

# HHLA2 and TMIGD2: new immunotherapeutic targets of the B7 and CD28 families

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**Keywords:** angiogenesis, B7 family, CD28 family, HHLA2, immunosuppression, immunotherapy, ligand, receptor, tumor microenvironment, TMIGD2

We and others recently discovered HHLA2 as a new B7 family member and transmembrane and immunoglobulin domain containing 2 (TMIGD2) as one of its receptors. Based on a new study we propose that HHLA2 may represent a novel immunosuppressive mechanism within the tumor microenvironment and hence could be a target for cancer therapy. TMIGD2 may be another therapeutic target.

HHLA2 (B7H7/B7-H5/B7y) has recently been identified as a new member of the B7 family member.<sup>1-3</sup> HHLA2 was initially discovered as a gene in the Immunoglobulin (Ig) superfamily when screening the human genome for human endogenous retroviral (HERV) long terminal repeat (LTR) sequences which provide polyadenation signals.<sup>4</sup> Hence the name, HHLA2, is short for HERV-H LTR-associating 2. HHLA2 orthologs appear to be present in a wide range of species such as fish, frog, giant panda, monkey and human, but not in laboratory mouse and rat strains. The HHLA2 protein has amino acid similarity of 23 to 33% to the other human B7 family molecules and phylogenetically it is most similar to B7-H3 and B7x (B7-H4/B7S1). The predicted structure of HHLA2 is a type I transmembrane molecule with three extracellular Ig domains. This is unique as most other B7 family members contain only two Ig domains while human B7-H3 has four Ig domains (Fig. 1A).

HHLA2 functions as a T cell coinhibitory molecule as it suppresses proliferation and cytokine production of both human CD4<sup>+</sup> and CD8<sup>+</sup> T cells.<sup>1,5</sup> HHLA2 is constitutively expressed on the surface of human monocytes and is induced on B cells after stimulation.<sup>1</sup>

Unlike PD-L1 and B7-1 though, HHLA2 is not inducible on T cells. The differences in expression on immune cells suggest that HHLA2 could be involved in immune regulation at a different functional level than other B7 family members. Using immunohistochemistry with an HHLA2 monoclonal antibody, we have recently found that HHLA2 is not expressed in most human tissues, except the placenta, kidney, intestine, gall bladder, and breast.<sup>6</sup> Expression of HHLA2 in the placenta and the intestines is interesting as it may help fetal-maternal immune tolerance or control intestinal inflammation, respectively. Importantly, we have shown many human cancers overexpress HHLA2 including cancers from the breast, lung, thyroid, melanoma, pancreas, ovary, liver, bladder, colon, prostate, kidney, and esophagus.<sup>6</sup> Moreover, in a small cohort of human triple-negative breast cancer (TNBC) patients, higher expression of HHLA2 on tumor cells was associated with increased lymph node metastases.<sup>6</sup> The wide expression of HHLA2 in human cancers and its association with more invasive disease in the TNBC cohort suggest that HHLA2 potentially plays an important role in tumor evolution and metastases through immune suppression.

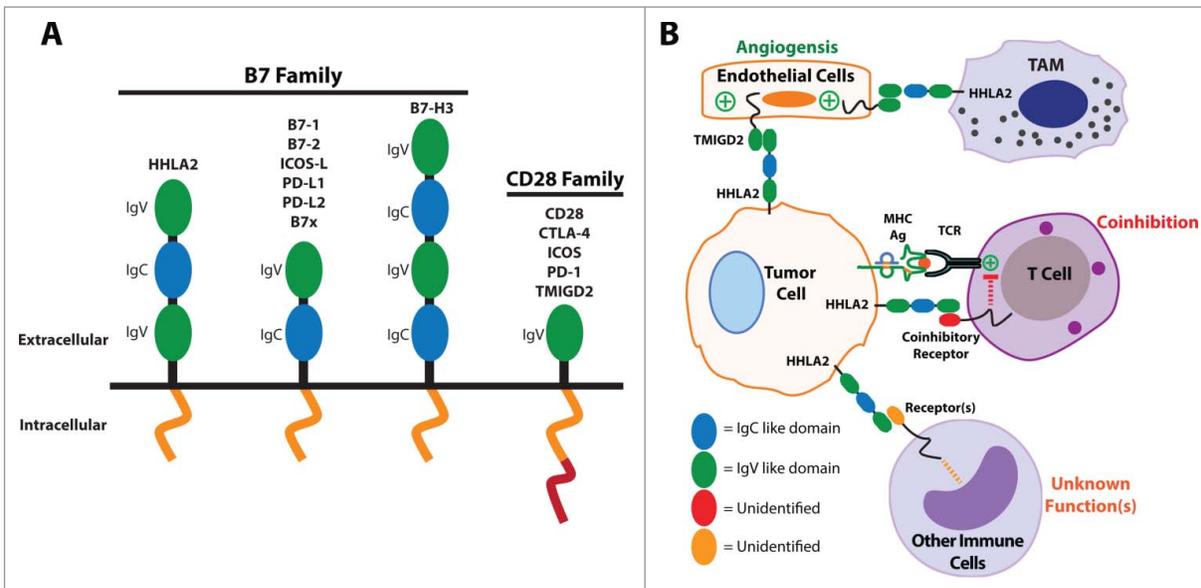
There are at least two mechanisms upregulating HHLA2 expression. One mechanism is inflammatory stimulation.<sup>1,2</sup> HHLA2 expression can be increased on monocytes and macrophages and is induced on B cells by stimulation with LPS and IFN- $\gamma$ .<sup>1,2</sup> The second mechanism is the gene copy-number variation.<sup>6</sup> We compared gene dosage in the basal subtype of TNBC using the cBioPortal for the Cancer Genomics database.<sup>7</sup> In this subtype, 32% had HHLA2 gene copy-number variations and the majority (95%) of these variants were either amplifications or gains of HHLA2 gene copy number,<sup>6</sup> suggesting this could be another mechanism of overexpression.

