Review

Molecular Cancer Therapeutics

New B7 Family Checkpoints in Human Cancers

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Abstract

T cells are the main effector cells in immune response against tumors. The activation of T cells is regulated by the innate immune system through positive and negative costimulatory molecules. Targeting immune checkpoint regulators such as programmed cell death 1 (PD-1)/PD-1 ligand 1 (PD-L1) and CTL antigen 4 (CTLA-4) has achieved notable benefit in a variety of cancers, which leads to multiple clinical trials with antibodies targeting the other related B7/CD28 family members. Recently, five new B7 family ligands, B7-H3, B7-H4, B7-H5, B7-H6, and B7-H7, were identified. Here we review recent understanding of new B7 family checkpoint molecules as they have come to the front of cancer research with the concept that tumor cells exploit them to escape immune surveillance. The aim of this article is to address the structure and expression of the new B7 family molecules as well as their roles in controlling and suppressing immune responses of T cells as well as NK cells. We also discuss clinical significance and contribution of these checkpoint expressions in human cancers. *Mol Cancer Ther; 16(7); 1203–11. ©2017 AACR.*

Introduction

Adaptive immune responses are of two major types: humoral immunity directed against extracellular invaders and cellular immunity directed against intracellular invaders. Cancer is characterized by the accumulation of a variable number of genetic alternations and the loss of normal cellular regulatory processes (1). These events have long been known to result in the expression of neoantigens, differentiated antigens, or cancer testis antigens, which distinguish them from their normal counterparts (2). Cellular immunity contains T-cell–mediated immune responses, which function as nonredundant effector cells in anticancer immunity. T-cell activation is regulated by the innate immune system through positive and negative costimulatory molecules. Members of the B7 family have been shown to be important for regulating T-cell responses.

In the past decade, in the context of growing scientific understanding of the interplay of cancer and the immune system, new agents targeting B7:CD28 family checkpoints have been developed and tested in advanced solid tumors. The significance of modulating these pathways was highlighted in 2011 by the FDA approval of ipilmumab, an antibody targeting CTL antigen 4 (CTLA-4) in advanced melanoma. The ability to successfully target checkpoint regulators has since led to multiple clinical trials with antibodies targeting the pathways of the B7 family members. The growing B7 family now comprises 10 members, which are CD80 (also known as B7.1), CD86 (also known as B7.2), B7-H1 (also known as PD-L1 or CD274), B7-DC (also known as PD-L2 or CD273), B7-H2 (also known as ICOSL), B7-H3 (also known as CD276), B7-H4 (also known as B7S1, B7x, or Vtcn1), B7-H5 (also known as VISTA, GI24, Dies1 or PD-1H), B7-H6 (also known as NCR3LG1), and B7-H7 (also known as HHLA2). Compelling evidence indicates that B7 molecules not only provide crucial positive signals to stimulate and support T-cell action, but also offer negative signals that control and suppress T-cell responses.

In this review, we will focus on recent understanding of new B7 family checkpoint molecules, as tumor cells exploit them to oppose immune attack. The aim of this article is to address the structure and expression of new B7 family checkpoint molecules as well as their roles in controlling and suppressing immune responses. We also discuss the clinical significance and contribution of those checkpoint expressions in human cancers.

B7-H3

The expressions of B7-H3 and its receptor

B7-H3 (also known CD276) is a type I membrane protein with its sequence similarity to the extracellular domain of PD-L1 (also known as B7-H1). Our laboratory first discovered murine B7-H3 (3), which contains one IgV and one IgC domains (4). In humans, but not mice, B7-H3 has an alternate isoform containing a tandem repeat of IgV and IgC domains (VCVC) and this isoform is the more common form expressed (Table 1; ref. 3). *B7-H3* is widely expressed in both lymphoid and nonlymphoid organs at RNA level, but the expression of B7-H3 protein is more restricted to cell types such as activated dendritic cells (DC), monocytes, T cells, B cells, and NK cells. Recent studies found that aberrant B7-H3 was expressed on a wide variety of cancers, including lung (5), prostate (6), kidney (7), ovary (8), endometrium (9), colorectum (10), liver (11), and breast (12).

In addition to membrane B7-H3, B7-H3 also exists in a soluble form, which is cleaved from membrane B7-H3 by proteinase. Zhang and colleagues found that B7-H3 release from cells was blocked by addition of a matrix metalloproteinase inhibitor (MMPI), which concomitantly caused the accumulation of B7-H3 on the cell surface (13). The duplication in 4IgB7-H3 generates a new conserved region in the first IgC domain, which might disable 4IgB7-H3 from releasing soluble form, whereas 2IgB7-H3 presents both membrane and soluble forms (14). Circulating

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Table 1. Sketch of new B7 family molecules

