1432 Discovery of HIO-3, a tumor-produced protein that binds the human IgG1 Fc CH3 domain and suppresses antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC)

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Leaders in Humoral Immuno-Oncology

Abstract

Human cancers employ a number of mechanisms to evade host immune responses against antigens generated from aberrant over-expression, mutations and/or epigenetic alterations. Humoral immunity utilizes antibodies and immune-effector cells as well as the complement system to mediate the killing of dysregulated cancer cells. We refer to these anti-cancer mechanisms as Humoral Immuno-Oncology (HIO). HIO immunosuppression is mediated by tumor-produced proteins called HIO factors. The prototype HIO factor, CA125, was previously shown to bind IgG-type antibodies and suppress their antibody-dependent (ADCC) and complement-dependent (CDC) cytotoxic activities. Using a combination of patient serum analysis and literature searches, we screened a number of samples and proteins that are produced by tumors to determine if they could impact HIO. Herein, we describe the characterization of HIO-3, a tumor antigen that binds IgG1-type antibodies and inhibits their immune-effector activity. Through a combination of truncation and substitution mutagenesis, we identified a unique motif within the CH3 domain of IgG1 essential for HIO-3 binding, resulting in the inhibition of ADCC activity via suppression of CD16a Fc receptor engagement with IgG1 Fc domain. Amino acid substitutions in this domain were able to abrogate HIO-3 binding and overcome ADCC suppression. These findings highlight a novel mechanism by which tumors can suppress the host's immune system for survival and offers new concepts for engineering antibody-based therapies that are refractory to HIO-3. Moreover, the findings here offer clinical design opportunities to improve upon approved antibody-based therapies in HIO-3 expressing cancers.

Fig. 1- HIO-3 directly binds IgG1 and inhibits ADCC activity

A) Biotinylated IgG1 binds immobilized HIO-3. B) Biotinylated HIO-3 binds immobilized IgG1. C) Dose-dependent inhibition of ADCC by HIO-3. D) HIO-3 inhibits ADCC of therapeutic Abs.

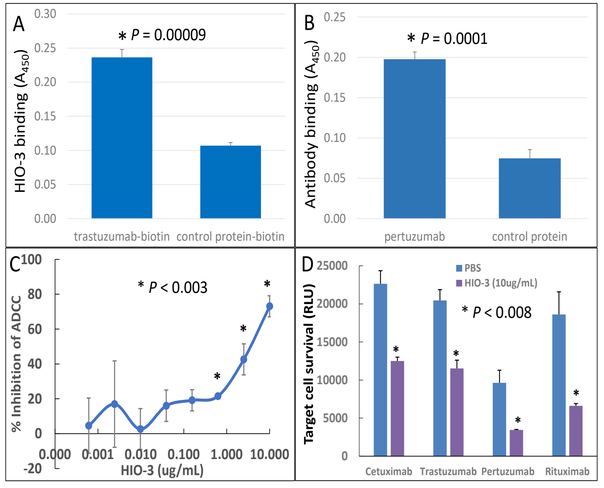


Fig. 2- HIO-3 blocks ADCC and CDC via suppressed CD16a Fc receptor and C1q protein engagement with IgG1 Fc domain

A) Biotinylated CD16a binds immobilized cetuximab and is inhibited by HIO-3. B) Biotinylated C1q binds immobilized cetuximab and is inhibited by HIO-3.

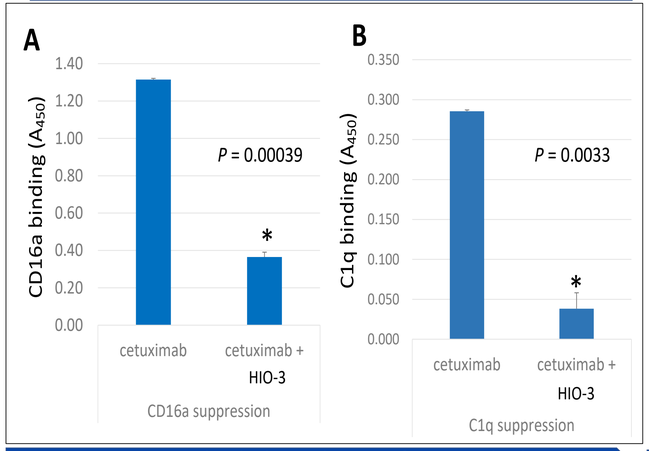


Fig. 3- HIO-3 binds the Fc domain of IgG1 antibodies

A) Immobilized full length and IgG1 antibody fragments were probed with biotinylated-HIO-3 and binding was found to be localized to the Fc domain. B) Probing of immobilized dimeric (hu Fc) and monomeric Fc subdomains suggests HIO-3 binding recognizes tertiary structures of dimerized IgG1 Fc heavy chains.

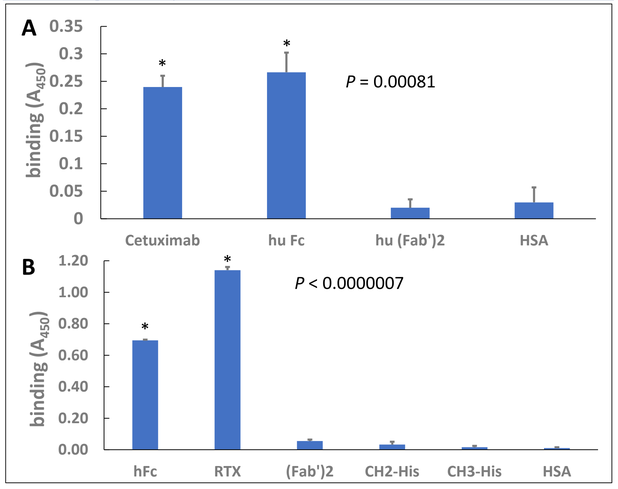


Fig. 4- ELISA mapping of HIO-3 binding domain within IgG1 CH3

(A) IgG1 Fc GST fusion construct structure. B) Competition assay method. C) Assay results measuring HIO-3 competitive binding. ELISA plates were coated with rituximab (RTX) and probed with HIO-3-His and various GST-Fc substitution and deletion fragments shown in Fig.5.

