Published in final edited form as:

Gynecol Oncol. 2014 July ; 134(1): 181–189. doi:10.1016/j.ygyno.2014.03.553.

B7-H4 as a potential target for immunotherapy for gynecologic cancers: A closer look

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Abstract

B7-H4 is a transmembrane protein that binds an unknown receptor on activated T cells resulting in inhibition of T-cell effector function via cell cycle arrest, decreased proliferation, and reduced IL-2 production. B7-H4 is up-regulated on the surface of cancer cells and immunosuppressive tumor-associated macrophages (TAMs) in a variety of human cancers.

Notably, B7-H4 expression levels inversely correlate with patient survival in ovarian cancer, making B7-H4 an attractive candidate for therapeutic intervention. Here, we summarize the experimental data and methodologies that have revealed B7-H4's mRNA and protein expression and function in both mice and humans since its discovery in 2003, with a specific focus on B7-H4's role in ovarian cancer. We also underscore the discrepancies in published data due to high variability in methodology and use of different antibodies, most of which are not commercially available. Finally, since B7-H4 is expressed on tumor cells and TAMs in various cancer types, directing therapeutics against B7-H4 could have tremendous synergistic outcomes in favorably altering the tumor micro-environment and eliminating cancer cells. We highlight the therapeutic potential of targeting B7-H4, both by comparing other negative immune modulators such as PD-1 and CTLA-4 and by identifying novel methods to target B7-H4 directly or indirectly to overcome B7-H4-mediated T-cell inhibition.

Keywords

Ovarian cancer; B7-H4; Targeted therapies

Introduction

Ovarian cancer is the fifth most common cause of cancer-related death among women, accounting for approximately 14,000 deaths and 22,000 new cases estimated in 2014 in the United States [1]. Most women are asymptomatic and diagnosed late in the cancer's progression, with 5-year survival rates of only 18–45% in stages III–IV [2]. Few therapies are effective and most women ultimately die of the disease [3]. The past few decades have brought targeted treatments for many cancer types. For ovarian cancer, the most effective new treatment has been bevacizumab [4], and while effective, results have been modest [5]. Given the limitations in current treatments and the correlation of endogenous immune responses with improved survival in ovarian cancer, research in targeted immunotherapy is of great interest.

One of the first publications showing immunogenicity of ovarian cancer observed that patients with detectable tumor infiltrating T cells (TILs) had a 38% 5-year overall survival rate compared to 4.5% for patients who did not [6]. A recent meta-analysis reviewed 10 studies that investigated CD3⁺ or CD8⁺ T cells in ovarian cancer, and all studies showed significantly longer survival with intraepithelial TILs [7]. While these studies suggest the importance of the immune system in the control of ovarian cancer, many mechanisms of immune evasion exist. This review focuses on the expression of one inhibitory protein, B7-H4 that facilitates escape from the host T-cell response, augments tumor cell proliferation [8] and anti-apoptotic ability [9], and promotes tumorigenesis [8–10].

Identification

B7-H4 is a transmembrane protein in the B7 family identified in 2003 by three separate groups, each performing searches in Expressed Sequence Tagged (EST) databases for sequences similar to B7 family molecules [11–13]. In June 2003, Sica et al. identified B7-H4 from a human EST search and predicted B7-H4 to be a 282 amino acid, type I transmembrane protein that includes an extracellular domain, hydro-phobic transmembrane domain, and a very short, 2 amino acid long intracellular domain [11]. Almost simultaneously, Prasad et al. also identified B7-H4 by the name of B7S1 [13], and Zang et al. identified mouse B7-H4 with the name B7 $\times$ [12].

After the initial discovery, B7-H4 (DD-O110) was again independently identified in 2004–2005 by Salceda et al., in a genomic effort to discover genes up-regulated in tumors, specifically looking for targets for breast and ovarian cancers [9]. The authors predicted the protein core to be 28.8 kDa, yet by western blot it is in the 40–80 kDa range because of glycosylation [9].

B7-H4 homology

B7-H4 protein is 25% homologous with other B7 molecules and is evolutionarily conserved in lower vertebrates including bony fish [14]. B7-H4 has 87% amino acid identity with mouse B7-H4 [11]; however several key differences exist between the two. First, murine VTCN1 (the gene encoding B7-H4) encodes a 283 amino acid protein, while human VTCN1 encodes a 282 amino acid protein. Second, in 2003 Prasad et al. demonstrated that

murine B7-H4 is anchored to the cell membrane via glycosyl phosphatidylinositol (GPI) linkage since the protein lacked an obvious transmembrane region and its cell surface expression was sensitive to phosphatidylinositol-specific phospholipase C (Pi-PLC) treatment [13]. However, Salceda et al. was unable to release human B7-H4 with Pi-PLC from a breast cancer line, and concluded that human B7-H4 is not GPI-linked [9]. A third difference was described by Zhang et al. in a recent publication that found a functional nuclear localization sequence (NLS) that is necessary for shuttling of B7-H4 protein between the nucleus and cytoplasm in human cells in vitro [8]. The murine B7-H4 sequence, however, did not contain a putative NLS [8], although a more in depth study of the potential shuttling capacity of mouse B7-H4 is needed.