New B7 family ligand	Ligand alias	Structure	Expression		Putative receptors	Role in T/NK-cell responses
			Immune cells	Tumor		
B7-H3	CD276	IgV-IgC-IgV-IgC or IgV-IgC	+	+	?	Activation/ inhibition
B7-H4	B7S1, B7x, or Vtcn1	IgV-IgC	+	+	?	Inhibition
B7-H5	VISTA, Gl24, Dies1 or PD-1H	IgV-IgC	+	−	?	Inhibition
B7-H6	NCR3LG1	IgV-IgC	−	+	NKp30	Activation
B7-H7	HHLA2	IgV-IgC-IgV	+	+	CD28H (TMIGD2, IGPR-1)/?	Activation/ inhibition

serum B7-H3 levels are significantly higher in patients with lung cancer (15), colorectal carcinoma (16), hepatocellular carcinoma (HCC; ref. 17), renal cell carcinoma (RCC; ref. 18), and glioma (19) than those in healthy volunteers.

B7-H3-Ig protein binds a counter-receptor on activated T cells (3, 4), indicating that its putative receptor is expressed on activated T cells. Moreover, Zhang and colleagues (20) found that a putative receptor for B7-H3 was detected on monocytes and peritoneal macrophages from septic patients, but not on monocytes from healthy donors, suggesting that its receptor on monocytes and macrophages is induced by disease environment.

B7-H3 acts as a costimulatory/coinhibitory molecule

It was reported that B7-H3 exerted a costimulating effect on the proliferation of both $CD4^+$ and $CD8^+$ T cells when it was first discovered (4). As a costimulatory molecule, B7-H3 signaling induces cellular immunity and selectively enhances IFNγ production in the presence of TCR signaling (4, 21). However, other groups have showed that both murine and human B7-H3 acts as a coinhibitory molecule (22–25). Suh and colleagues (22) found that murine B7-H3 inhibited T-cell proliferation mediated by antibody to T-cell receptor or allogeneic antigen-presenting cells. B7-H3–deficient mice develops more severe airway inflammation than do wild-type mice in conditions in which Th cells differentiate toward Th1 rather than Th2. Recently, Veenstra and colleagues provided strong evidence that B7-H3 might play an inhibitory role on T-cell proliferation (26). The inhibition may govern through NFAT, NFκB, and AP-1 factors, three major signaling pathways through which TCR regulates gene transcription (27). Collectively, these results suggest that the immunologic function of B7-H3 remains controversial, with conflicting costimulatory and coinhibitory functions. This could be because B7-H3 has more than one possible binding partner that determines its alternative function.

The clinical significance and contribution of B7-H3 expression in human cancers

B7-H3 expression is significantly associated with poor outcome in patients with RCC (28–30), lung cancer (31), prostate cancer (32), colorectal carcinoma (33, 34), gallbladder cancer (35), esophageal squamous cancer (36), cervical cancer (37), osteosarcoma (38), and breast cancer (39). Thus, B7-H3 expression might be a feasible and effective means to predict the prognosis in cancer patients. B7-H3 in human RCC is a direct target of miR-187 (28). Lower miR-187 expression levels are associated with higher RCC grade and stage. Downregulation of miR-187 might play roles in RCC progression via deregulating B7-H3 expression in RCC. Crispen and colleagues found that B7-H3 expression by RCC cells or RCC vasculature was detected in 17% and 95% of specimens, respectively (29). The presence of either tumor cell or diffuse tumor vasculature expression of B7-H3 is present in 46% of specimens and is associated with multiple adverse clinical and pathologic features. Moreover, the presence of either tumor cell or diffuse tumor vasculature B7-H3 expression is significantly associated with an increased risk of death from RCC. This finding was confirmed by Shi and colleagues (30), indicating that B7-H3 is a cancer-specific endothelial marker of potential importance for the development of tumor-specific, vascular-targeted therapy, although the role of B7-H3 on tumor vasculature still remain unknown.

B7-H3 in lung cancer modulates the expression of FASN, a fatty acid synthase, specifically (40). Furthermore, deletion of B7-H3 downregulates the mRNA and protein levels of SREBP-1, a transcription factor governing the expression of FASN. In addition, expression levels of B7-H3 and FASN exhibit a positive correlation in clinical lung cancer tissues. These data suggest that B7-H3 hijacks SREBP-1/FASN signaling mediating abnormal lipid metabolism in lung cancer (40). Kentaro and colleagues (41) showed that the association of B7-H3 expression in lung cancer with survival differed by smoking history. High B7-H3 expression is associated with decreased lung cancer–specific survival in moderate/heavy-smoking patients, but not in non/light-smoking patients, indicating the potential effectiveness of anti-B7-H3 therapy for smokers' lung adenocarcinoma (41).

