C7

Identification of Novel Liquid Biopsy Biomarker for Monitoring the Immune Set Point in Both Solid Tumor and Hematological Malignancy Patients

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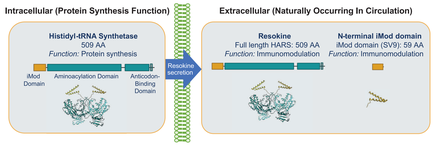
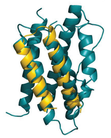
Introduction

- A number of non-canonical functions of proteins generated from tRNA synthetase genes have been reported, demonstrating diverse roles for these proteins outside of protein synthesis. These include roles in regulating inflammatory responses, angiogenesis and mTOR signaling (Wakasugi & Schimmel, 1999; Park et al., 2008, Arif et al., 2017).
- The gene for histidyl-tRNA synthetase (HARS) gives rise to a number of splice variants, many of which have lost their catalytic activity, but which retain the N-terminal domain of 59 amino acids, sometimes referred to as the WHEP domain (Xu et al., 2012, Lo et al., 2014). This domain was appended to HARS during evolution of multicellular organisms and is not essential for protein synthetic activity (as in prokaryotic organisms) but is retained with high homology across mammalian species.
- Proteins derived from the HARS gene, both full-length and splice variants, are present in human circulation and play a role in modulating immune responses. We have termed the extracellular HARS proteins as Resokine proteins, to differentiate them from the intracellular enzyme involved in protein synthesis. Resokine proteins, all of which contain the N-terminal HARS domain (the immunomodulatory domain, or iMod domain), have activity to modulate T cell activity and levels of iMod domain proteins are elevated in cancer, both in human plasma and in syngeneic tumor models in mice. In addition, monoclonal antibodies to the iMod domain are capable of inhibiting tumor growth in mice.

Resokine Proteins: Extracellular Histidyl-tRNA Synthetase Gene Products With Immune Modulation Activity



iMod Domain Structural Similarity to Cytokines



Resokine iMod domain

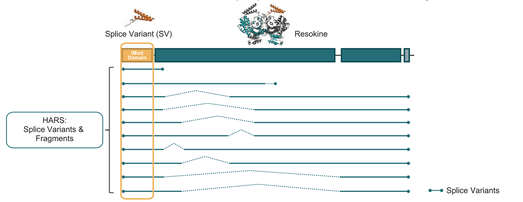
4α helical cytokine

Examples include

IL-2
IL-4
IL-6
IL-10
IL-13
IL-23
Interferon Type 1/2

- Resokine (the extracellular proteins derived from the HARS gene) is secreted from cells and circulates naturally in all individuals tested. The N-terminal "iMod" domain has structural similarity to 4 alpha–helical bundle cytokines. Overlay shown is with human IL-6, but structure is similarly conserved among other members of the family.
- The N-terminal iMod domain is conserved among all the characterized splice variants arising from the HARS gene, and thus constitutes a potential epitope that can be targeted by antibodies to neutralize all forms of Resokine.

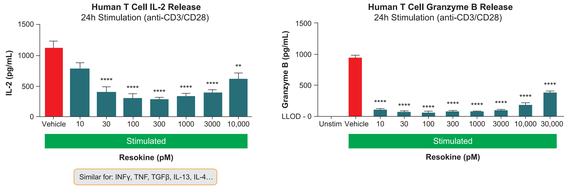
iMod Domain: Universal Epitopes Across the Resokine Family



Resokine Is a Novel Immunomodulator

- To test in vitro effects of Resokine on T cell activation, recombinant Resokine was purified and verified to be > 95% pure with low/undetectable endotoxin contamination. Resokine was then tested by addition to T cells undergoing activation in vitro with antibodies to CD3/CD28.

Resokine Reduces Cytokine and Granzyme B Release During T Cell Activation



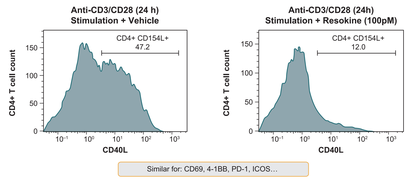
Similar for: INFγ, TNF, TGFβ, IL-13, IL-4...

$^{****}p < 0.0001$; $^{***}p < 0.001$; $^{**}p < 0.01$

$^{****}p < 0.0001$

Resokine Inhibits Up-regulation of CD40L and Other Cell Surface Activation Markers

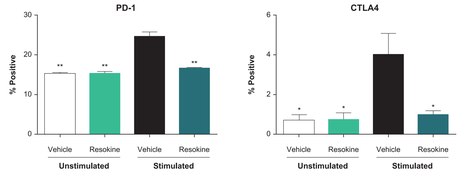
24h Stimulation (anti-CD3/CD28)



Similar for: CD69, 4-1BB, PD-1, ICOS...

- Human T cells were isolated from fresh human blood via PBMC by standard techniques. T cells were activated by antibodies to CD3 (plate bound) and CD28 (soluble) using sub-optimal stimulation conditions in the presence of Resokine or buffer vehicle. After 24 hours, supernatant was collected for the measurement of cytokines or granzyme B by ELISA, and cell surface markers were analyzed by flow cytometry. Data shown for CD4 positive T cells.