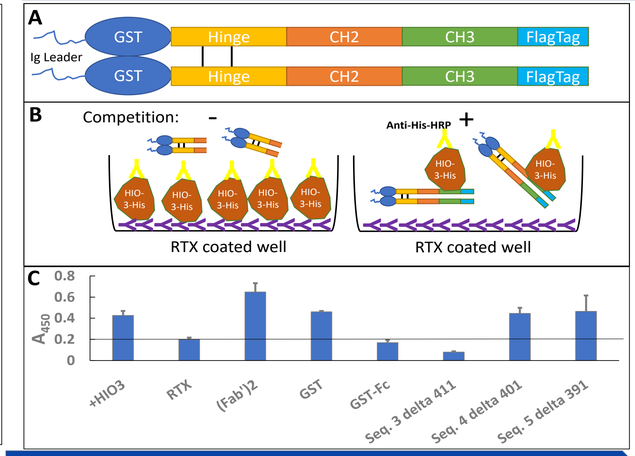


Fig. 5- IgG1 Fc domain mapping of the HIO-3 binding domain

Amino acid sequence ID numbers (left) of IgG1 GST Fc deletion and truncation mutants to map the HIO-3 binding domain. Bold sequences indicate substitution mutations. The right column summarizes Fc regions and residues required for HIO-3 binding.

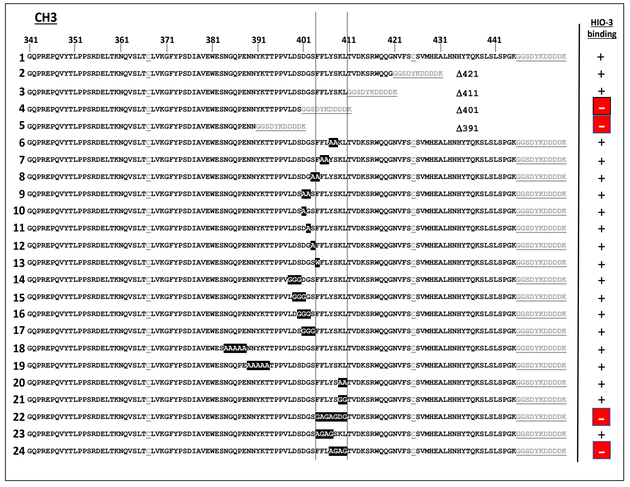
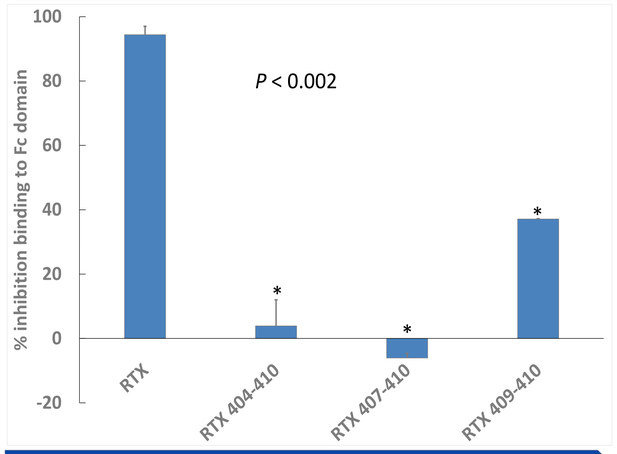
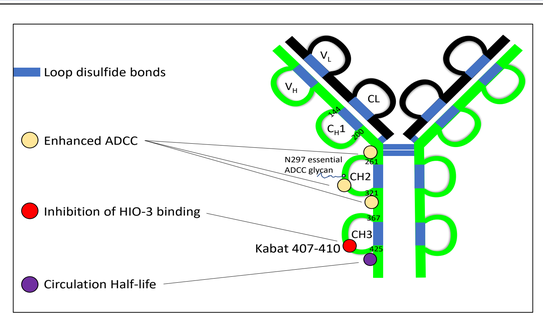


Fig. 6- HIO-3 binding is localized to residues 407-410 in Fc domain

Rituximab (RTX) antibodies were engineered with amino acid substitutions within the HIO-3 binding domain and tested for HIO-3 binding via binding competition assays. RTX-407-410 variant was found to be completely devoid of HIO-3 binding, localizing binding to CH3.



Conclusions & Future Directions



- HIO-3 is a secreted tumor protein that inhibits IgG1 ADCC and CDC
- HIO-3 IgG1 Fc binding reduces CD16a Fc receptor and C1q-protein IgG1 binding
- Fc substitution mutations localized HIO-3 binding to the CH3 residues 407-410
- CH3 407-410 motif does not overlap with any domains previously known to alter ADCC or CDC functions nor IgG1 circulating half-life (FcRn)
- HIO-3 refractory antibodies (e.g., cetuximab, daratumumab, rituximab, trastuzumab) are being tested in HIO-3 expressing cancer models as next gen antibody therapies