B7-H3 expression in prostate cancer correlates with ERG-positive disease and androgen receptor (AR) expression. There is an AR-binding site upstream of B7-H3 and the presence of androgens decreases B7-H3 expression in LNCaP, suggesting potential direct AR regulation on B7-H3 expression (32). B7-H3 in colorectal cancer downregulates the expression of E-cadherin and β-catenin, while upregulates N-cadherin and Vimentin expression, implying that B7-H3 promotes the EMT in colorectal cancer cells (34). More importantly, B7-H3 promotes the EMT by activating the PI3K–Akt pathway and upregulating the expression of Smad1 (34).

B7-H3 in osteosarcoma cells is a direct target of miR-124 (38). Overexpression of miR-124 decreases B7-H3 mRNA and protein level and inhibits B7-H3 3′-UTR reporter activity. Treatment of osteosarcoma cells with miR-124 mimics inhibits cell growth and invasion *in vitro*, which can be abrogated by transfected by B7-H3 expression vector (38). The data suggests the potential application of miR-124 as a novel onco-miRNA via downregulation of B7-H3 in osteosarcoma.

In addition to the above mentioned contributions, B7-H3 also contributes to some cancer metabolism. Lim and colleagues showed that B7-H3 promoted aerobic glycolysis (also known as the Warburg effect) in breast cancer as well as melanoma, evidenced by increased glucose uptake and lactate production (42).

B7-H3 increases the protein levels of HIF1α and its downstream targets, LDHA and PDK1, key enzymes in the glycolytic pathway. Furthermore, B7-H3 promotes reactive oxygen species–dependent stabilization of HIF1α by suppressing the activity of the stress-activated transcription factor Nrf2 and its target genes, including the antioxidants SOD1, SOD2, and PRX3. Metabolic imaging of human breast cancer xenografts in mice confirmed that B7-H3 enhanced tumor glucose uptake and tumor growth (42).

B7-H4

The expression of B7-H4 and its receptor

B7-H4 (also known as B7-S1, B7x, or Vtcn1) is another type I membrane B7 family member that was discovered by our laboratory (43), Chen and colleagues (44), as well as Allison and colleagues (45), simultaneously. It contains one IgV and one IgC domains (Table 1), a highly evolutionarily conserved molecule that bears 87% amino acid identity between human and mouse. Human B7-H4 is mapped on chromosome 1 comprised of six exons and five introns. Murine B7-H4, similar to human B7-H4 structure, is mapped on chromosome 3 (46). *B7-H4* mRNA is widely distributed in mouse and human peripheral tissues. However, protein expression is more restricted and can be induced on antigen-presenting cells (APCs) after *in vitro* stimulation. Recent studies found that aberrant B7-H4 was expressed on a broad spectrum of cancers, including stomach (47), kidney (48), ovary (49), lung (50), uterus (51), breast (49), prostate (6), and skin (52).

Similar to B7-H3, B7-H4 can also exist in soluble form, which is generated by proteolytic cleavage mediated by the metalloproteinase nardilysin (53). Soluble B7-H4 is detected at higher levels in the sera from patients with gastric cancer (54), kidney cancer (55, 56), lung cancer (57–59), liver cancer (60), ovarian cancer (61), and osteosarcoma (62). In addition, serum B7-H4 is associated with tumor metastasis. Therefore, soluble B7-H4 may be a valuable blood marker for predicting the progression and prognosis of patients with broad spectra of cancers.

Until now we have not identified its counter receptor. However, it is agreed that its putative receptor is induced by activation. In addition to activated T cells, MDSCs are also reported to express its putative receptor. Moreover, B7-H4 binds to MDSCs more potently than activated T cells, indicating that these two cell types may express different B7-H4 receptors or the same receptor at the highly distinct levels (63).

B7-H4 acts as a coinhibitory molecule

B7-H4 is a novel B7 ligand that plays an important role in the T-cell–mediated immune response as a negative regulator. The ligation of B7-H4 to unidentified receptors results in the inhibition of TCR-mediated T-cell proliferation, cell-cycle progression, and IL-2 production, indicative of its negative regulator for T-cell responses (43–45).

Soluble B7-H4 is a decoy molecule to block the inhibitory functions of membrane B7-H4 (64). In a mouse model of rheumatoid arthritis, transgenic expression of soluble B7-H4 or genetic deletion of B7-H4 accelerates the progression of collagen-induced arthritis, accompanied by enhanced T- and B-cell–mediated autoimmune responses as well as increased activity of neutrophils. Expression *in vivo* of an agonist, a B7-H4-Fc fusion protein, profoundly suppresses disease progression in the mouse model, leading to exacerbation of collagen-induced arthritis. Gradual loss of membrane-tethered B7-H4 from APCs combined with an increased release of soluble B7-H4 occurs in parallel to natural type I diabetes development, potentiating hyperproliferation of diabetogenic T cells (53), implying that soluble B7-H4 is a decoy molecule.

