CD8^+$ T cells.[38] The studies by the Metelitsa lab revealed that human type I NKT cells exert anti-tumor functions in a neuroblastoma model by directly killing $CD1d^+$ tumor-associated macrophages.[39] Interestingly, NKT cells killed up to 90% of myeloid cells pulsed with tumor lysate, which exceeded the levels of cytotoxicity against myeloid cells pulsed with α-GalCer or the lysate from normal PBMCs.[39] The levels of cytotoxicity against myeloid cells pulsed with α-GalCer or the lysate from normal PBMCs were similar. This indicated that type I NKT cells do not have lytic activity against antigen-presenting cells complexed with CD1d and α-GalCer. This contradicts previous studies that showed human type I NKT cell clones have cytotoxicity against monocyte-derived DCs pulsed with α-GalCer.[40] Type I NKT cells also play a role in the reprogramming of pro-tumor M2 macrophages into anti-tumor M1 macrophages.[41] Thus, type I NKT cells can regulate the function of myeloid cells by multiple mechanisms and thus play a critical role in tumor immunity.

The main mechanism of protection provided by type I NKT cells was originally thought to be solely due to the production of IFN-γ. An exception to this paradigm was found when our lab discovered a new class of type I NKT cell agonist called beta-mannosylceramide (β-ManCer).[42] This lipid agonist differs from α-GalCer not only in structure, but also in its mechanism of protection, which is dependent on TNF-α and nitric oxide synthase (NOS) instead of IFN-γ.[43] Although β-ManCer stimulated lower levels of cytokine production than α-GalCer, it did not induce long-term anergy of NKT cells and provided protection against lung metastasis.[42] This was a potentially important discovery and has clinical relevance because β-ManCer can be administered repeatedly, unlike α-GalCer which induces long-term functional anergy. Interestingly, α-GalCer and β-ManCer-loaded tetramers conjugated with different fluorochromes stained the same type I NKT cell population, indicating that they were not acting through different cells.[44] Furthermore, it was shown that combinatorial treatment with suboptimal doses of α-GalCer and β-ManCer can synergize and provide superior protection against tumors compared to treatment with either alone.[43] The unique properties of β-ManCer may warrant its clinical development, especially in cases where α-GalCer is not effective.

Type II NKT cells in tumor immunity

In light of all the evidence of the anti-tumor functions of NKT cells, the field was surprised when it was discovered that NKT cells could also suppress tumor immunity.[25] The first clue was in an immunogenic fibrosarcoma tumor model in which subcutaneous tumors grew, regressed and then recurred but failed to recur in CD1d KO mice devoid of NKT cells.[25] This effect was due to IL-13 production by NKT cells which induced myeloid-derived suppressor cells (MDSC) to produce TGF-β and subsequently suppressing $CD8^+$ T cell-mediated immunosurveillance.[45] A conundrum existed in this pathway because it was dependent on IL-13 and not IL-4, and they both signal through IL-4 Rα.[25] When the full mechanism was elucidated, it was found that signals from the TNF-α and IL-4Rα/STAT6 pathways synergized to upregulate IL-13Rα2, which responds to only IL-13, not IL-4 and subsequently induces the production of TGF-β.[46] Thus, it became clear why IL-4 was not sufficient.

The defining characteristics of type I NKT cells having anti-tumor properties and type II NKT cells suppressing tumor immunity were developed collectively among several labs using a variety of tumor models.[47–50] These studies used a combination of knockout (KO) mouse models, Jα18 KO mice which lack only type I NKT but not type II NKT cells, or CD1d KO mice which lack both type I and type II NKT cells. In tumor challenge experiment, the suppressive activity was maintained in Jα18 KO mice but was lost in CD1d KO mice, indicating the importance of type II NKT cells in suppressing tumor immunity. Furthermore, our lab and others have shown that sulfatide, the prototypical type II NKT cell agonist discovered by Vipin Kumar's lab,[22] promotes tumor growth.[47,49,51]

