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Phenotypical and potential functional characteristics of different immune cells expressing CD28H/B7-H5 and their relationship with cancer prognosis

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Summary

CD28H and B7-H5 have been identified as receptor–ligand pairs in the B7/CD28 family, and have co-stimulatory activity in immune cells. Here, we have systematically reviewed the research reports concerning the CD28H/B7-H5 pathway. It was found that CD28H is mainly expressed in T cells and natural killer (NK) cells with naive and poorly differentiated properties, and repeated antigen stimulation leads to permanent loss of CD28H. In tumors, CD28H is mainly expressed in tissue-resident memory (T_{RM}) lymphocyte T cells, which is associated with improved tumor prognosis. B7-H5 is a ligand for CD28H and is widely expressed in tumor cells. B7-H5 expression is closely related to the prognosis of the tumor. Studies have shown that high expression of B7-H5 in tumor is related to a worse prognosis for lung cancer, osteosarcoma, oral squamous cell carcinoma (OSCC), breast carcinoma, human clear cell renal cell carcinoma (ccRCC), intrahepatic cholangiocarcinoma (ICC), bladder urothelial carcinoma (BUC) and colorectal cancer (CRC), but is associated with a better prognosis for pancreatic ductal adenocarcinoma (PDAC) and glioma. Controversial views exist in studies on gastric cancer prognosis.

Keywords: cancer immunotherapy, cancer prognosis, CD28H/B7-H5, immune checkpoints, phenotype

Introduction

CD28H was previously named 'transmembrane and immunoglobulin domain containing 2' (TMIGD2, gene ID 126259), and has been identified as a novel adhesion molecule involved in angiogenesis in 2012 [1]. In an extensive whole-genome search, CD28H was identified based on preferential expression in T cells and homology to CD28 [2]. The human CD28H gene, constituted by five exons, is located on chromosome 19q13.3. However, no coding gene for CD28H has been found in mice or rats [2]. In human peripheral blood cell subsets, CD28H expression in granulocytes, monocytes, bone marrow dendritic cells (MDCs) and B cells is negligible [2,3]. By contrast, high levels of CD28H were detected in T cells, natural killer (NK) cells, plasmacytoid dendritic cells (PDCs) and innate lymphoid cells (ILCs) [2–4]. In human peripheral tissues, CD28H transcripts are preferentially enriched in lymphoid organs. The spleen and thymus have the highest levels of CD28H transcript [2], and the liver is also rich in CD28H transcripts. In human lungs, approximately 20% of $CD8^+$ T cells express CD28H, while almost all $CD8^+$ T cells express CD28H in the human small intestine [5]. There is no difference in the moderately expression of CD28H in the tonsils and spleen [3]. CD28H is expressed in tumor-infiltrating lymphocytes (TILs) in different tissues, including pancreatic carcinoma, ovarian carcinoma, breast carcinoma, colorectal carcinoma, lung cancer, melanoma and glioma [3,5].

The B7/CD28 family regulates lymphocyte responses through co-stimulation and co-inhibition [6–8] (Fig. 1) and can be phylogenetically divided into three groups [9,10]. The first group consists of B7-1/B7-2 / CD28/cytotoxic T lymphocyte antigen 4 (CTLA-4) and inducible co-stimulator ligand L/inducible co-stimulator ligand (ICOSL/ICOS); group II contains programmed cell death ligand 1/programmed cell death 1 (PD-L1/PD-L2/PD-1); the third group includes B7-H3, B7x (B7-H4) and HERV–H long terminal repeat (LTR)-associating protein 2 (HHLA2) (B7H7/B7-H5)/CD28H (TMIGD2). Identifying the CTLA-4 and PD-1/PD-L1 pathways has had a major