The clinical significance and contribution of B7-H4 expression in human cancers

B7-H4 expression is significantly associated with poor outcome in patients with RCC (65), lung cancer (66), colorectal carcinoma (67), cholangiocarcinoma (68), glioma (69), pancreatic cancer (70), oral squamous cell carcinoma (71), esophageal squamous cell carcinoma (72), gallbladder cancer (35), ovarian serous carcinoma (73), and gastric cancer (74). B7-H4 expression in RCC is associated with adverse clinical and pathologic features, including constitutional symptoms, tumor necrosis, and advanced tumor size, stage, and grade. Patients with B7-H4–expressing RCC tumors are also three times more likely to die from RCC compared with patients with B7-H4–negative tumors (65). In addition, tumor vasculature endothelial cells show B7-H4 positive, indicating that B7-H4 represents a target for attacking tumor cells as well as tumor neovasculature to facilitate immunotherapeutic treatment of RCC tumors. For clear cell RCC (ccRCC), B7-H4 expression has critical impact on the prognosis of the patients, particularly on the recurrence of the carcinoma in patients with clinical stage T1 RCC (75). In terms of coexpression of PD-L1 and B7-H4, RCC with positive expressions of B7-H4 and PD-L1 is markedly related to advanced TNM stage, high grade, and larger tumor size than patients with B7-H4–negative and PD-L1–negative in RCC tissues (76). Furthermore, the patients with positive expressions of both PD-L1 and B7-H4 were found to be markedly correlated with the overall survival of the patients and tended to have an increased risk of death when compared with negative expression groups. Coexpression of B7-H4 and PD-L1 decreases overall survival. Therefore, the coexpressions of PD-L1 and B7-H4 in RCC patients indicate that these markers may be a predictor of tumor development and death risk.

B7-H4 in lung cancer promotes tumor cell proliferation, invasion and migration, and cell apoptosis (66). Knockdown of B7-H4 by shRNA is accompanied by a marked increase in Bax and caspase-3/caspase-8, but a decrease in Bcl-2, cyclin D1, and activation of AKT, demonstrating a strong promoting role of B7-H4 in lung tumor growth, progression, and metastasis.

In the context of colorectal carcinoma, overexpression of B7-H3 and B7-H4 in HCT-116 cells induces T cells to secrete TGFβ1, while B7-H3 and B7-H4 are the direct target genes of miR-143 (67). TGFβIL-21 elevated the expression of miR-155 in colorectal cancer cells through SMAD3 and SMAD4. The upregulated miR-155 attenuated miR-143 by inhibiting its direct target, the transcription factor CEBPB. The result reveals the mechanism by which TGFβ1 leads to T-cell–mediated tumor evasion through an increase in B7-H3 and B7-H4 expression (67).

Higher soluble B7-H4 in RCC patients correlates with advanced tumor stage (56). Fukuda and colleagues (77) found that elevated preoperative serum levels of PD-L1 and B7-H4 were correlated with less differentiated tumors, higher invasive and metastatic potential, a worse response to anti-VEGF therapy, and shorter overall survival, indicating that investigating preoperative serum

levels of PD-L1 and B7-H4 might not only be useful to assess the biological aggressiveness of RCCs, but also to predict the efficacy of anti-VEGF therapy and the eventual prognosis.

B7-H5

The expression of B7-H5 and its receptor

B7-H5, also known as V domain–containing Ig suppressor of T-cell activation (VISTA), GI24, Dies1, and PD-1 homolog (PD-1H), is a type I membrane protein with the extracellular domain homologous to PD-L1 (Table 1). Murine B7-H5 was identified in 2011 (78, 79). The extracellular Ig domain of murine B7-H5 shares significant sequence homology with PD-L1 and PD-L2. The expression of murine *B7-H5* mRNA is detected in peritoneal macrophages, splenic $CD11b^+$, monocytes, $CD11c^+$ DCs, $CD4^+$ T cells, and $CD8^+$ T cells, but at a lower expression level in B cells, while B7-H5 protein is highly upregulated on APCs and $Foxp3^+$ $CD4^+$ regulatory T cells, but not on B cells, NK cells, or granulocytes. Similar to murine B7-H5, human B7-H5 is predominantly, if not exclusively, expressed in hematopoietic tissues or in tissues that contain significantly numbers of infiltrating leukocytes (80). Human B7-H5 is not expressed on B cells or NK ($CD56^{hi}$) cells, but highly expressed on myeloid cells with reduced expression on $CD4^+$ and $CD8^+$ T cells. Interestingly, expression of B7-H5 is particularly high in human placenta, which may be indicative of a functional role for B7-H5 in allofetal tolerance.

So far its receptor has not identified. However, B7-H5-Ig suppresses proliferation of T cells, but not B cells, and blunts the production of T-cell cytokines and activation markers, suggesting that its receptor is expressed on T cells, not B cells. In addition, both naïve and memory T cells are sensitive to B7-H5–induced suppression upon activation (78, 80), implying that its putative receptor is induced by activation.