B7-H4 expression

Expression of mRNA, but not protein on normal cells

In healthy individuals, B7-H4 mRNA is detected at low levels in many non-lymphoid tissues such as the placenta, lung, liver, ovary and spleen [11,15]. However, B7-H4 protein expression seems to be limited due to tight translational control in peripheral tissues in both humans [15,16] and mice [13]. In human tissue, B7-H4 mRNA was found in spleen, lung, and thymus by northern blot analysis and in human placenta, liver, skeletal muscle, kidney, pancreas, prostate, testis, ovary, and small intestine by RT-PCR. However, consistent with many publications, immunohistochemical analysis using a human B7-H4 mAb (hH4) was negative [11], suggesting that B7-H4 protein is not constitutively expressed on healthy peripheral tissues in humans.

The endogenous expression of B7-H4 on immune cells in healthy individuals and mice is also not fully understood. By flow cytometric analysis (clone hH4), human T cells, B cells, monocytes, and DC do not express B7-H4 on the cell surface, but could be mildly induced by supra-physiological, in vitro stimulation with LPS, PHA, IFN- γ , PMA, or ionomycin after three days [11]. Although this study showed that human T cells express B7-H4 protein, this observation has not been repeated in following studies under physiologic conditions. In human ovarian cancer patients, B7-H4 was not seen on CD3⁺ TILs or CD3⁺ T cells in ascites fluid [16].

However, there is a difficulty in correctly assessing cell surface B7-H4 expression on tumor cells and immune cells. Along with the recent finding that B7-H4 shuttles between the nucleus and cytoplasm [8], part of the inconsistency in B7-H4 expression noted in the literature is due to the use of dissimilar methodologies. He et al. clearly summarize clinical studies showing B7-H4 expression by IHC, ELISA, and RT-PCR, but do not discuss surface expression by flow cytometry since this method is rarely used [17]. Additionally, many different antibodies have been utilized, most of which are not commercially available and their differences are highlighted by specificity, technical use, and publication in Table 1.

In 2003, Choi et al. prepared mAbs (hH4.3 and hH4.2) against human B7-H4 that showed no staining of normal human tissue by IHC, even though they detected mRNA in placenta, kidney, liver, lung, ovary, testis, and spleen [15]. However, they did show staining in 85% (22 of 26) of freshly isolated ovarian tumors, 31% (5 of 16) of lung cancer tissue, and 0%

(0/17) of melanomas. Microscopically, B7-H4 was found either in cytoplasm or plasma membrane [15], consistent with Zhang's 2013 study that describes B7-H4's ability to shuttle between the nucleus and the cytoplasm.

In 2005, Salceda et al. developed antibody A57.1 for IHC on human samples that revealed intense circumferential membrane and cytoplasmic staining of ovarian serous adenocarcinoma and breast ductal adenocarcinoma [9]. No B7-H4 staining was seen on normal ovarian tissue and less intense staining, restricted to apical cytoplasmic membrane of ductal and lobular cells was seen in normal breast tissue. Tringler et al. used the same antibody (A57.1) for IHC to show that B7-H4 was expressed in cytoplasm with pronounced circumferential membranous component in the majority of serous cystadenocarcinomas, while serous tumors of low malignant potential, serous cystadenomas, and endometriosis show apical membranous staining [18]. Yet, B7-H4 was rarely expressed in mucinous carcinomas and was consistently negative in normal ovarian tissue. Other studies using A57.1 for IHC detected B7-H4 in 95.4% of primary breast cancers [19,20].

In 2011, Qian et al. developed two novel mAbs to the extracellular domain of B7-H4 (4H8 and 3E8) [21,22]. By IHC using 4H8, the highest proportion of stained cells and the highest intensity staining were seen in human tumor tissues from ovary, uterus, breast, and colon compared to corresponding normal tissues. Similar to Simon et al. [23], staining of B7-H4 was also associated with stage of disease. This effect was not seen in cancer from liver or stomach, and the differences were not significant in cancers such as thyroid, kidney, and prostate [21]. Using 3E8, B7-H4 expression was higher in human tumors from uterus, breast, and colon, and again was associated with stage [21]. IHC showed that normal breast, uterus, and ovary tissues had low staining scores (<2) with predominantly apical membranous staining, whereas most tumors in these tissues showed overexpression stained in diffuse pattern either in membrane or in cytoplasm of cells. By flow analysis, they also showed B7-H4 expression on leukemic cells, opening the door to potentially target hematological as well as solid malignancies.