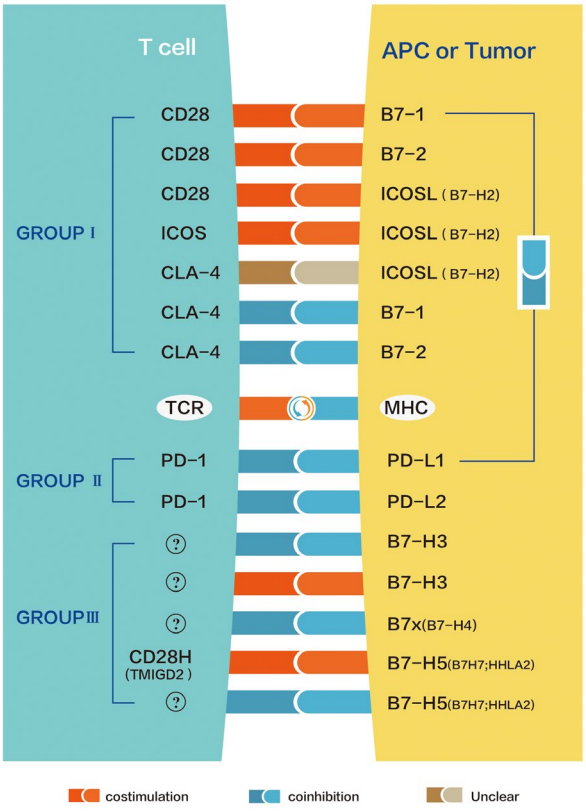

Fig. 1. The B7/CD28 family regulates T lymphocyte responses through co-stimulation and co-inhibition. CD28 and cytotoxic T lymphocyte antigen 4 (CTLA-4) compete for the same ligands B7-1 and B7-2. Inducible co-stimulator ligand L (ICOSL) is a ligand for inducible co-stimulator ligand (ICOS) and can also bind to CD28 and CTLA-4 [65]. Programmed cell death 1 (PD-1) is a common co-inhibitory receptor for PD ligand 1 (PD-L1) and PD-L2. B7-H3 has unknown co-inhibitory and co-stimulatory receptors. B7x has an unknown co-suppressor receptor. B7-H5 possesses the co-stimulatory receptor CD28H and an unknown co-inhibitory receptor [9]. In addition, B7-1 can interact with PD-L1 to inhibit the T cell response [66–68].

impact on the immunotherapy of cancer, resulting in improved treatment and survival of cancer patients [11,12]. B7-H5, which has also been called B7H7 [13], has no specific function, is a ligand for CD28H and was formerly known as human endogenous retrovirus-H long terminal repeat-associating 2 (HHLA2) [14]. It is the only member of the B7 family that can be found in humans but not in mice. By binding to unknown receptors, B7-H5 has been reported to be effective in inhibiting the proliferation of human CD4 and CD8 T cells and inhibiting the production of T cell cytokines, including interferon (IFN)-γ, tumor necrosis factor (TNF)-α, interleukin (IL)-5, IL-10, IL-13, IL-17A and IL-22 [9]. At the same time, it can also co-stimulate T cells by the CD28H/B7-H5 pathway [2]. Similarly to the B7-H3, both play a T cell co-inhibitory role as well as a co-stimulatory one. Based on homology searches, B7-H5 was revealed as a new candidate for the B7 family in 2001 [15]. B7-H5 was known as a binding protein for CD28H by screening for receptor sequences of more than 2300 transmembrane proteins [2]. As well as B7-H3 [16], which has two repeats of immunoglobulin (Ig)V-IgC, B7-H5 differs from other known B7 members with two Ig domains and its extracellular region has three characteristic Ig domains (IgV-Igc-IgV), with the first IgV domain having the highest homology to other B7 family members [2]. B7-H5 is preferentially transcribed in peripheral tissues [2], with particularly high levels of B7-H5 mRNA in the testes, colon, lungs, kidneys and pancreas. B7-H5 is transcriptionally low in liver and skeletal muscle. In immune cells, B7-H5 is not transcribed in T cells, whereas B cells, dendritic cells and macrophages are abundant in B7-H5 mRNA. Studies have shown that the expression of B7-H5 on B cells is induced by inflammatory stimuli [9]. At the same time, studies have suggested that there is no expression of B7-H5 on freshly isolated monocytes or T, B, NK cells on human peripheral blood mononuclear cells (PBMCs), but B7-H5 expression is present on monocyte-derived macrophages and dendritic cells [2]. These results suggest that B7-H5 is largely an inducer of antigen-presenting cells (APCs) response to inflammation. B7-H5 is also expressed on activated myeloid cells, and widely expressed in human cancer tissues [2], including pancreatic ductal adenocarcinoma (PDAC) [17,18], lung cancer [19], osteosarcoma [20], breast cancer [21], oral squamous cell carcinoma (OSCC) [22], human clear cell renal cell carcinoma (ccRCC), intrahepatic cholangiocarcinoma (ICC), bladder urothelial (ccRCC) [23,24], ICC [25], bladder urothelial carcinoma (BUC) [26], colorectal cancer (CRC) [27], glioma [28] and gastric cancer [29,30].