B7-H5 acts as a coinhibitory molecule

B7-H5 is a negative immune checkpoint protein, which suppresses T-cell activation. A soluble B7-H5-Ig fusion protein or B7-H5 expression on APCs inhibits T-cell proliferation and cytokine production *in vitro*. A B7-H5–specific mAb interferes with B7-H5–induced suppression of T-cell responses by B7-H5–expressing APCs *in vitro* (78). Furthermore, anti-B7-H5 treatment exacerbates the development of the T-cell–mediated autoimmune disease experimental autoimmune encephalomyelitis in mice. These suggest that B7-H5 negatively regulates T-cell activation.

More interestingly, $CD4^+$ T cells in mice lacking B7-H5 exhibited a dramatically increased response to antigen stimulation and delivery of a B7-H5–specific agonist mAb directly inhibited $CD4^+$ T-cell activation both *in vitro* and *in vivo* (81), indicating that in addition as a coinhibitory ligand on APCs that suppress T-cell responses, B7-H5 also functions as a coinhibitory receptor on $CD4^+$ T cells.

The clinical significance of B7-H5 expression in human cancers

Few studies on B7-H5 expression in human cancers have been reported. Lines and colleagues examined B7-H5 expression on human colon and lung cancer lesions by immunofluorescence (82). B7-H5 expression is confined predominantly to the infiltrating CD11b-positive myeloid cells in the TME of colon cancer lesions, whereas infiltrating $B7\text{-}H5^+$ cells in some cases, but not others, expressed CD11b in lung cancer cells.

In the context of pancreatic carcinoma, Byers and colleagues (83) showed that membranous B7-H5 protein was expressed on normal ductal epithelium within the pancreas, not other cell types from the normal pancreas. In adenocarcinoma, B7-H5 protein was decreased or absent. Interestingly, B7-H5 expression in intraductal papillary mucinous neoplasms varied with grade. Normal ducts adjacent to tumors were highly positive, indicating that B7-H5 expression was restricted to ductal cells in the normal pancreas and the expression was downregulated in pancreatic adenocarcinomas (83). The study suggests that loss of the B7-H5 signal may contribute to immune evasion of pancreatic adenocarcinoma.

In some cancer cell lines, Patricia and colleagues detected promoter methylation that controls B7-H5 expression (84). In gastric cancer, myofibroblasts in the TME overexpress B7-H5, whereas B7-H5 expression loss is a recurrent event in tumor cells, caused by promoter methylation and/or miR-125a-5p overexpression (84). These findings highlight B7-H5 as a novel player in gastric cancer, with distinct roles within tumor cells and in the TME. Future studies in larger cohorts of cancer patients will be needed to identify tumor characteristics that may be associated with B7-H5 expression in the TME and in the tumor cells.

B7-H6

The expression of B7-H6 and its receptor

B7-H6 (also known as NCR3LG1) is a ligand for NK-cell–activating receptor NKp30 (85). B7-H6 contains two Ig domains (Table 1) and its sequence is homologous to the other B7 family members. *B7-H6 mRNA* is not found in 48 normal tissues under steady-state conditions, while several subsets of human primary lymphoma, leukemia, ovarian cancer, brain tumors, breast cancers, RCC, and various sarcomas potentially express high amounts of *B7-H6*. Human B7-H6 protein is not expressed in normal tissues, but in contrast, is expressed on various primary human tumors, including leukemia, lymphoma, and gastrointestinal stromal tumors (86). The absence of *B7-H6* mRNA in normal tissues, coupled with its relative abundance among tumor cells, indicates that its expression is upregulated by tumor transformation, which has been proved by Baratin and colleagues (87). However, B7-H6 can be induced at the surface of $CD14^+CD16^+$ proinflammatory monocytes and neutrophils upon stimulation by ligands of Toll-like receptors or proinflammatory cytokines such as IL-1β and TNFα (88).

B7-H6 expression is regulated by several factors in tumor cells. In various tumor cell lines, B7-H6 surface protein and mRNA expression are downregulated upon treatment with pan- or class I histone deacetylase (HDAC) inhibitors as well as after siRNA-mediated knockdown of HDAC2 or 3 (89). B7-H6 downregulation in tumor cell lines is associated with decreased B7-H6 reporter activity and reduced histone acetylation at the B7-H6 promoter. In certain primary lymphoma and HCC samples, *B7-H6* mRNA levels are elevated and correlated with HDAC3 expression. Furthermore, treatment of tumor cells with almost all standard tumor therapeutics, including chemotherapy, radiotherapy, nonlethal heat shock, and cytokine therapy (TNFα) can upregulate the expression of B7-H6 in tumor cells and enhance tumor sensitivity to NK-cell cytolysis (90). Pharmacologic inhibition or siRNA/shRNA–mediated knockdown

of c-Myc or N-Myc significantly decreases B7-H6 expression in a variety of tumor cells, including melanoma, pancreatic carcinoma, and neuroblastoma cell lines (91). In tumor cell lines from different origin and primary tumor tissues of HCC, lymphoma, and neuroblastoma, mRNA levels of c-Myc positively correlate with B7-H6 expression.