The relationship between type I and II NKT cells was further complicated by the observation that Jα18 KO mice were more suppressed than WT mice. This led to the finding that type I NKT cells could regulate the function of type II NKT cells, and vice versa.[51] Indeed, when type II NKT cells were stimulated by sulfatide, they decreased the tumor protection induced by α-GalCer-stimulated type I NKT cells.[51] Thus, type I and type II NKT cells form a new regulatory axis in which they cross-regulate each other and the one that dominates can set the tone for subsequent responses by conventional T cells.[51] This metastable balance is somewhat analogous to the axis originally discovered between Th1 and Th2 $CD4^+$ T cells that so profoundly influenced immunology. The cross-regulation between type I and II NKT cells can be extended to other immune cells. For example, type II NKT cells enhance the tumor promoting functions of MDSCs via IL-13, while type I NKT cells suppress the pro-tumor effects of MDSCs by converting them into immunostimulatory APCs.[52] The contradictory effects of type I and II NKT cells in regard to $CD8^+$ T cells in cancer are quite evident. Type I NKT cells can activate $CD8^+$ T cells and enhance their resistance against MDSC, while type II NKT cells suppress the anti-tumor activity of $CD8^+$ T cells.[34,38,45,46,53,54] Because of the relationship between type I and II NKT cells, the balance between the two subsets plays a significant role in tumor immunity.

NKT cells in GI cancers

The outcome for patients diagnosed with GI cancers is generally considered poor, especially for patients with pancreatic and/or liver cancer[55,56] because they are often diagnosed at late stages. The studies of GI cancers have revealed the importance of type I NKT cells in regulating anti-tumor immunity. The role of type I NKT cells in GI cancers is complicated and differs among the different types of cancers. For instance, type I NKT cells function to enhance tumor immunity against gastric and pancreatic cancers[57–60] but suppresses anti-tumor immunity in intestinal cancer,[61] and in liver and colon cancer it is controversial because type I NKT cells can promote or suppress tumor growth[62–68] (see Figure 1). A list of the studies that are directly relevant to the role of type I NKT cells in GI cancers is listed in Table 1.

NKT cells in liver cancer

The studies investigating the role of NKT cells in liver cancer have been controversial and difficult to dissect, because there are studies that show hepatic NKT cells playing both pro-tumorigenic and anti-tumorigenic roles.[63,64,67–69] To add another layer of complexity, there are major differences between human and murine hepatic NKT cells. The percentage of hepatic type I NKT cells in adult mice is large (10–30% of liver lymphocytes) compared to the percentages found in humans which is quite variable and ranges from 0.05 to 1%.[70] Furthermore, the studies by the Derek Doherty lab showed that human hepatic type I NKT cells from normal and tumor-

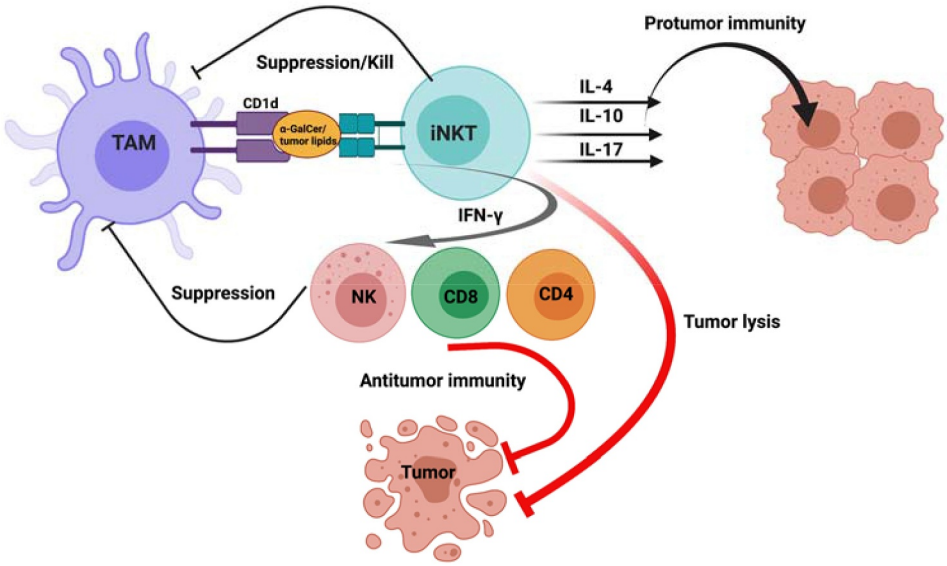

Figure 1. Type I NKT cell antitumor and protumor functions. Initially, tumor-associated macrophages (TAMs) presenting α-GalCer or tumor-derived lipids activate type I NKT cells. In antitumor immunity, activated NKT cells can directly lyse tumor cells and/or secrete TH1 cytokines such as IFN-γ to induce NK, CD4 and CD8 T cell antitumor functions. In contrast, activated NKT cells can secrete TH2 cytokines such as IL-4 and IL-10 and Th17 cytokine IL-17 which contribute to protumor immunity. **Created with BioRender.com**

Table 1. A summary of the studies demonstrating the role of type I NKT cells in GI cancers and method of identification. Selected publication criteria based on keyword search: liver, colon, intestinal, pancreatic, gastric, esophageal, cancer, and NKT cell (No such study in esophageal cancer was found.)