Phenotypical characteristics of different immune cells expressing CD28H

Phenotypical characteristics of naive T cells expressing CD28H

It has been shown that CD28H is widely expressed in naive T cells, and studies have shown that only 6–15% of naive T cells are $CD28H^-$ [3]. By comparing expression of CD31, which is the most recent thymic transplant marker [31], on $CD28H^+$ and $CD28H^-$ naive T cells, it was found that CD31 was expressed on most $CD28H^+$ but not $CD28H^-$ naive T cells. Furthermore, $CD28H^-$ naive T cells express higher levels of the T helper type 1 (Th1) regulatory transcription factor T-bet, as well as the effectors IFN-γ and TNF-α. Similar expression levels of IL-8 and IL-2 were found in naive and memory T cells of $CD4^+CD28H^+$ and $CD4^+CD28H^-$ [3]. It can

be concluded that CD28H$^+$ naive T cells exhibit more abundant naive characteristics than CD28H$^-$ naive T cells.

Phenotypical characteristics of activated T cells expressing CD28H

Activation of T cells results in irreversible loss of CD28H. Twenty per cent of memory T cells express CD28H [3], while almost all T cells with a senescent phenotype (CD7^{low}CD27$^-$CD28$^-$CD57$^+$) do not express CD28H [2,32–35]. Studies have shown that IFN-γ and CD57 levels in CD28H$^-$ T cells are higher than in CD28H$^+$ T cells [3]. CD57 can be used as a marker for T cell terminal differentiation [36]. Based on the expression of CCR7 and CD45RA, CD8$^+$ T cells can be divided into different subpopulations [37], including naive T cells (CD45RA$^+$CCR7$^+$), central memory T cells (CD45RA$^-$CCR7$^+$) and effector memory T cells (CD45RA$^-$CCR7$^-$; CD45RA$^+$CCR7$^-$). Nearly all (97·5%) naive T cells (CD45RA$^+$CCR7$^+$) were found to constitutively express CD28H [2]. Approximately half (52·9 and 41·5%) of the T cells with central memory (CD45RA$^-$CCR7$^+$) and effector memory (CD45RA$^-$CCR7$^-$) phenotypes were CD28H$^+$ [2]. Most (73·6%) CD45RA$^+$ effector memory cells (CD45RA$^+$CCR7$^-$), possibly terminally differentiated effector T cells, lose expression of CD28H [2,37,38]. A similar finding was detected in the CD4$^+$ T cell subset [2]. By staining CD28H on T cells and various other T cell surface markers, it was found that CD28H$^-$ T cells have higher lateral scatter and CD45RO is expressed in most of them, which do not express CD45RA [2]. Furthermore, expression of activation-induced molecules, such as PD-1, was shown to be increased in CD28$^-$ T cells, while expression levels of CD62L and CD27 were decreased [39,40]. Similarly, almost all CD4$^+$ T helper cells producing effector cytokines or forkhead box protein 3 (FoxP3)$^+$CD4$^+$ T regulatory cells did not express CD28H. These results led to the conclusion that less differentiation and effector function are shown in CD28H$^+$ memory T cells, and the senescence of T cell may lead to the loss of CD28H expression. In human peripheral tissues, It is worth noting that all CD8$^+$ T cells in the human small intestine were CD28H$^+$ and they all express CD103 and CD69 [5], which is the main feature of tissue-resident memory (T_{RM}) T cells, which have recently been identified as a new subset of memory T cells [41–43]. In human lungs, approximately 20% of CD8$^+$ T cells express CD28H, but in T_{RM} cells there are more CD28H$^+$ T cells (27·4 *versus* 11·1%) [5]. In the spleen, although few CD8$^+$ T cells showed a T_{RM} phenotype, CD28H expression was highest in T_{RM} cells (33%) [5]. These findings indicate that in human tissues T cells expressing CD28H are mainly T cells with a T_{RM} phenotype. In this study, it was demonstrated that IL-2-stimulated T cells gradually lost CD28H expression during division, while stimulation of IL-7 plus IL-15 preserved the expression of CD28H [5]. Regardless of the stimulation by OKT3 or IL-15, stimulation with TGF-β can attract higher expression of CD28H in activated CD8$^+$ T cells [5]. It was also found that the expression of CD103$^+$CD69$^+$ T_{RM} cells in activated CD8$^+$ T cells was significantly increased upon TGF-β stimulation [5]. Furthermore, T_{RM} cells in activated CD8$^+$ T cells have higher CD28H expression and a higher percentage of CD28H-positive cells. Stimulation with TGF-β also increased the percentage of cells expressing CD28H (72·5 *versus* 89·1%) and increased the CD28H expression in T_{RM} cells [5]. These results indicate that it is likely that IL-15 and TGF-β are able to maintain CD28H expression in T cells exposed to epithelial stimulation.