B7-H6, similar to B7-H3 and B7-H4, has another form that is generated by ectodomain shedding mediated by the cell surface proteases, a disintegrin and metalloproteases. Soluble B7-H6 is selectively detected in the sera of patients with gram-negative sepsis and is associated with membrane vesicles that cosedimented with the exosomal fraction (88). However, the level of soluble B7-H6 is comparatively low/absent. In a subset of patients with stage IV melanoma, significant elevated levels of soluble B7-H6 compared with healthy controls were observed (92). Moreover, high concentration of soluble B7-H6 detected in the sera from patients with high-risk neuroblastoma (HR-NB) is detected, which is associated with NK-cell malfunction (93). Therefore, the concentration of soluble B7-H6 in the serum may be clinically useful as biomarkers for risk stratification.

Its putative receptor is NKp30 on NK cells. NK cells are lymphocytes of the innate immune system that participate in the elimination of tumor cells (78). NKp30 recognizes B7-H6 expressed on tumor, but not healthy cells. B7-H6 contacts NKp30 through the complementarity-determining region (CDR)-like loops of its V-like domain in an antibody-like interaction (78). This structure of the complex of NKp30 and B7-H6 provides a template for designing molecules to stimulate NKp30-mediated cytolytic activity for tumor immunotherapy.

The role of B7-H6 in NK-cell immune responses

NK cells eliminate B7-H6–expressing tumor cells either directly via cytotoxicity or indirectly by cytokine secretion. These highlight the role of tumor-induced self-molecule B7-H6 in alerting innate immunity. One of the mechanisms by which tumor cells escape immune surveillance is to impede NK-mediated recognition of B7-H6. Tumor cells can shed membrane B7-H6 (92) to impair NK-cell function. In a fraction of patients with ovarian cancer, soluble B7-H6 in the TME is detected and NKp30 expression on tumor-associated NK cells is substantially reduced as compared with the autologous peripheral blood NK cells (94). Moreover, the presence of soluble B7-H6 is associated with impaired expression of NKp30. Functionally, those tumor-infiltrating NKp30^{low} NK cells display an impaired IFNγ production and cytolytic activity when tested against target cells expressing surface B7-H6. Collectively, the defective expression and function of NKp30 may be induced by the chronic engagement of this receptor partially with soluble B7-H6. In the context of HR-NB, serum concentration of soluble B7-H6 correlates with the downregulation of NKp30, bone marrow metastases, and chemoresistance. In addition, soluble B7-H6 contained in the serum of HR-NB patients inhibites NK-cell functions *in vitro* (93). Thus, inhibiting this proteolytic shedding process increases membrane B7-H6 on tumor cells, enhancing NKp30-mediated activation of NK cells.

The clinical significance and contribution of B7-H6 expression in human cancers

In ovarian cancer, positive B7-H6 staining was predominantly observed on the membrane and in the cytoplasm of the ovarian cancer cells (95). B7-H6 expression in the ovarian cancer tissues is significantly correlated with distant metastasis status and FIGO stage. The overall survival rate of the subgroup with lower B7-H6 expression is significantly better than that of the subgroup with higher B7-H6 expression, suggesting B7-H6 expression is involved in the progression of human ovarian cancer. In astrocytoma, B7-H6–positive expression is significantly associated with World Health Organization grade (96).

Preclinical work suggests that B7-H6 may serve as a promising target for cancer immunotherapy. Zhang and colleagues reported that chimeric NKp30-expressing T cells responded to B7-H6^{+} tumor cells (97) and those T cells produced IFNγ and killed B7-H6–expressing tumor cells *in vivo*. More importantly, this response was dependent upon ligand expression on target cells but not on MHC expression, highlighting the significance of B7-H6 in killing tumor cells.

B7-H7

The expression of B7-H7 and its receptors

B7-H7 was identified by screening the expressed sequence tag (EST) database and termed HERV–H LTR–associating 2 (HHLA2) in 1999 (98). In 2003, B7-H7 has been discovered as the newest member of the B7 family, which shares 10%–18% amino acid identity and 23%–33% similarity to other human B7 proteins (85, 99). It phylogenetically is most similar to B7-H3 and B7-H4. The gene has an open reading frame (ORF) of 414 amino acids with three immunoglobulin-like domains (IgV-IgC-IgV; Table 1). B7-H7 is the only B7 family member that is found in humans but not in mice.

