Targeting the HHLA2:KIR3DL3 pathway in kidney cancer

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Disclosure Information

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HHLA2 is an immunomodulatory B7 protein that is highly expressed in clear cell kidney cancer.
Clear cell kidney cancer expresses HHLA2 or PDL1, but rarely both.

Bhatt, Mahoney et al.  
Cancer Immunol Res. 2021
In the rare tumor expressing both HHLA2 or PDL1, expression was non-overlapping.

Bhatt, Mahoney et al. Cancer Immunol Res. 2021
We identified and characterized KIR3DL3 as a receptor for HHLA2

- cell microarray technology at Retrogenix covering all 16 members of KIR family
- soluble HHLA2-Fc fusion protein binds to TMIGD2 and KIR3DL3 overexpressed on cells
HHLA2 has both immune stimulatory and immune inhibitory receptors.
KIR3DL3 is an inhibitory receptor for HHLA2 that mediates an alternative immunoinhibitory pathway to PD1

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KIR3DL3-HHLA2 is a human immunosuppressive pathway and a therapeutic target

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The B7 family ligand HERY-H LTR-associated protein 2 (HHLA2) is an attractive target for cancer immunotherapy because of its coinhibitory function, overexpression in human cancers, and association with poor prognoses. However, the knowledge of the HHLA2 pathway is incomplete. HHLA2 has an established positive receptor transmembrane and immunoglobulin (Ig) domain containing 2 (TMIGD2) but a poorly characterized negative receptor human killer cell Ig-like receptor, three Ig domains, and long cytoplasmic tail (KIR3DL3). Here, KIR3DL3 and TMIGD2 simultaneously bound to different sites of HHLA2. KIR3DL3 was mainly expressed on CD56+ NK and terminally differentiated effector memory CD8+ T (CD8+ TMEMA) cells. KIR3DL3-CDB8+ TMEMA acquired an NK-like phenotype and function. HHLA2 engagement recruited KIR3DL3 to the immunological synapse and coinduced CD8+ T and NK cell function and killing, inducing immune-evasive HHLA2+ tumors. KIR3DL3 recruited SHP-1 and SHP-2 to attenuate Vav1, ERK1/2, AKT, and NF-κB signaling. HHLA2+ tumors from human kidney, lung, gallbladder, and stomach were infiltrated by KIR3DL3+ immune cells. KIR3DL3 blockade inhibited tumor growth in multiple humanized mouse models. Thus, our findings elucidated the molecular and cellular basis for the inhibitory function of KIR3DL3, demonstrating that the KIR3DL3-HHLA2 pathway is a potential immunotherapeutic target for cancer.
Challenges for HHLA2:KIR3DL3 pathway development

• HHLA2, KIR3DL3, TMIGD2 are **not expressed in rodents**
  → We rely on developing humanized mouse models to test therapeutics and resistance mechanisms

• The biology of HHLA2 in carcinomas such as RCC may be very different from sarcoma or Hodgkin lymphoma cell lines that constitutively express HHLA2.
  → HHLA2 expression on kidney cancer cells is **lost when cultured in vitro**
What in the tumor microenvironment supports HHLA2 expression?

- HHLA2⁺ RCC lose HHLA2 expression in ex vivo culture

HHLA2 expression on CA9⁺ isolated tumor cells from patient’s Nx

Tomonari Shigemura, Kathleen Mahoney
What in the tumor microenvironment supports HHLA2 expression?

- HHLA2 negative human cell lines begin to express HHLA2 in xenograft/SCID mouse models

Huang, El Ahmar, Bagheri, Freeman, Signoretti, Mahoney
HHLA2 is regulated differently than PD-L1. Interferon-\(\gamma\) upregulates PD-L1 but not HHLA2.

- qRT-PCR of 786-O kidney cancer cells after 48 hours of stimulation

Konge, Bhatt
Future Directions in lab:

• Understand pathways regulating HHLA2 expression may help direct choices of combination therapy or selection of patient populations
  → Whole genome CRISPR in RCC cells to determine regulators

• Developing testable humanized mouse models may help determine what is optimal patient population to treat
  → Is this pathway more effective in tumors that are sensitive to CD8 or NK cell killing?
Future Directions for HHLA2:KIR3DL3 therapeutic development

• Analyze HHLA2 expression on patient samples post PD-1 and TKI therapy

• Optimally develop immunohistochemistry as potential companion biomarker

• Determine what is optimal patient population to treat
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We have optimized HHLA2 IHC for assaying human and xenograft expression?

A204+: Human Rhabdomyosarcoma cell line +ve for HHLA2

A204-: HHLA2 CRISPRed out. Negative by flow cytometry
We have optimized HHLA2 IHC for assaying human and xenograft expression?

ccRCC
Tissue Sample 1:
All tumor

With little normal kidney staining

556.1 mAb
E1U6X mb (rabbit)

20x magnification

Mahoney, El Ahmar, Bagheri, Freeman, Signoretti