Phenotypical characteristics of NK cells expressing CD28H

CD28H expression was observed on the cell surface of most freshly isolated human NK cells [4]. Based on CD56 expression, human NK cells can be divided into two subpopulations, CD56^{bright} and CD56^{dim} NK cells, which have different phenotypes and properties [44]. Studies have shown that CD56^{bright} subpopulations express a larger proportion of CD28H [4], and most CD56^{bright} NK cells have a phenotype of CD56^{bright}CD16$^-$KIR$^-$NKG2A$^+$ CD57$^-$, a phenotype representing a less mature NK cell population [4]. The expression of KIR and NKG2A is not related to the expression of CD28H in CD56^{dim} NK cells [4]. Based on CD57 expression, CD56^{dim} NK cells can also be categorized [45]. CD57$^+$ NK cells exhibit maturation, terminal differentiation and reduced proliferative capacity [45]. The CD56^{dim}CD57$^-$NK cell subset expressed a higher proportion of CD28H than the CD56^{dim}CD57$^+$NK cell subset [4]. *In-vitro*-expanded and -activated NK cells are highly cytotoxic, and have been used in clinical and basic research for decades [46,47]. A combination of CD28H and 2B4 results in synergistic activation of freshly isolated NK cells for degranulation, target cell lysis and expression of cytokines and chemokines [4]. CD28H is an addition to the NK cell co-activator family that functions synergistically with 2B4 and NKp46, but does not interact with natural killer group 2D (NKG2D), CD2 or DNAX accessory molecule-1 (DNAM-1) [4]. During prolonged activation of IL-2, expression of CD28H is turned off. CD28H can also enhance NK-cell activation through CD16 for antibody-dependent cellular cytotoxicity (ADCC). Co-engagement of CD16 with CD28H enhances its NK-mediated cell degranulation and cytotoxicity. Unlike co-acting receptors that require synergy, CD16 signaling in NK cells is sufficient to activate cytotoxicity [48,49]. Other activated receptors that enhance ADCC have also been reported, such as 2B4, CD2, NKG2D and DNAM-1 [48–51].

Mutation of tyrosine 192 on the cytoplasmic tail of CD28H abolished NK cell activation by CD28H. As B7-H5 is widely expressed in tumor tissues, the CD28H chimeric antigen receptor ($CD28H^{-}$CAR) has been designed [4], which consists of full-length CD28H fused to the cytoplasmic domain of the T cell receptor ζ chain. Notably, $CD28H^{-}$ CAR expression in NK cells triggers the cleavage of $B7\text{-}H5^{+}$ human leukocyte antigen (HLA)-E^{+} tumor cells by overcoming the inhibition of the HLA-E receptor NKG2A. Both the CD28H and the cytoplasmic domains of the ζ chain are required for this activity. Thus, CD28H is a potent activating receptor for NK cells, which broadens their anti-tumor activity, and is a potential component of NK-based CAR for cancer immunotherapy.