Janakiram and colleagues showed that B7-H7 protein was detected in trophoblastic cells of the placenta and the epithelium of gut, kidney, gallbladder, and breast, but not in most other organs. In the immune system, B7-H7 protein is constitutively expressed on human monocytes/macrophages, but not on immature DCs, resting T or B cells. However, expression on both mature DCs and monocytes is modestly upregulated by inflammatory signals like LPS, IFNγ, and poly I:C (99, 100). B7-H7 protein was widely expressed in human cancers from the breast, lung, thyroid, melanoma, pancreas, ovary, liver, bladder, colon, prostate, kidney, and esophagus (101).

B7-H7 was previously called B7-H5 by Zhu and colleagues (100), but now B7-H5 is referred to PD-1H protein and B7-H7 is referred to HHLA2. B7-H7 does not interact with other known members of the CD28 family or the B7 family, but does bind a putative receptor that is constitutively expressed not only on resting and activated T cells but also on APCs (99). In 2013, Zhu and colleagues (100) found that CD28 homolog (CD28H) bound to B7-H7 on APCs. In 2015, Janakiram and colleagues (101) found that B7-H7-Ig bound to cells expressing Transmembrane and Immunoglobulin Domain Containing 2 (TMIGD2) and TMIGD2-Ig bound strongly to 3T3 cells expressing B7-H7. TMIGD2 contains an N-terminal signal peptide, an extracellular IgV-like domain, three potential sites for N-linked glycosylation, a transmembrane region, and a cytoplasmic tail with four potential sites for phosphorylation and a possible site for SH3 domain recognition. By sequence analysis, TMIGD2, the immunoglobulin-containing and proline-rich receptor-1 (IGPR-1), and CD28H are the same molecule, which further confirmed that CD28H is one of B7-H7 putative receptors. CD28H is expressed on naïve T cells as well as endothelium.

B7-H7 acts as a costimulatory/coinhibitory molecule

It was reported that the interaction between CD28H and B7-H7 on APCs costimulated human T-cell proliferation and cytokine production via a pathway involving AKT phosphorylation (100). In contrast, other groups have proposed the opposite function of B7-H7. In the presence of TCR signaling, B7-H7 inhibits proliferation of both $CD4^+$ and $CD8^+$ T cells (99). In addition, B7-H7 significantly reduces cytokine production by T cells, including IFNγ, TNFα, IL-5, IL-10, IL-13, IL-17α, and IL-22. Thus, the ligation of B7-H7 to T cells suppresses T-cell responses. Similar to B7-H3, both a T-cell coinhibitory role and a costimulatory role have been reported for this ligand (99, 100). One explanation is that B7-H7 has two counter receptors and CD28H is a costimulatory receptor. With repetitive T-cell activation, expression of CD28H is gradually lost, allowing expression of a second receptor to become dominant. B7-H7 on APCs or tumor cells can interact with this second receptor and exert a coinhibitory function. Future study will be required to discover the receptors on activated T cells in which B7-H7–expressing tumor cells interact with to impair antitumor immunity.

The clinical significance of B7-H7 expression in human cancers

In the context of breast cancer, high B7-H7 expression is significantly associated with regional lymph node metastasis and stage (101). In lung cancer, highly frequent expression of B7-H7 is associated with clinical–pathologic characteristics and clinical outcome (102). B7-H7 is expressed in the majority of osteosarcoma tumors and its expression is associated with metastatic disease and poorer survival, suggesting that B7-H7 may be a novel immunosuppressive mechanism within the osteosarcoma tumor microenvironment (103).

Tumor-expressed B7-H7 could protect the tumor from immune surveillance via its interaction with unidentified receptors on activated T cells and other immune cells, and it may also promote angiogenesis within the TME via its interaction with endothelial-expressed CD28H. Interestingly, therapies targeting B7-H7 could not only enhance antitumor immune responses, but may also inhibit tumor angiogenesis (104).

Conclusion

On the basis of these functional and clinical observations, blocking some of the B7:CD28 pathways (CTLA4/CD28 and PD-L1/PD-1) has yielded some therapeutic success in human malignancies. However, either CTLA4 inhibitor or PD-1 inhibitors did not give rise to high response rate in the treatment of patients with some cancer types, such as colorectal carcinoma. In addition, PD-1 inhibitor can induce upregulation of other immune checkpoint, Tim-3 (105). Therefore, it is quite urgent to identify other checkpoint inhibitors to be used in monotherapy or combotherapy with PD-1/PD-L1 inhibitors.