Liver cancer:			
Publication	Year	Role of NKT cells	NKT cell identification
-Kenna *et al.*	2003	Protumor	Tetramers
-Miyagi *et al.*	2003	Antitumor	Tetramers
-Margalit *et al.*	2005	Antitumor	CD3 and DX5 microbeads
-Bricard *et al.*	2009	Antitumor and protumor	Tetramers
-Xiao *et al.*	2013	Antitumor	Vα24 qRT-PCR
-Wolf *et al.*	2014	Protumor	Tetramers
-Ma *et al.*	2018	Antitumor	Tetramers
Colon cancer:			
Publication	**Year**	**Role of NKT cells**	**NKT cell indentification**
- Park *et al.*	2005	Protumor	Indirect; CD1d KO mice
- Tachibana *et al.*	2005	Antitumor	Vα24 IHC
- Ambrosino *et al.*	2007	Antitumor	Jα18 KO mice
- Hattori *et al.*	2007	Antitumor	α-GalCer treatment
- Yoshioka *et al.*	2011	Antitumor	α-GalCer/CD1d protein FC
- Izhak *et al.*	2013	Antitumor	Tetramers
- Tie *et al.*	2017	Antitumor	Tetramers
- Wang *et al.*	2017	Antitumor	Tetramers
- Wang *et al.*	2019	Antitumor and protumor	Tetramers
- Krijgsman *et al.*	2019	Protumor	$CD3^+CD56^+$ FC
- Darcy *et al.*	2020	Protumor	Tetramers
- Wang *et al.*	2020	Antitumor	Tetramers
- Aoyama *et al.*	2021	Antitumor	$CD3^+NK1.1^+$ FC
Pancreatic cancer:			
Publication	**Year**	**Role of NKT cells**	**NKT cell indentification**
- Nagaraj *et al.*	2006	Antitumor	α-GalCer treatment
- Janakiram *et al.*	2017	Antitumor	Vα24, NK1.1/TCRβ IHC
Gastric cancer:			
Publication	**Year**	**Role of NKT cells**	**NKT cell indentification**
-Yanagisawa *et al.*	2002	Antitumor	$CD3^+Vα24^+Vβ11^+$ FC
- Peng *et al.*	2016	Antitumor	$CD3^+CD56^+$ FC
- Xu *et al.*	2018	Antitumor	Vα24Jα18 microbeads
- Melo *et al.*	2020	Antitumor	$CD3^+Vα24^+Jα18^+$ FC

Type of GI cancers; the tumor type studied in the listed publications are all adenocarcinomas.

bearing livers produced IFN-γ but not IL-4 when stimulated with α-GalCer.[70] In contrast, murine hepatic NKT cells stimulated with α-GalCer have the ability to produce both Th1 and Th2 cytokines.[71–73]