Phenotypical characteristics of tumor-infiltrating lymphocytes expressing CD28H

CD28H is expressed in TILs of different tumor tissues, including pancreatic carcinoma, ovarian cancer, breast carcinoma, colorectal carcinoma, lung carcinoma, melanoma and glioma [3,5]. Although TILs contain a lower percentage of CD28H than PBMCs, CD28H is still highly expressed in TILs. Unlike human peripheral blood, all $CD28H^{+}$ cells in TILs are memory-phenotype or antigen-experienced T cells. They express CD45RO but do not express CD45RA, and the majority of them express ICOS. $CD28H^{+}$ TILs do not express the aging-related molecule killer cell lectin like receptor G1 (KLRG1) or CD57, which is similar to the compartment in peripheral blood [2]. It is worth noting that after short-term cell stimulation *in vitro*, $CD28H^{+}$ T cells express less CCR7 and produce more IL-5 and less IFN-γ [5]. Consistently, in TILs, $CD28H^{+}$ cells produced less CD107a, perforin and granzyme B [5]. All these results indicate that most $CD28H^{+}$ T cells are effector/memory cells with younger and less differentiated phenotypes [3,5]. According to the expression of CD28H, CD69 and CD103, TILs from pancreatic cancer can be further divided into three $CD8^{+}$ T cell subpopulations, namely $CD28H^{-}$ T_{RM}, $CD28H^{+}$ T_{RM} and non-T_{RM}. According to gene enrichment analysis, cytokine IL-2 is mainly transcribed on $CD28H^{+}$ T_{RM} cells. In $CD28H^{+}$ T_{RM} cells the expression of CD161 and CCR6, which are important for differentiation of memory T cells, was higher. CD49a has been recently associated with highly cytotoxic T_{RM} cells, and exhibits significantly lower transcription levels in the $CD28H^{+}$ T_{RM} subpopulation [52]. $CD28H^{+}$ T cells express higher levels of IL-7R (CD127), which is related to long-term survival of T cells. Flow cytometric analysis of TILs from diverse tumor tissues indicated that $CD28H^{+}$ T_{RM} cells expressed less CD49a but had higher levels of IL-7R. These results indicate that the $CD28^{+}$ T cell subset consists of young T_{RM} cells with lower cytotoxicity [5].

Relationship between expression of CD28H and cancer prognosis

CD28H is expressed in TILs in different tumor tissues, including pancreatic carcinoma, ovarian cancer, breast carcinoma, colorectal carcinoma, lung carcinoma, melanoma and glioma [3,5]. Recent research shows that many human TILs have the characteristics of T_{RM} cells and may also be positively associated with cancer patient survival [53–55] and prognosis [56,57]. Physiologically, it is crucial for maintaining the integrity of epithelia that T_{RM} cells interact with epithelial cells [58,59]. T_{RM} cells respond quickly to attack pathogens at the barrier site before recruiting T cells from the blood [60]. Recent clinical studies have suggested that T_{RM} cells play a critical role in human cancer immunity [56,57,61]. It has been proposed that $CD28H^{+}$ T cells are characteristic of T_{RM} cells in human TILs, and experiments have shown that there is a significant positive correlation between the percentage of T_{RM} cells and $CD28H^{+}$ T cells in $CD8^{+}$ TILs from pancreatic cancer [5]. This means that amplification of $CD28H^{+}$ TILs can directly affect the number of T_{RM} cells in TILs, which may help to improve the prognosis of cancer.

B7-H5 expression in cancer and relationship with cancer prognosis

Pancreatic ductal adenocarcinoma

Reports on the prognosis of PDAC suggests that CD28H/B7-H5 is a co-stimulatory pathway that can improve the prognosis of PDAC [17,18]. Investigations found that when tumor specimens were co-cultured with T cells, PDAC cells with higher expression of B7-H5 induced a stronger immune reaction, indicated by increased proliferation of T lymphocytes and high cytokine release. Accordingly, patients with stronger expression of B7-H5 had significantly longer overall survival (OS) [17]. In Han's study of 92 patients with PDAC [18], B7-H5 was rarely detected in normal acinar, islet and ductal cells, but was widely expressed from early pancreatic pre-cancerous lesions to invasive PDAC. In the low-grade pancreatic intra-epithelial neoplasia (PanIN), the overall B7-H5 positive rate was 95% (19 of 20) and in intraductal papillary mucinous neoplasm (IPMN) was 70·73% (29 of 41). In this population, B7-H5 expression was detected in 77·17% (71 of 92) of PDAC patients and was significantly associated with a better survival rate [18]. It has been shown that B7-H5 can act as a co-stimulatory factor through its receptor CD28H. Therefore, overcoming the weak expression of CD28H in T lymphocytes which have infiltrated tumor tissue, or up-regulating expression of B7-H5 on PDCA cells, may constitute a new mechanism to improve the function of PDCA immunotherapy [17].