To further underscore the discrepancies in B7-H4 expression reported, in 2006 using hH4.1, Kryczek et al. showed that 100% of freshly resected ovarian tumor cells, as well as tumor cell lines, exclusively express B7-H4 intracellularly, with <5% surface B7-H4 by flow cytometry [16]. Yet other publications have shown in vivo surface expression. A recent publication by Dangaj et al. showed tumor cell surface expression of B7-H4 in all 15 fresh primary solid tumors and ascites samples, as well as on tumor-associated macrophages, using a commercially available antibody. This study also demonstrated up-regulation of B7-H4 expression in OVCAR5 tumor cells after in vivo growth (4–38%), which then returned to undetectable levels after short term culture [24]. Chen et al. also showed that surface expression of B7-H4 on a lung cancer cell line was only seen after in vivo implantation [25]. Together these studies demonstrate that the surface expression of B7-H4 on tumor cells is inconsistent in the literature and difficult to analyze, possibly due to different antibodies used or dynamic expression as further discussed below.

TAMs/regulation of surface expression in the tumor microenvironment

The cell surface expression of B7-H4 protein on tumor-associated macrophages (TAMs) [16] in ovarian cancer [24] is more consistently seen. Greater than 70% of CD14⁺ tumor macrophages freshly isolated from tumor ascites express B7-H4 by flow cytometry, and a similar percentage is seen by immunofluorescence for tumor macrophages [16].

Mechanistically, surface expression of B7-H4 is likely dynamic and influenced by its microenvironment. Exposure to tumor-associated macrophages (TAMs) induces membrane bound B7-H4 expression on a lung cancer cell line [25], and is likely to occur in other in vitro models once formally examined. In the tumor microenvironment, high levels of secreted IL-6 and IL-10 cause up-regulation of B7-H4 expression on the surface of TAMs [16,26,27]. Although its mechanism is not fully understood, B7-H4 expression on macrophages is stimulated by T-regulatory cells (Tregs), and not by conventional T cells [26]. Tregs induce IL-6 and IL-10 production by macrophages that leads to auto-crine signaling that augments B7-H4 expression on macrophages [16]. Of note, high serum levels of IL-6 are seen in serous endometrial cancers, a more aggressive form of endometrial cancer compared to endometrioid cancers [28].

In support of their in vitro work, in 2007 Kryczek et al. demonstrated that B7-H4 expression in macrophages was significantly correlated with Treg cell numbers in patients with ovarian cancer [27]. Additionally, Treg count and macrophage B7-H4, but not tumor B7-H4, were negatively associated with patient outcome. Although up-regulation of B7-H4 expression on tumor cells was not seen in the 2006 report by Kryczek, a study by Chen et al. in 2011 demonstrated that IL-10 secreted by TAMs induces B7-H4 expression of tumor cells in a lung carcinoma mouse model in vivo [10]. Therefore, a stronger evaluation of the impact of cytokines or other factors in the induction of B7-H4 protein expression is necessary to fully understand the mechanism of B7-H4 up-regulation in the tumor microenvironment.

B7-H4 receptor

The receptor for B7-H4 is yet to be identified. Initially, BTLA was thought to be the B7-H4 ligand since activated T cells from BTLA-deficient mice did not bind B7-H4 [12], but in 2005, BTLA and B7-H4 were shown to not directly interact [29]. It is accepted, however, that the putative B7-H4 receptor is expressed on activated T cells. The initial three B7-H4 papers in 2003 all used either mouse or human B7-H4 fusion proteins to investigate the putative receptor's function and binding on both resting and activated mouse and human T cells [11–13]. By flow cytometric analysis, 20–30% of activated T cells bound recombinant B7-H4 extracellular domain fusion protein after 68 h (but not 44 h) of stimulation with anti-CD3 mAb [11]. Using the B7-H4 fusion protein, Zang et al. showed that T cells express a B7-H4 receptor by flow analysis after T cell stimulation distinct from CD28, CTLA-4, ICOS, or PD-1 [11,12].

B7-H4 function

B7-H4 inhibits T-cell function

A growing body of literature suggests that B7-H4 on the cell surface binds the putative B7-H4 receptor on activated CD4⁺ and CD8⁺ T cells resulting in the inhibition of effector function via cell cycle arrest, decreased proliferation, and reduced IL-2 production. Various studies have reinforced the role of B7-H4 in diminishing both CD4⁺ and CD8⁺ T-cell functionality. Sica et al. first showed that a B7-H4 immunoglobulin fusion protein (B7-H4.Ig) decreased the proliferation, production of IL-2 and IL-10, and cytolytic activity of murine CD4⁺ T cells 48–72 h after stimulation with anti-CD3 antibodies [11]. Exposure to B7-H4.Ig also caused a significant inhibition of cell division, exhibiting an increase in the G₀/G₁ phase of the cell cycle while apoptosis was not affected. In addition to studies utilizing soluble B7-H4.Ig fusion proteins, B7-H4 expressed on the cell surface also functionally inhibits proliferation and cytokine production of murine T cells [12]. Similarly, Wang et al. later showed the most profound inhibition of IL-2 secretion at 16 h in the presence of B7-H4 showing decreases of 75.4%, 72.8%, and 71.5% in CD3⁺, CD4⁺, and CD8⁺ T cells, respectively [30].

