Clinical Cancer Research

A New B7:CD28 Family Checkpoint Target for Cancer Immunotherapy: HHLA2

Yanping Xiao and Gordon J. Freeman

HHLA2 is a newly identified B7 family member that modulates T-cell functions through interaction with TMIGD2 and possibly a second receptor, with coinhibition in two studies and costimulation in one study. HHLA2 is expressed on a variety of human cancers, and its coinhibitory function makes it a candidate for cancer immunotherapy. *Clin Cancer Res; 21(10); 2201–3. ©2015 AACR.*

See related article by Janakiram et al., p. 2359

In this issue of *Clinical Cancer Research*, Janakiram and colleagues (1) report that human endogenous retrovirus-H long terminal repeat-associating protein 2 (HHLA2) had limited expression in normal human tissues but was widely expressed in human cancers. They also identified transmembrane and immunoglobulin domain containing 2 (TMIGD2) as one of the receptors for HHLA2.

With the success of PD-1 pathway antagonists in cancer immunotherapy, there is great interest in identifying other B7:CD28 family immunosuppressive pathways that could be targeted to enhance antitumor immunity. HHLA2 was discovered in 1999 as a new member of the immunoglobulin (Ig) superfamily (2), and recent work has emphasized its immunologic activity and similarity to the B7 family, with alternative names of B7-H5 and B7H7 (3–5). HHLA2 is a membrane protein with three Ig-like domains (IgV-IgC-IgV; refs. 2, 4, 5), whereas other members of the B7 family generally have only two Ig domains (IgV-IgC). HHLA2 is somewhat more closely related to B7-H3 and B7-H4 and shares 10% to 18% amino acid identity and 23% to 33% similarity to B7 family members (4). HHLA2 mRNA is highly expressed in kidney, colon, small intestine, and lung (2, 5). By immunohistochemistry, HHLA2 protein in normal human tissues is expressed in the epithelium of kidney, gut, gallbladder, and breast as well as placental trophoblast cells (1). In the immune system, HHLA2 protein is constitutively expressed on human monocytes/macrophages. HHLA2 is not expressed on immature dendritic cells, but expression on both dendritic cells and monocytes is modestly upregulated by inflammatory signals like lipopolysaccharide, IFNγ, and poly I:C. HHLA2 is not expressed on resting T or B cells and is upregulated on activated B cells (4, 5).

Zhao and colleagues (4) used HHLA2–Ig fusion protein to show that resting T cells expressed a receptor for HHLA2. They reasoned that because the *HHLA2* gene was lost in mice and rats, the receptor should also be lost due to coevolution. Janakiram and colleagues (1) tested Ig family members expressed in humans but not in mice and rats for binding to HHLA2–Ig and identified TMIGD2 as a receptor for HHLA2. Zhu and colleagues (5) approached the problem from the receptor side, identifying TMIGD2 as a membrane protein with 10% amino acid identity with CD28, CTLA-4, ICOS, and PD-1, hence the name CD28H. They identified HHLA2 as a ligand for TMIGD2 in a high-throughput screen of 2,300 individually transfected membrane genes for binding to TMIGD2–Ig (5). TMIGD2 has one extracellular IgV-like domain, a transmembrane region, and a proline-rich cytoplasmic domain with two tyrosine signaling motifs (1, 5, 6). HHLA2 does not interact with other known members of the CD28 or B7 gene families (4, 5).

Using a TMIGD2 mAb, Zhu and colleagues (5) showed that TMIGD2 protein is constitutively expressed on all naïve T cells and the majority of natural killer (NK) cells, but not on T regulatory cells or B cells. TMIGD2 expression was slowly lost with repetitive stimulation of T cells. Consistent with this, TMIGD2 was expressed on only about half of memory T cells, and TMIGD2 negative T cells had a terminally differentiated, senescent phenotype. This pattern of HHLA2 receptor expression on resting T cells is consistent with the results of Zhao and colleagues; however, they also showed HHLA2 receptor expression on antigen-presenting cells (APC) where TMIGD2 is not expressed, suggesting the possibility of a second receptor. TMIGD2 has also been shown to be expressed in endothelial and epithelial cells and function to reduce cell migration and promote capillary tube formation during angiogenesis (6).

Three studies have shown that HHLA2 regulates human T-cell functions. All used plate-bound HHLA2–Ig and anti-CD3 to stimulate purified human T cells. Two groups reported inhibition of T-cell proliferation and cytokine production (INFγ, TNFα, and others; refs. 4, 7), whereas the other reported increased T-cell proliferation and cytokine production (5). The group reporting costimulatory activity also found stimulatory activity for a plate-bound anti-TMIGD2 mAb and anti-CD3. In addition, an anti-HHLA2 mAb that blocked interaction with TMIGD2 reduced allogeneic T-cell response. The costimulatory function of HHLA2 and TMIGD2 interaction was also observed *in vivo*, using a human xenograft model of graft versus host disease or a humanized mouse model (5).