Lung cancer

In a retrospective study of lung cancer [19], it was found that B7-H5 is not observed in most normal lung tissue, but expressed in more than 60% of cases of non-small-cell lung carcinoma among different subtypes. High TILs intensity and epidermal growth factor receptor (EGFR) mutation status were independently associated with B7-H5 expression in lung adenocarcinoma by multivariate analysis. B7-H5 may thus be a new target for lung cancer immunotherapy.

Osteosarcoma

In a study of the prognosis of osteosarcoma [20], it was found that B7-H5 expresses in most of osteosarcoma samples. Studies have shown that B7-H5 is more common in patients with advanced disease and metastatic tissue. Higher expression of B7-H5 may lead to an increased capability to survive after leaving the primary tumor and entering the blood circulation. Patients whose tumors were ≥ 25% or ≥ 50% B7-H5-positive had a significantly worse 5-year event-free-survival. In conclusion, higher levels of B7-H5 expression in the tumor resulted in a worse prognosis.

Breast cancer

In studies of breast cancer [21], clinical features of patients with locally advanced breast cancer were collected. All these patients were first treated surgically, and followed by chemotherapy, radiation therapy or both. Triple-negative breast cancer with high B7-H5 expression is associated with lymph node positive and higher cancer stages at diagnosis, indicating that tumors with high B7-H5 expression are more aggressive and lead to worse prognosis.

Oral squamous cell carcinoma

In a study on OSCC [22], it was found that B7-H5 expression is increased in dysplasia and OSCC. Higher expression levels of B7-H5 indicate poor OS. The study also found that B7H5 was positively correlated with the expression levels of T cell immunoglobulin and mucin domain-containing protein 3 (TIM3), lymphocyte-activation gene 3 (LAG3), B7H3 (CD276), B7H4 and V-domain immunoglobulin-containing suppressor of T cell activation (VISTA). These molecules are negative immunological checkpoint molecules with increased expression in OCSS. Based on these relationships, we can speculate that B7-H5 acts as an immunosuppressive molecule in OCSS.

Human ccRCC

In studies into human ccRCC [23,24], it was found that the expression of B7-H5 mRNA is higher in human ccRCC tissues compared with adjacent normal tissues according to TCGA data, and the staining intensity of B7-H5 in human ccRCC tissues was significantly higher than that in adjacent normal tissues by immunohistochemistry study. It has been suggested that the OS rate of patients with high expression of B7-H5 in human ccRCC is significantly lower than that of patients with low expression of B7-H5 in human ccRCC, and the high B7-H5 expression in human ccRCC tissues was significantly positively correlated with larger tumor size and late tumor–node–metastasis (TNM) stage.

ICC

In a study on ICC [25], B7-H5 was identified as an independent prognostic indicator for OS. B7-H5 was used as an inhibitory checkpoint and it was found that B7-H5 expression was more frequent in ICC than PD-L1 (49·0 *versus* 28·1%). Patients with over-expression of B7-H5 were more likely to relapse and metastasize because B7-H5 expression was positively correlated with serum carcinoembryonic antigen and CA19-9 levels. Two tumor biomarkers usually reflect the primary tumor and ICC cycle [62]. It was also suggested that B7-H5 may promote tumor progression by binding to CD28H, and was identified as an independent prognostic indicator for OS.

Bladder urothelial carcinoma

In a study on BUC [26], it was found that the expression of B7-H5 in BUC tissues was significantly up-regulated compared to normal bladder tissue by immunohistochemical staining. In BUC tissues, the expression of B7-H5 was significantly related to tumor stage, tumor size, tumor grade and lymph node metastasis. B7-H5 expression is an independent prognostic factor for tumor metastasis in BUC, and could be a useful diagnostic indicator.

CRC

In a study on human CRC [27], it was found that B7-H5 expression is up-regulated in CRC patients, and B7-H5 is an independent prognostic factor for OS in CRC patients. B7-H5 expression level was significantly related to the depth of invasion and $CD8^+$ T cell infiltration status, and predicted a high mortality rate. High B7-H5 expression corresponds to deeper depth of invasion and fewer numbers of $CD8^+$ cells, and predicts poor prognosis in patients with CRC. Studies also point out that high expression of B7-H5 is closely related to poor 5-year recurrence-free survival and OS in patients with BUC.