Similar results have emerged using human cell cultures. Ou et al. [31] was one of first studies to show B7-H4-mediated functional inhibition of human T cells. Much like previous reports using murine T cells, immobilized B7-H4.Ig fusion protein inhibited T-cell proliferation, arrested T cells in G₀/G₁ of the cell cycle, and induced T-cell apoptosis in activated CD4⁺ and CD8⁺ T cells after 3–5 days in the presence of B7-H4 [31]. The diminished functional capacity of human T cells in the presence of cellular B7-H4 at both endogenous and over-expressed levels of B7-H4 protein has also been assessed, confirming the findings that B7-H4 mediates functional inhibition of human T cells [16].

Role in autoimmunity and cancer

Disruption of the restricted regulation of B7-H4 expression is implicated in autoimmune diseases and cancer. Loss of endogenous B7-H4 expression may play a role during induction and/or promotion of auto-immune diseases including rheumatoid arthritis and diabetes [32]. For example, Wei et al. demonstrated B7-H4 expression in murine pancreatic Langerhans islet-cells by IHC, and showed that B7-H4 deficient mice had a more aggressive form of diabetes in the presence of adoptively transferred, transgenic islet-reactive T cells, while overexpression of B7-H4 in the islet decreased the progression of diabetes [33]. However, the endogenous role of B7-H4 in normal mice is not well elucidated, as B7-H4^{-/-} mice do not spontaneously develop autoimmunity and do mount normal T cell responses against viral infection [34].

While loss of B7-H4 expression has been studied in the context of autoimmunity, overexpression of B7-H4 is seen in various types and stages of ovarian, lung, ductal breast, renal cell, uterine, and endometrial cancers [15,35]. In the vast majority of breast and ovarian cancers, B7-H4 mRNA is at least 2-fold higher than mRNA found in normal tissue [9]. B7-H4 protein is detected in half of early stage and two-thirds of late stage ovarian tumor samples [36], but not in corresponding normal ovarian tissue [9,19,24]. By protein

ELISA from ovarian cancer patient tissue lysates, Simon et al. showed that patients with higher stage disease had increased B7-H4 protein expression: 48% for stage I, 55% for stage II, and 67% for stages III–IV, while normal and benign tumors had very low expression [36]. Similar to correlations found in ovarian cancer lysate samples [36], B7-H4 expression in tumor tissues from breast, uterus, ovary, colon, and pancreas showed a statistically significant increase in percentage B7-H4 expressed and stage [21]. There were 54.1% overexpression in stages III–IV, and 36.6% and 43.8% overexpressed in stages I and II respectively.

Other effects on cancer cells: apoptosis, tumorigenesis, metastasis

Although B7-H4 research has been focused on B7-H4's effects as an immune-modulatory signaling molecule, several papers indicate distinct intracellular effects such as decreased apoptosis, enhanced proliferation, and facilitated metastasis in B7-H4-expressing cells themselves.

B7-H4 may protect cancer cells from apoptosis as knockdown of B7-H4 in a breast cancer cell line increased apoptosis, and overexpression of B7-H4 protected epithelial cells from anoikis [9]. Additionally, ovarian cancer cell lines transfected with B7-H4 have increased proliferation, cell adhesion, migration, and invasion in vitro [38]. An in vivo model of a B7-H4-engineered ovarian cancer cell line demonstrated increased tumor growth compared to its parental cell line in immune-deficient mice, pointing to a possible functional role of B7-H4 in tumorigenesis even in the absence of host immunity [9,38]. A recent study also showed that overexpression of wild-type B7-H4 in HEK293 cells enhanced proliferation by promoting G₁/S phase transition [8]. This data was further validated by Zhang et al.'s discovery of B7-H4's functional nuclear localization sequence (NLS) where mutation of the NLS abrogated the nuclear effects of B7-H4, deeming the NLS necessary to promote G₁/S transition, decrease apoptosis, and increase tumor growth in vivo [8]. B7-H4 deficient mice also point to B7-H4's role in metastasis. In a 4T1 metastatic breast cancer model, B7-H4^{-/-} mice have fewer lung nodules, enhanced survival, and decreased infiltration of immunosuppressive cells (tumor associated macrophages and Tregs) [39], suggesting that B7-H4 plays a role in helping metastasizing cancer cells escape local antitumor immune responses.