Receptors for HHLA2 can be found on a wide variety of immune cells, including T cells, B cells, monocytes, and dendritic cells.<sup>1</sup> We and others have independently identified one of receptors for HHLA2, TMIGD2,<sup>6</sup> also called CD28 homolog (CD28H),<sup>2</sup> through a bioinformatics analysis/immunological approach and a high-throughput screening, respectively. Like HHLA2, this molecule is expressed in humans and monkeys but not in mice or rats. This molecule was initially reported as an endothelial adhesion molecule which was renamed Immunoglobulin-containing and Proline-rich

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Submitted: 02/27/2015; Revised: 02/27/2015; Accepted: 02/27/2015

<http://dx.doi.org/10.1080/2162402X.2015.1026534>



**Figure 1.** The B7 and CD28 families and the significance of HHLA2 and TMIGD2 within the tumor microenvironment. **(A)** A structural representation of the B7 and CD28 family members. **(B)** A proposed model for the roles of HHLA2 and TMIGD2 within the tumor microenvironment. Tumor-expressed HHLA2 can interact not only with an unidentified receptor on activated T cells that leads to coinhibition, but also with TMIGD2 on endothelium that stimulates tumor angiogenesis. Additionally, tumor-expressed HHLA2 can bind to other immune cells and likely affects their functions in ways that are not yet understood. Finally, tumor-associated macrophages (TAM) may express HHLA2 and interact with TMIGD2 on endothelium.

Receptor-1 (IGPR-1).<sup>8</sup> TMIGD2 protein can be detected in cells of epithelial and endothelial origins, and is able to enhance angiogenesis *in vitro* when overexpressed by endothelial cell lines.<sup>8</sup> Furthermore, TMIGD2 is reported as a stimulatory receptor expressed primarily on naive T cells.<sup>2</sup> Like other CD28 family members, TMIGD2 is an Ig superfamily member with an extracellular IgV-like domain, a transmembrane region, and a cytoplasmic tail.<sup>6</sup> The cytoplasmic tail contains tyrosine residues which can be phosphorylated<sup>2</sup> and a proline-rich domain which associates with multiple Src homology 3 (SH3)-containing signaling molecules.<sup>8</sup> Together, these studies suggest that TMIGD2 has multiple functions

depending on the cell type and signaling pathways.

In summary, we have shown that the HHLA2 pathway could represent a novel immunosuppressive mechanism within the tumor microenvironment and is an attractive target for human cancer therapy. HHLA2's overexpression may be advantageous to cancer growth and survival through different mechanisms (Fig. 1B). Tumor-expressed HHLA2 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 microenvironment via its interaction with endothelial-expressed TMIGD2. The blockade of the B7-1/

B7-2/CTLA-4 and PD-L1/PD-L2/PD-1 pathways within the B7 and CD28 families to enhance anti-tumor immunity has been exploited with therapeutic success.<sup>9,10</sup> Interestingly, therapies targeting HHLA2 could not only enhance anti-tumor immune responses, but may also inhibit tumor angiogenesis. Further studies are required to dissect TMIGD2's expression patterns and functions in order to develop new therapies targeting TMIGD2.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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