Glioma

In a recent study on gliomas 28, B7-H5 expression was not present in glioblastoma multiforme. As the degree of

malignancy of the tumor increases, the expression of B7-H5 in glioma gradually decreases until it disappears. Down-regulated B7-H5 predicts a poor prognosis for gliomas. Patients with elevated B7-H5 have better OS. Using B7-H5 as an immunostimulant may become a valuable method for clinical treatment of glioma.

Gastric cancer

In a study on gastric cancer prognosis [29], it was found that B7-H5 expression is increased in gastric cancer tissue specimens compared to normal gastric tissue specimens by analyzing of B7-H5 expression data of gastric cancer tissue samples in the database. In addition, studies have found that high B7-H5 expression in tumor tissue is associated with advanced clinical stage, deep tumor invasion, lymph node metastasis, distant metastasis and short OS. High expression of B7-H5 has been shown to be a poor independent prognostic factor for OS in patients with gastric cancer. Different conclusions emerged in another study on the prognosis of gastric cancer [30]. It was found that normal epithelial cells showed higher B7-H5 expression than tumor cells by using immunohistochemistry, which is similar to the expression of B7-H5 in PDAC. This study also compared the expression of B7-H5 mRNA in the blood of patients with gastric cancer and normal subjects. The results suggest that the level of B7-H5 mRNA in the blood of patients is significantly lower than that of normal people. This study indicated that B7-H5 expression in blood was inversely correlated with tumor invasion depth, distant metastasis and disease stage. At the same time, it is suggested that high expression of B7-H5 in the blood leads to a higher 5-year survival rate.

Conclusions

As a new member of the B7/CD28 family, CD28H interacts with its ligand B7-H5 and regulates T cell responses. Current studies have indicated that CD28H and its ligand B7-H5 are the second signaling molecules of T lymphocytes [63]. Here, we mainly summarize the phenotypical characteristics of different immune cells expressing CD28H (Table 1). The results suggest that CD28H was not expressed on monocytes, granulocytes, B cells or MDCs. High levels of CD28H are expressed on naive T cells, ILCs, NK cells and PDCs in human peripheral blood. Moderate levels of CD28H are expressed on memory T cells. There was irreversible loss of CD28H expression with activation of immune cells after repeated stimulation, which is related to the replicative aging of $CD28H^+$ T cells [2,64]. In NK cells, CD28H is a strong activator which can be used for the lysis of $B7\text{-}H5^+$ tumor cells [4]. In human tissues,

Table 1. Phenotypical characteristics of different immune cells expressing CD28H

Immune cells	Naive T cells		Effector memory T cell		Central memory T cell	NK cell
CH28H expression	$CD28H^{+1}$	$CD28H^{-2}$	$CD28H^+$	$CD28H^-$	$CD28H^+$	$CD28H^+$
CD45RA	Most[3,2]	Little[3,2]	Medium[3,2]		Medium[2]	
CA45RO		Most[2]		Most[2]		
CCR7	Most[2]		Medium[2]		Medium[2]	
CD31	High[4,3]	Low[4,3]				
T-bet	Low[3]	High[3]				
IFN-γ	Low[3]	High[3]	Low[7]	Low[7]	Low[3]	
TNF-α	Low[3]	High[3]				
CD28	High[3]	Low[3]				
IL-2	Similar[4,3]	Similar[3]				
CXCL8	Similar[3]	Similar[3]				
CD7		Low[2,3,32–35]		Low[2,3,32–35]		
CD27		Little[2,3,32–35]		Little[2 3,32–35]		
CD28		Little[2,3 32–35]		Little[2,3,32–35]		
CD57	Low[3]	Most[2,3,32–35]	Low[3]	Most[2,3 32–35]	Low[3]	
KIR						Little[4]
NKG2A						Most[4]
CD56						Little[4]

[1]$CD28H^+$: immune cells express CD28H-positive.

[2]$CD28H^-$: immune cells express CD28H-negative.

[3]Most, medium and little: percentage of $CD28H^+$ or $CD28H^-$ immune cells expressing different cell surface molecules.

[4]High, similar and low: comparison of expression levels of different cell surface molecules in $CD28H^+$ and $CD28H^-$ T cells.