Soluble protein

B7-H4 can also exist in a soluble form in serum of diseased patients. Levels of soluble, serum B7-H4 correlate with tumor stage, poor prognosis, and pathological types [17], and this topic was covered more thoroughly in a review paper by Fauci et al. [40]. In short, B7-H4 is elevated in ovarian cancer patients when compared with healthy controls, and not significantly elevated in other types of cancer such as colon, breast, lung, and prostate [23]. In early stage patients, adding B7-H4 to CA125 diagnostic testing improved detection of ovarian cancer from 52% to 65% [36], and in combination with other serum markers, B7-H4 was useful in predicting short-term (1 year) survival [41].

Therapeutics

Cutting the brakes: CTLA-4 and PD-1

Comparison of B7-H4 to other negative immune molecules is warranted. CTLA-4 is expressed on T cells and its inhibitory effect occurs in secondary lymphoid tissues, PD-L1 is on APCs and its effect is mostly in peripheral tissues during inflammatory response, yet B7-H4 likely has more of a local effect at tumor site and may be a better target for ovarian and gynecologic cancers [42,43]. The CTLA-4 antibody ipilimumab (Yervoy) was FDA approved for melanoma in 2011, and is currently being investigated as a single or combinatorial therapy in clinical trials (clinicaltrials.gov). Hodi et al. showed modest antitumor effects in patients with stage IV ovarian cancer patients, 1/9 patients with a regression of disease, and 3/9 had stable disease [44]. PD-L1 pathway in ovarian cancer is of interest as higher PD-L1 expression is associated with poorer prognosis [42]. A Phase I trial in 2012 by Brahmer et al. demonstrated responses in only 1/17 ovarian cancer patients and these samples were not evaluated for expression of PD-L1 [43]. Moving forward, synergistic targeting of multiple negative immune-modulatory pathways may be necessary to fight disease.

Cutting the brakes: B7-H4

Although not yet targeted clinically, the advantage of targeting B7-H4 may be several folds since B7-H4 is expressed on tumor cells and TAMs in various cancer types. Additionally, since B7-H4 plays a role in immune evasion (Fig. 1) and tumorigenesis, eliminating or diminishing the effects of B7-H4 may positively affect the host immune response and negatively affect tumor cell survival simultaneously. Although no clinical studies targeting B7-H4 have been performed to date, potential methods discussed here include siRNA, immunotoxins, blocking antibodies, or T-cell based immunotherapy. Other potential ways to target B7-H4 that are not discussed in detail here are via antibody drug conjugate (ADC) and a B7-H4-specific vaccine.

Oligonucleotides, siRNA, shRNA

Oligonucleotides, short hairpin (shRNA), or small interfering (siRNA) RNA are used to decrease expression of protein in vitro (Fig. 2), yet these tools are difficult to use in clinical therapy. To reduce B7-H4 expression, Kryczek et al. designed a B7-H4-specific morpholino that specifically blocked B7-H4 expression in macrophages, resulting in increased T-cell proliferation and reduced tumor volumes in mice with tumor associated antigen (TAA)-specific T cells [16]. Knockdown of B7-H4 expression in breast cancer cell lines showed increased apoptosis of tumor cells in the absence of immune cells in vitro, while knockdown in a nonsmall-cell lung cancer cell line leads to enhanced Jurkat T cell proliferation, decreased apoptosis, and cell cycle progression [45]. The current challenge is to identify and develop safe delivery methods for these molecules to pursue clinical use (Fig. 2).

Immunotoxins

Immunotoxins are chimeric proteins that contain a targeting moiety (such as an antibody) linked to a toxin [46]. Immunotoxins have been studied for cancer treatment as well as

autoimmune disease. SS1P immunotoxin (anti-mesothelin linked to a truncated form of *Pseudomonas* exotoxin) has shown modest activity in Phase I clinical trials in patients with mesothelioma, ovarian and pancreatic cancers [47], and anti-Lewis Y immunotoxin has shown one complete remission in a patient with metastatic breast cancer [48]. Development of an immunotoxin with a targeting moiety against B7-H4 could be promising (Fig. 2). However, as described in the next section, finding human/ humanized antibodies against B7-H4 has been problematic.

Blocking antibodies

One of the best therapeutic methods to efficiently disrupt the functionality of cell surface proteins in the tumor microenvironment is the use of monoclonal antibodies (mAbs), a strategy that has shown promise targeting other negative immune modulators such as PD-L1, PD-1, and CTLA-4. Blocking the putative B7-H4 receptor on T cells from engaging with B7-H4 on the surface of tumor cells or macrophages could be achieved using an anti-B7-H4 antibody (Fig. 2).