B7-H3, although, has a coinhibitory function and a costimulating function on T cells, the expression on either tumor cells or diffuse tumor vasculature is significantly associated with an increased risk of death and bad outcome. Targeting B7-H3 not only enhances antitumor immunity but also inhibits tumor angiogenesis. These highlight the potential usage of this pathway for cancer immunotherapy. Antibodies targeting B7-H3 are being

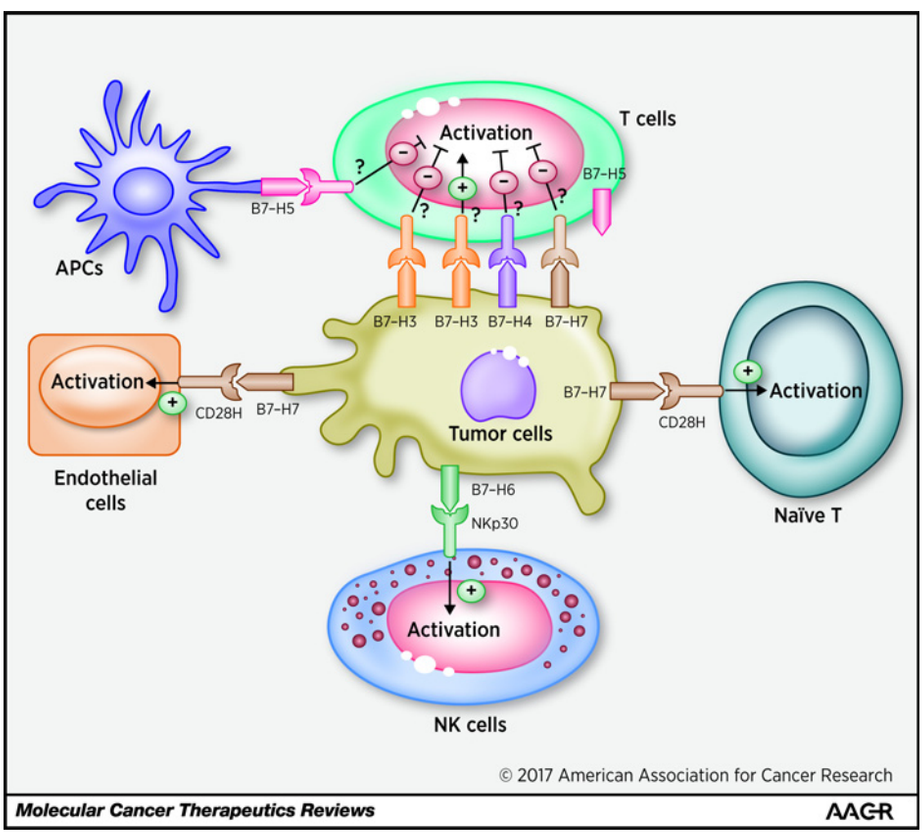

Figure 1.
A proposed model for the roles of new B7 family members within the TME. B7-H3–expressing tumor cells can play a coinhibitory role and costimulatory role in T-cell activation. Tumor-expressed B7-H4 and B7-H7 can interact with an unknown receptor on activated T cells that result in coinhibition. B7-H7 can also bind with CD28H on endothelium and naïve T cells that leads to tumor angiogenesis and T-cell activation, respectively. B7-H5 can function as a coinhibitory ligand expressed on APCs and a coinhibitory receptor expressed on T cells within TME. Tumor-expressed B7-H6 can interact with NKp30 to activate NK cells.

tested in a phase I/II clinical trials, indicating it represents a promising target for cancer immunotherapy.

B7-H4 plays a significant role in the "immune escape" theory of tumors. RCC with coexpressions of B7-H4 and PD-L1 were markedly related to advanced disease stage than patients with B7-H4-negative and PD-L1-negative in RCC tissues. Although no anti-B7-H4–targeting therapies have been investigated in any disease indication clinically, B7-H4 remains a high priority candidate for targeted inhibition or elimination in cancer immunotherapy. Therefore, generation of a highly specific anti-human B7-H4 antibody would open the door to robust preclinical studies for the treatment of tumors.

B7-H5 is predominantly expressed on myeloid cells, not tumor cells within TME. B7-H5 delivers a coinhibitory impact on T-cell response. In the CT26 colon cancer model, targeting B7-H5 and PD-L1 simultaneously achieve optimal tumor-clearing therapeutic efficacy, whereas targeting each molecule alone was less effective (106). These lay a foundation for designing B7-H5–targeted approaches either as a monotherapy or in combination with PD-1/PD-L1 inhibitors for cancer immunotherapy.

B7-H6 is exclusively expressed on tumor cells, and its binding partner is NKp30, which is one of the receptors involved in NK-cell activation. Basically, checkpoint inhibitors targeting the other B7 family molecules strongly rescue effector T-cell functions. One combotherapy strategy targeting effector T cells and NK cells will yield the best therapy efficacy.

B7-H7 is widely expressed in human malignancies and its expression is associated with poor prognostic factors. Targeting B7-H7 not only benefits antitumor immunity but also inhibits tumor angiogenesis. Collectively, B7-H7 pathway represents a novel immunosuppressive mechanism within TME and an attractive target for human cancer therapy.

Although the expression patterns of new B7 family molecules indicate that cancer may use redundant mechanisms to compromise immune attack (Fig. 1), some molecules are unique, such as B7-H5 and B7-H6. In the future, immunotherapy will focus on the effect of combined B7-H ligand.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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