Resokine Inhibits Up-regulation of PD-1 and CTLA-4



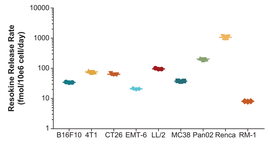
Human T cells, 24h unstimulated, stimulated (αCD3/αCD28) 24 h + Resokine @ 1 nM

- Results demonstrate that Resokine has immunomodulatory activity in vitro through reduction in the extent of T cell activation. This activity is dependent on the iMod domain because the recombinant iMod domain alone, or fused to immunoglobulin Fc, has equivalent activity in these assays (data not shown). Because Resokine circulates naturally in all individuals tested, and has immuno-modulatory activity, we propose that Resokine may act as an "immune setpoint" controlling the extent of T cell activation achieved with a given stimulus.

Resokine Levels Are Elevated in Cancer

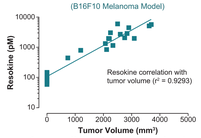
- Resokine levels were determined from mouse or human samples using specific immunoassays with 2 non-competing antibodies to Resokine. Assays were carried out on the MSD platform and verified using multiple formats. Upper and lower limits of quantification were established for each assay as shown in the figures.
- Plasma samples from human cancer patients were obtained commercially from Conversant Bio. Plasma samples from healthy human volunteers also came from Conversant Bio, as well as Innovative Research, and some additional donated samples.

Resokine Is Released From Cancer Cell Lines in Culture



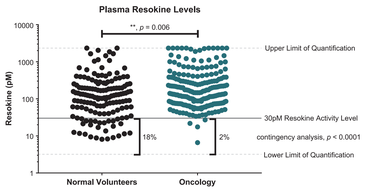
Circulating Resokine Levels Correlate With Tumor Volume in Mice

(B16F10 Melanoma Model)



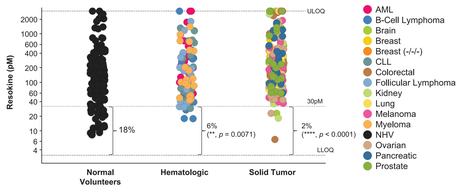
- Resokine is released from cancer cell lines in culture. Levels vary from cell line to cell line. In vivo tumor growth results in increased levels of Resokine in the circulation of C57Bl6 mice bearing B16F10 tumors.
- Resokine levels in serum from normal C57Bl6 mice ranged from 70 to 250pM (n = 10). Significantly higher levels (450-3000pM) were found in the serum of B16F10 tumor-bearing mice ($p < 0.001$).
- Resokine levels are increased in proportion to tumor growth in mice, with levels increasing over 10-fold in very large tumors.

Cancer Patients Have Higher Plasma Resokine Levels



Plasma; Resokine ELISA; NHV: n = 148; Oncology: n = 215; Mann-Whitney

Overall Resokine Levels Are Elevated in 15/15 Cancer Types



Normal Volunteers n = 148; Hematology n = 100; Oncology n = 215
Fisher's exact test; Plasma samples

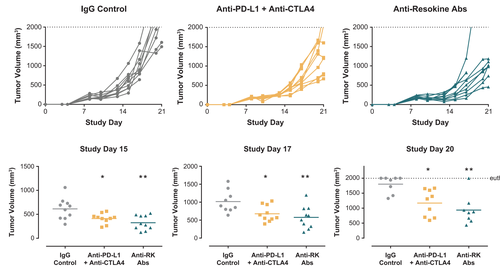
- Human plasma samples from patients with tumors show a higher mean level of Resokine than normal healthy volunteers, and notably few patient samples have levels of Resokine below 30pM, a level with functional activity in vitro. Extended data sets show this pattern appears to be retained across multiple tumor types.
- Resokine levels in healthy volunteers (n = 148) ranged from 8pM to > 2333pM with 18% of the individuals possessing a level below 30pM. In contrast, levels measured across patients (n = 466) with all tumor types tested ranged from 20pM to > 2333pM (above the upper limit of quantification) with only 4% of the patients possessing low levels, defined as < 30pM; ($p < 0.0001$).

Is enhanced Resokine secretion used by tumors as an additional mechanism to down-regulate anti-tumor immune responses?

Since levels of circulating Resokine are sufficient to modulate T cell activity, we hypothesize that the enhanced release of Resokine from tumor cells may further increase the threshold stimulation required to generate an active immune response. There is the potential for this to be an additional mechanism by which tumor cells may regulate immune responses.

Anti-Resokine Antibodies Have Anti-Tumor Activity

B16F10 Mouse Melanoma Syngeneic Model



$^{*}p < 0.05$, $^{**}p < 0.01$

- Anti-tumor activity was initially tested in the B16F10 syngeneic tumor model in C57Bl6 mice. B16F10 tumor cells ($1x10^5$ cells in 0.1mL PBS/20% matrigel) were implanted subcutaneously on the right flank on day 0. Antibody therapy was administered IP on days -1, 6, and 13 (200microgram/antibody/mouse). Anti-mouse PD-L1 and anti-mouse CTLA4 antibodies were from BioXcell. Tumor volumes were measured at indicated intervals over 21 days. Statistics are 1-way ANOVA, followed by Dunnett's.

Conclusions

- Resokine proteins are extracellular proteins derived from the HARS gene, including full-length HARS and a number of splice variants
- Resokine proteins contain an N-terminal domain, which we have termed the iMod domain
- This domain has immunomodulatory activity both in vitro (inhibition of T cell activation) and in vivo
- Levels of circulating Resokine are elevated in cancer patients across multiple tumor types
- Levels are also increased in mice bearing syngeneic tumors, and correlate with tumor volume
- Antibodies to Resokine have demonstrated anti-tumor activity in the B16F10 syngeneic tumor model

References

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