Several studies to date have utilized anti-B7-H4 antibodies for in vitro and in vivo studies, many of which are described in Table 1. Several anti-mouse B7-H4 antibodies have demonstrated rescue of T-cell function in the presence of B7-H4 in vitro. Prasad et al. showed augmented IL-2 production and increased T-cell proliferation post-T-cell activation in the presence of B7-H4 and antibody. Sica et al. developed an anti-mouse B7-H4 mAb that showed a partial neutralization of the inhibition of T-cell proliferation post-incubation with B7-H4-transfected cells [11]. Another anti-mouse B7-H4 antibody (3E8) showed reversal of B7-H4-mediated decreases in cytokine secretion post-murine T-cell activation [22]. Additionally, anti-mouse B7-H4 antibodies are able to augment T-cell responses in vivo [11,13] and decrease tumor burden in a syngeneic B7-H4-expressing murine lung cancer model [49].

Although not as widely available or studied as anti-mouse B7-H4 antibodies, anti-human B7-H4 antibodies have been efficacious in rescuing B7-H4-mediated functional inhibition of T cells in vitro. Xue et al. utilized an anti-human B7-H4 blocking antibody to significantly attenuate the T-cell inhibitory effects of B7-H4 expressed by human bone marrow-derived mesenchymal stem cells (hBMSCs) [50]. However, in vivo analysis of the ability of an anti-B7-H4 mAb to reduce human tumor burden in a xenograft model has not been assessed. A review by He et al. [17] highlighted the fact that efficient neutralizing antibodies specific for human B7-H4 are not yet available.

A recently published paper by Dangaj et al. demonstrates potential for a novel therapeutic B7-H4 by identifying and characterizing recombinant single chain variable fragments (scFv) isolated from a yeast display library [24]. These anti-B7-H4 scFvs specifically rescued T-cell function from B7-H4-mediated T-cell inhibition, as demonstrated by increased IFN- γ secretion, up-regulation of CD69 expression, and augmented T-cell proliferation in response to anti-CD3 stimulation and inhibition through recombinant, human B7-H4 protein. These scFvs also specifically abrogated B7-H4-mediated functional inhibition of HER-2 TCR-engineered T cells in vitro in the presence of B7-H4-expressing APCs pulsed with HER-2 peptide or a B7-H4-engineered tumor cell line. Delayed growth of human B7-H4⁺ ovarian

cancer tumor was observed with intraperitoneal injections of the anti-B7-H4 scFv in mice [24].

Although promising, moving forward with clinical trials utilizing scFv alone as systemic therapy may be difficult due to problems with tumor penetration, rapid renal clearance, and lack of effector functions. However, scFvs are versatile and can be conjugated to a myriad of efficacious tools including radio-isotopes for imaging, endotoxins, nano-particles, or even T-cell signaling domains.

T-cell therapy

In spite of the association between intratumoral T cell accumulation and improved overall survival in ovarian cancer [6], targeting of solid epithelial tumors such as ovarian malignancies with naturally-occurring tumor-reactive T cells has not been highly efficacious [51–54]. An alternative approach is to genetically modify T cells to express chimeric antigen receptors (CARs), proteins that combine antibody recognition and TCR signaling [55]. The promise of CAR T-cell therapy has been shown in the treatment of B-cell malignancies [56,57], but to date, hasn't been effective in ovarian cancer.

The first CAR T cell trial conducted utilized T cells targeting alpha-folate receptor in ovarian cancer [58], however, no reduction in tumor burden was seen. Lack of clinical efficacy was thought to be attributed to, in part, poor CAR T cell persistence after infusion due to the lack of T-cell co-stimulatory domains in the CAR and the development of an inhibitory factor in the serum of patients that reduced CAR activity [58,59]. This approach has been revitalized for ovarian cancer using a CAR that harnesses the potent pro-survival signals of 4-1BB (CD137) domain to improve T-cell persistence in vivo [59], and will soon begin in a Phase I clinical trial [60].

It is feasible that failures in prior T-cell based immune therapies for ovarian cancer may also be related to the lack of understanding of the negative effects on anti-tumor T-cells of inhibitory molecules, such as B7-H4. Therefore, it is conceivable that CAR T-cell approaches directly targeting B7-H4 may be effective by simultaneously destroying B7-H4⁺ tumor cells/TAMs and eliminating negative-immune modulating cells from the tumor microenvironment. Although not yet tested in the clinic, an anti-B7-H4-specific CAR could be generated through combination of an anti-B7-H4 single chain variable fragment (scFv) with optimal T cell co-stimulation domains (Fig. 3). Alternatively, the activity of CAR T cells directed against a tumor antigen, such as alpha folate receptor, may be bolstered by co-administration of a B7-H4 blocking antibody.