In liver cancer, the distinction that stimulated $CD4^+$ type I NKT cells secrete higher levels of Th2 cytokines and have lower cytolytic activity than $CD4^-$ type I NKT cells is evident. In patients with liver cancer, the proportion of tumor infiltrating $CD4^+$ type I NKT cells is increased compared to healthy donor liver.[65] Furthermore, the number of $CD4^-$ type I NKT cells are decreased in liver cancer.[65] This supports other studies that showed $CD4^-$ type I NKT cells are potent producers of IFN-γ and suppress tumor growth.[70,74,75] Interestingly, the combination of intratumoral type I NKT cells and IFN-γ are potential independent prognostic factors, for which the levels are inversely proportional to the risk of recurrence and proportional to survival after curative resection in patients with HCC.[66] The effect of type I NKT cells on hepatic tumor cells is site specific. When mice are treated with α-GalCer, the growth of disseminated BNL 1MEA.7 R.1 (BNL) liver cancer cells in the liver of BALB/c mice were suppressed but the treatment had no effect on the growth of subcutaneous BNL tumors.[69] In the same study, it was shown that hepatic NKT cells were rapidly activated after α-GalCer administration compared to splenic NKT cells. Moreover, in a preclinical setting the adoptive transfer of splenic NKT cells stimulated by DCs presenting HCC-derived antigens resulted in complete resolution of subcutaneous Hep3B hepatic tumors.[76] This suggests that splenic and hepatic NKT cells are functionally different, and the sources of stimulation are critical factors in liver cancer. The studies from the Tim Greten lab have also demonstrated the importance of the microbiome in regulating NKT cell-mediated anti-tumor responses in liver cancer. They demonstrated that primary bile acids upregulated the chemokine CXCL16 in the liver, which is recognized by CXCR6 expressed on NKT cells. Thus, the interaction between CXCL16 and CXCR6 led to the accumulation of liver NKT cells and suppression of liver tumor growth. Microbes that converted primary to secondary bile acids abrogated this activity.[67]

NKT cells in colon cancer

Similar to the role of NKT cells in liver cancer, the studies investigating the role of NKT cells in colon cancer have demonstrated both pro-tumor and anti-tumor roles. For example, in a study with tissues from 103 patients with colorectal cancer (CRC) it was shown that higher $Vα24^+$ NKT cell tumor infiltration in colorectal carcinomas is an independent prognostic factor for favorable prognosis.[62] In contrast, it has been demonstrated that high peripheral blood $CD16^+$ NKT-like ($CD3^+CD56^+$) cells are associated with shorter disease-free

survival of patients with CRC.[77,78] It is important to note that the $CD3^+CD56^+$ cell population can include both NKT and conventional T cells, because activated T cells can also express CD56.[77] Together, these studies would suggest that it is the non-NKT activated $CD3^+$ T cells expressing CD56 and CD16 in PBMCs that correlate with poor prognosis, not the type I NKT cells themselves.

The yin and yang nature of NKT cells in colon cancer are also evident in mouse models. In two models of hypercholesterolemia, the ApoE KO mouse and the C57BL/6 mouse fed a high cholesterol diet, the number of colorectal tumors was increased after treatment with the potent carcinogen, azoxymethane.[79] In this study, hypercholesterolemia inhibited the differentiation of hematopoietic stem cells into terminally differentiated type I NKT cells in the thymus, in the colon submucosa and at the early stages of tumorigenesis, subsequently impairing immunosurveillance against colorectal neoplasia.[79] Additionally, in support of the anti-tumor functions of type I NKT cells, a subcutaneous mouse model of colon cancer demonstrated that the anti-tumor effect of whole-body hyperthermia was increased after treatment with α-GalCer.[80] In contrast, recent studies have revealed that IL-10-producing type I NKT cells accumulate in pre-cancerous colon polyps.[64] Moreover, the IL-10 producing NKT cells were identified as a unique subset expressing low levels of the transcription factor Yin Yang 1 (YY1), which is required for the full function of the transcription factor, PLZF.[64]

The ability of type I and type II NKT cells to cross-regulate each other is apparent in colon cancer. The studies in our lab have shown that both Tregs and type II NKT cells can suppress tumor immunity in a subcutaneous CT26 colon cancer mouse model.[51] In wild-type (WT) mice, type I and type II NKT cells cancel each other's functions, so Tregs are the primary regulator of tumor immunity, similar to the situation in CD1d KO mice (lack both type I and type II NKT cells).[51] Interestingly, in the absence of type I NKT cells (Jα18 KO mice), blocking Tregs did not reduce tumor growth because now type II NKT cells could suppress instead of the Tregs.[51] This demonstrates the ability of type II NKT cells to suppress protection against colon cancer, as well as their regulation by type I NKT cells that regulate the regulators.

The role of NKT cells in colonic pre-cancer polyps has also been studied.