IFN = interferon; TNF = tumor necrosis factor; IL = interleukin; CXCL8 = C-X-C motif chemokine ligand 8; KIR = killer immunoglobulin-like receptors.

Table 2. Relationship between B7-H5 expression and tumor pathology and prognosis

Cancer type	Samples	B7-H5 expression	Pathological correlates	Clinical outcomes	Refs
Pancreatic ductal adenocarcinoma	136	68% (high[1]) 32% (low)	No correlation	Better OS	[17]
	92	77% (positive[2])	Highest expression in low-grade pancreatic intra-epithelial neoplasia (PanIN)	Better survival rate	[18]
Lung cancer	195 (cohort 1) 197 (cohort 2)	61% (positive) 64% (positive)	High TIL intensity and EGFR mutation status	Decreased OS (not statistically)	[19]
Osteosarcoma	55	68% (positive)	High expression in metastatic tissue	Worse five-year event-free-survival	[20]
Breast cancer	50	56% (high)	Related to regional lymph node metastasis and advanced disease	Worse prognosis	[21]
Oral squamous cell carcinoma	210	65% (high)	No correlation	Poor OS	[22]
Human clear cell renal cell carcinoma	87	Unclear	Related to larger tumor size and advanced TNM stage	Poor OS	[23]
	92	95% (positive)	Tumor size, clinical stage and histological grade	Poor OS	[24]
Intrahepatic cholangiocarcinoma	153 (cohort 1) 65 (cohort 2)	49% high) 68% (high)	More likely to relapse and metastasize	Poor OS	[25]
Bladder urothelial carcinoma	212	84% (positive) 26% (high)	Significantly related to tumor stage, tumor size, tumor grade and lymph node metastasis	Poor OS and 5-year recurrence-free survival	[26]
Colorectal carcinoma	63	48% (high)	Deeper infiltration depth	Poor OS	[27]
Glioma	669	Unclear	Low malignancy	Better OS	[28]
Gastric cancer	124	53% (high)	Related to advanced clinical stages, deep tumor infiltration, lymph node metastasis and distant metastasis	Poor OS	[29]
	111	50% (high)	inversely related to the depth of tumor invasion, distant metastasis and disease stage	Higher 5-year survival rate	[30]

[1]High: high B7-H5 expression.

[2]Positive: B7-H5 express positive.

OS = overall survival; TNM = tumor–node–metastasis.

CD28H expression identifies resident memory $CD8^+$ T cells with less cytotoxicity. The role of T_{RM} cells has recently become an active subject of investigation, and several recent clinical studies have suggested that T_{RM} cells play a vital role in human cancer immunity. The close relationship between CD28H and T_{RM} cells suggests a role for CD28H in tumor immunotherapy. As the ligand for CD28H, B7-H5 interacts with CD28H to promote T cell responses, and it has also been demonstrated to deliver a suppressive signal to T cells via an unknown receptor 9. This shows that CD28H/B7-H5 pathway both play a T cell co-inhibitory as well as a co-stimulatory role. In contrast to B7-1 and B7-2, which are limited on professional APCs, B7-H5 is preferentially transcribed in peripheral tissues. This is likely to be essential for CD28H/B7-H5 to co-stimulate newly produced effectors or effector/memory T cells in peripheral tissues. B7-H5 is not detected in T cells. However, B7-H5 is likely to be expressed on B cells, dendritic cells and macrophages as an inducing molecule of the inflammatory response. This is different from other B7s. B7-H5 is widely expressed in tumor cells (Table 2). High expression of B7-H5 in tumor leads to a better prognosis of PDAC and glioma, but leads to a worse prognosis of lung cancer, osteosarcoma, breast cancer, OSCC, human ccRCC, ICC, BUC and CRC. There are contradictions between the two studies [29,30] on the expression characteristics of B7-H5 in gastric cancer tumor tissues and their relationship with tumor prognosis, and further research is needed. Based on current research, B7-H5 and CD28H do not bind to other known B7 receptors or ligands, respectively, and the CD28H/B7-H5 pathway is not present in rats and mice. This is different from other B7/CD28 pathways. Much remains to be discovered about this new CD28H/B7-H5 pathway. The underlying mechanism regulating B7-H5 or CD28H is still unknown.

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Disclosure

The authors declare no conflicts of interest.

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