Additional therapies to resist the inhibitory effects of B7-H4 may prove useful in the field of T-cell based therapy. One example is the use of bi-specific antibodies that engage anti-CD3 expressed on T-cells and a second antigen expressed on tumor cells, such as anti-B7-H4, to facilitate the redirection of T cells to B7-H4 expressed on cancer cells and TAMs (Fig. 2).

Exploiting B7-H4 as a target with a T-cell based therapy, or overcoming its inhibitory effect in T cell-therapy, may be a therapeutic option in ovarian and other B7-H4⁺ malignancies in the near future.

Conclusion

In healthy individuals, B7-H4 mRNA is detected at low levels in many tissues [11,15] however, B7-H4 protein expression seems to be limited due to tight translational control in peripheral tissues in both humans [15,16] and mice [13]. This regulation of restricted and inducible B7-H4 expression, however, does not take place in cancer. Instead, B7-H4 is overexpressed in multiple stages of cancer and various types including ovarian, uterine, and endometrial cancers [15,35]. Still, its pattern of surface expression remains debated in the published literature. Within the tumor, B7-H4 protein is also expressed on the cell surface of immunosuppressive tumor-associated macrophages (TAMs) [16] and tumor cells of ovarian cancer [24]. Based upon its potential, restricted expression and functional role in cancer, B7-H4 remains a high priority candidate for targeted inhibition or elimination in ovarian cancer and other types of cancer.

To date, clinical trials utilizing an anti-human B7-H4 blocking antibody have not been conducted in any disease indication. Since many clinical strategies rely on antibody/scFv specificity for generation of therapies, including monoclonal antibodies, immunotoxins, radio-therapy, bi-specific antibodies, and chimeric antigen receptors, generation of a highly specific anti-human B7-H4 antibody would open the door to robust preclinical studies. Results from several studies utilizing various anti-mouse B7-H4 antibodies have seen success in rescuing T-cell function in vitro [11,13,22], augmenting T-cell responses in vivo [11,13], and reducing tumor burden in a murine lung cancer model [49], demonstrating a proof of concept for targeting B7-H4. However, effective anti-human B7-H4 antibodies are not yet available [17] and pre-clinical studies have not yet assessed the ability of an anti-human B7-H4 mAb to reduce human cancer burden in vivo. Novel anti-human B7-H4 scFvs that impact human tumor progression in vivo have recently been described [24] and may have implications if engineered as radio-isotopes for imaging, or as immunotoxins, nanoparticles, or CARs for therapy.

While the last decade has offered insight into the functional role of B7-H4 in tumor biology and immune evasion, no anti-B7-H4 targeted therapies have been investigated clinically. Many opportunities still exist to determine if stand-alone or combinatorial therapies to directly target or overcome B7-H4-mediated hypo-function of T cells in the ovarian tumor microenvironment can provide clinical benefit in women with ovarian cancer.

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HIGHLIGHTS

- B7-H4 expression levels inversely correlate with patient survival in ovarian cancer.
- Validation of B7-H4 surface expression in ovarian tumors is difficult due to discrepancies in reagents and methodologies among studies.
- Novel anti-B7-H4-targeted therapies are warranted, but have not yet been explored in clinical trials.

Fig 1.

B7-H4 attenuates optimal T-cell function. B7-H4 protein expressed on either the cancer cell or tumor-associated macrophages (TAMs) binds the putative B7-H4 receptor on tumor-associated antigen (TAA)-specific T-cells. Subsequent inhibitory signaling in the TAA-specific T cells cause cell cycle arrest, decreased proliferation, and reduced IL-2 production. This leads to sub-optimal cytotoxic effector functions of CD8+ TAA-specific T cells including reduced secretion of cytokines (IFN- γ , IL-2, and TNF) and perforin/granzyme compared to non-inhibited T cells.

Fig 2.

Rescue of T-cell function by anti-B7-H4 treatment modalities. Although not yet tested in clinical or pre-clinical models, several therapies targeting B7-H4 could have important anti-inhibitory effects in the tumor microenvironment. Anti-B7-H4 oligonucleotides could destroy B7-H4 mRNA and potentially eliminate B7-H4 protein from the surface of tumor or tumor-associated macrophages (TAMs). Anti-B7-H4 blocking antibodies could prevent B7-H4 ligation with the putative B7-H4 receptor on T cells, thereby hindering B7-H4 inhibitory signals in TAA-specific T cells. Anti-B7-H4 immunotoxins composed of an anti-B7-H4 blocking antibody could impede B7-H4 signaling and deliver an immunotoxin to B7-H4⁺ target cells. Finally, an anti-B7-H4/anti-CD3 bi-specific antibody could also dampen B7-H4 inhibitory signaling and direct T cells to tumor cells/TAMs. All these modalities would “rescue” tumor-associated antigen (TAA)-specific T-cell function from B7-H4-mediated functional inhibition.