In agreement with the pro-tumor functions of type I NKT cells in colon cancer, it has been recently shown that type I NKT cells increase intestinal polyp formation in a spontaneous mouse model of colon cancer using the $Apc^{Min/+}$ mice.[61,81] Additional studies identified a unique polyp type I NKT cell population ($CD4^+$, $NK1.1^-$, $CD44^{int}$ and $PD\text{-}1^{lo}$) that did not express PLZF, that were identified as IL-10 and IL-17 producers that suppressed Th1 immunity and promoted regulatory T cells (Tregs).[61] Interestingly, α-GalCer treatment can disrupt the natural tumor promoting function of type I NKT cells and reduce polyp development in the orthotopic $Apc^{Min/+}$ mouse model.[81] The treatment with α-GalCer induced TH1 skewing and cytokine production.[81]

NKT cells in pancreatic cancer

Currently, there is little information about the function of NKT cells in pancreatic cancer. The role of type I NKT cells in pancreatic cancer was first revealed when studies using α-GalCer-pulsed DCs showed anti-tumor functions in C57BL/6 mice inoculated with syngeneic Panc02 tumors.[82] In this study, the mice treated with α-GalCer-pulsed DCs exhibited a significant expansion of IFN-γ producing type I NKT cells, which correlated with a decrease in tumor growth.[82] More recently, a study by Janakiram *et al.* demonstrated the functional importance of NKT cells in pancreatic cancer by crossing the KPT model ($p48Cre^{/+}$-LSL-$Kras^{G12D/+}$) with CD1d KO mice that lack both type I NKT and type II NKT cells.[60] The KPT-CD1d KO mice displayed an increase in pancreatic intraepithelial neoplasia (PanIN) formation associated with an increase in M2 macrophages expressing microsomal prostaglandin E synthase-1 (mPGES-1) and 5-lipoxygenase (5-LOX) compared to KPT mice. Furthermore, the loss of NKT cells resulted in a decrease in the percentage of IFN-γ producing NK and $CD8^+$ T cells in the pancreas.[60]

NKT cells in gastric cancer

The role of NKT cells in gastric cancer first surfaced nearly 20 y ago by a study conducted by Yanagisawa *et al.* that demonstrated type I NKT cells derived from PBMCs of gastric cancer patients display a much lower proliferative response to α-GalCer than those of healthy volunteers.[83] Furthermore, a preclinical study showed that gastric cancer cell lines and primary tumors express CD1d.[57] As a result, type I NKT cells had a robust anti-tumor response against CD1d-positive gastric cancer *in vitro* and *in vivo* in the presence of α-GalCer.[57] In the same study, cisplatin upregulated CD1d expression in gastric cancer cells and enhanced their susceptibility to NKT-mediated cytotoxicity.[57] Recently, a transcriptomic study involving 876 patients with gastric cancer revealed that low CD1d expression is associated with poor prognosis.[84]

Conclusion

GI cancers are some of the deadliest cancers, and it is evident that NKT cells play an integral part in regulating tumor immunity. The role of NKT cells in GI cancers is complex and can differ depending on the type of cancer and location. For example, in liver cancer, α-GalCer treatment is effective in treating BNL 1MEA.7 R.1 tumors in the liver, but it is ineffective in treating the same tumors implanted subcutaneously.[69] To further complicate the role of NKT cells in GI cancers, there are data that show that NKT cells can have protumor and antitumor functions within the same type of cancer. In colon cancer, it has been demonstrated that IL-10 producing NKT cells accumulates in precancerous polyps[61,64] and that type I NKT cells increase polyp formation in a mouse model using $Apc^{Min/+}$ mice.[81] In contrast, in a study with colorectal cancer patient tissue samples it was shown that higher $Vα24^+$ NKT cell tumor infiltration in colorectal carcinomas is an independent favorable prognostic factor.[62]

Although several clinical trials with non-GI cancer patients have demonstrated that α-GalCer-based treatments are safe and can induce complete and partial clinical responses, unfortunately, the responses in the clinical trials have been inconsistent and unpredictable.[85–90] The contradictory roles of type I NKT cells in GI tumor immunity may exist in other types of cancers and help explain the inconsistent responses in the clinical trials using α-GalCer-based treatments.[85,87–89,91]

It has been demonstrated that type I NKT cells do have the potential to suppress tumor growth, but it is highly context dependent. In order to improve α-GalCer-based treatments, the type of cancer, location of the tumor, and a patient's baseline level and class of circulating NKT cells should be evaluated before starting treatments. Furthermore, the development and clinical testing of alternative type I NKT cell agonists that elicit a Th1-skewed response and type II NKT cell inhibitors to suppress protumor immunity is warranted and could lead to more consistent clinical responses.

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