These opposing results with seemingly similar assays are reminiscent of initial results with PD-L1 (B7-H1; refs. 8, 9) and might be explained by the complexity of interpreting whether a biologic result is due to receptor engagement or blockade. Alternatively, the opposite effects of HHLA2 on T-cell function may be explained by

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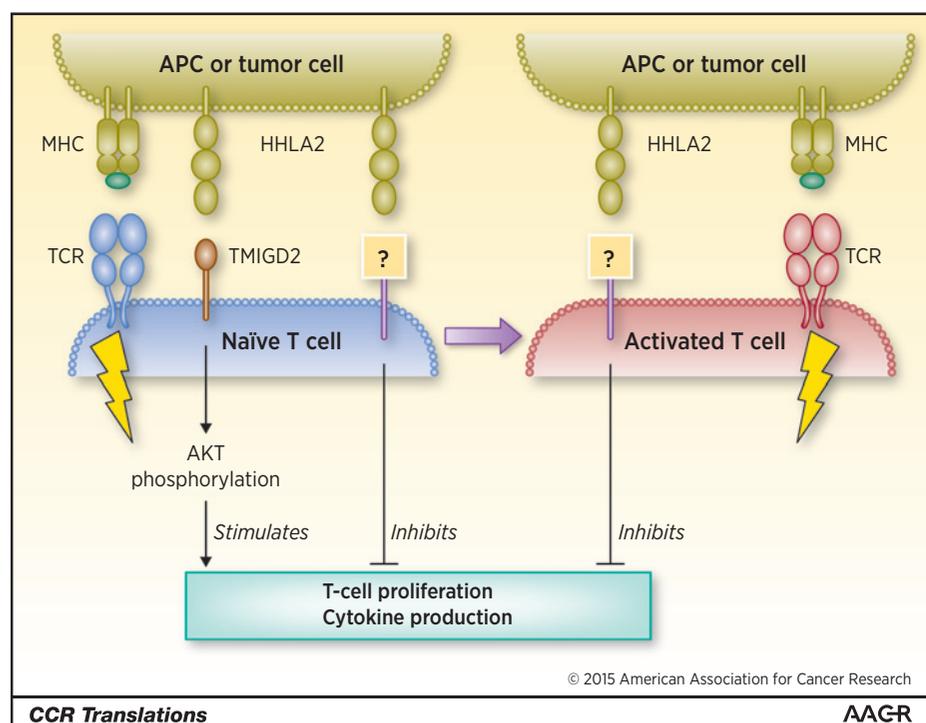
Figure 1.
Model for HHLA2 interaction with two receptors to regulate T-cell functions. Concomitant with T-cell receptor (TCR) signaling, TMIGD2 on naïve T cells interacts with HHLA2 on APCs and costimulates T-cell proliferation and cytokine production via a pathway involving AKT phosphorylation. With repetitive T-cell activation, expression of TMIGD2 is gradually lost, allowing expression of a second receptor to become dominant. HHLA2 on APCs or tumor cells can interact with this second receptor and exert a coinhibitory function.

HHLA2 binding to positive and negative receptors, as shown in Fig. 1. In this model, HHLA2 on APCs costimulates naïve T-cell proliferation and cytokine production through TMIGD2 via serine-threonine kinase AKT phosphorylation (5). As activated T cells lose TMIGD2 expression, a second receptor for HHLA2 on activated T cells exerts a coinhibitory function (4, 7). Identification of a second receptor would clarify the functions of HHLA2 on T-cell activation as well as its function in tumor environments where the interaction of activated T cells and tumor cells is involved.

Janakiram and colleagues show that HHLA2 is expressed in 20% to 70% of a wide range of human cancers from the breast, lung, thyroid, melanoma, pancreas, ovary, liver, bladder, colon, prostate, kidney, and esophagus but not on endometrial, gall-bladder, larynx, stomach, uterine, or lymphoma (1). Their data demonstrated that more than 50% of triple-negative breast cancer (TNBC) tumors have high HHLA2 expression and that patients with higher levels of HHLA2 on their tumors have a higher risk of disease spread and advanced stage. Analysis of the Cancer Genome Atlas database showed that TNBC had higher HHLA2 copy-number gains than other subtypes of breast cancer, which provides a possible mechanism for overexpression. Because HHLA2 can suppress T-cell function, the upregulation of HHLA2 expression on tumor cells and inducible HHLA2 expression on APCs provides a novel mechanism for tumor immune evasion.

Therefore, HHLA2 could be an attractive target for human cancer immunotherapy.

Further understanding the immunologic functions of the HHLA2 pathway will guide the selection of agents for cancer immunotherapy. Resolution of these functional differences will not come through the study of knockout mice or syngeneic mouse tumor models because HHLA2 and TMIGD2 do not exist in mice. Additional work with monomeric Fab, fusion proteins and mAbs mutated to not engage Fc receptor, HHLA2-transfected cells, nonrodent animal models, and others will all be welcome.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: Y. Xiao, G.J. Freeman
Writing, review, and/or revision of the manuscript: Y. Xiao, G.J. Freeman

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