Fig. 3.

Direct killing of B7-H4⁺ targets by anti-B7-H4 CARs. Genetic engineering of primary CD8⁺ T-cells to express anti-B7-H4 chimeric antigen receptors (CARs) that could directly kill both B7-H4⁺ tumor cells and tumor-associated macrophages (TAMs), resulting in destruction of both tumor and key components of the negative immune-modulatory tumor microenvironment. This opens the door for further destruction of non-B7-H4-expressing tumor cells by host tumor-associated antigen (TAA)-specific T cells that may have been previously inhibited by B7-H4.

Table 1

Uses and availability of published anti-B7-H4 antibodies

Antibody name	Antibody type	Notes	Use	Reference	Commercially available
8H4, 8H4.1	Hamster anti-mouse B7-H4 IgG mAbs		<i>Blocking antibody</i> in vivo/in vitro (autoimmunity model)	Sica et al. [11]	No
hH4	Mouse anti-human IgG1 mAb		Flow cytometry and IHC on human cells, flow cytometry of primary human ovarian cancer cells and TAMs	Sica et al. [11], Dangaj et al. [24]	BD Biosciences (MIH43-Ab derived from hH4)
hH4.3, hH4.2	Mouse anti-human B7-H4 IgG1 mAbs		IHC on snap frozen samples (not on paraffin embedded), Flow cytometry	Choi et al. [15]	BD Biosciences (MIH43-Ab derived from hH4)
hH4.1	Mouse anti-human B7-H4 IgG1 mAb		IF and flow cytometry	Kryczek et al. [16–26], Krambeck et al. [35]	BD Biosciences (MIH43-Ab derived from hH4)
Clone 9 and Clone 54	Rat anti-mouse B7-H4 IgG mAbs		Blocking Ab in vitro/in vivo, flow cytometry, IHC	Prasad et al. [13], Wei et al. [33], Chen et al. [49], Abadi et al. [39]	eBiosciences and Santa Cruz (clone 9)
A57.1	Mouse anti-human B7-H4 mAb		IHC on paraffin embedded samples, WB	Salceda et al. [9], Tringler et al. [19–20], Mugler et al. [20], Miyatake et al. (2007) <i>Gyn Onc.</i> , Simon et al. [23]	No
MIH29	Rat anti-mouse B7-H4 mAb		Flow cytometry, <i>blocking antibody</i> in mice in vivo	Kamimura et al. (2009) <i>Biochem & Biophys Res Comm</i> , Yamaura et al. [37]	BD Biosciences
3C8	Mouse anti-human B7-H4 mAb		Flow cytometry hBMDCs, IF, <i>neutralizing antibody</i> in vitro attenuated inhibitory effects on T-cells, IHC in human cancer tissues and IF in cancer cell lines	Xue et al. [50], Zhang et al. [8]	No
4H8	Mouse anti-mouse B7-H4 mAb	Binds extracellular portion of B7-H4	ELISA, IHC human tissue	Qian et al. [21]	No
3E8	Mouse anti-mouse IgM mAb	Specific to human and mouse B7-H4	ELISA, IHC human tissue, flow cytometry on cell lines	Qian et al. [22]	No
H74	Mouse anti-human B7-H4 IgG1 mAb	Cross-reactivity to other species unknown	IF and in vitro restoration of T-cell proliferation	Zhang et al. [8]	eBiosciences
G-18	Goat anti-human B7-H4 IgG pAb	Epitope maps near N-terminus	Recommended for WB, ELISA, and IF	Zhang et al. [8]	Santa Cruz, blocking peptide available

Antibody name	Antibody type	Notes	Use	Reference	Commercially available
		of human B7-H4; recommended for detection of mouse, rat and human B7-H4; cross reacts with equine, canine, bovine and porcine species			
H-108	Rabbit anti-human B7-H4 IgG pAb	Epitope maps near N-terminus of human B7-H4; recommended for detection of mouse, rat and human B7-H4; cross reacts with equine, canine, bovine and porcine species	Recommended for WB, IP, IF and ELISA; IHC	Zhang et al. [8], Li et al. (2012) <i>Acta Histochem</i>	Santa Cruz
A72.1/A57.1	Mouse anti-human mAb		WB, ELISA	Simon et al. [23]	No

This table illustrates the species specificity, isotype, method/use, commercial availability, and publication reference of anti-B7-H4 antibodies from the discovery of B7-H4 in 2003 until July 2013. Abbreviations: monoclonal antibody (mAb), polyclonal antibody (pAb), immunohistochemistry (IHC), immunofluorescence (IF), western blot (WB), human bone marrow-derived stem cells (hBMDCs), enzyme-linked immunosorbent assay (ELISA).