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The Resokine Pathway Is Implicated in the Pathology of Interstitial Lung Disease

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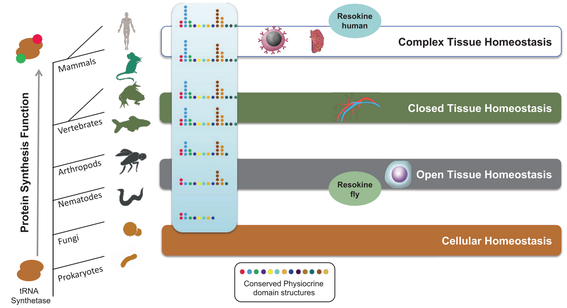
Rationale

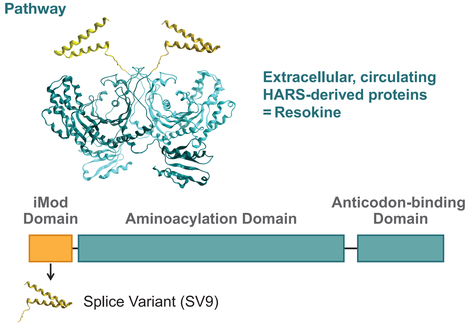
- The histidyl-tRNA synthetase (HARS) protein has long been identified as the sole target of Jo-1 autoantibodies (Jo-1 Abs), which are, in many cases, accompanied by interstitial lung disease (ILD). Extracellular HARS proteins (Resokine) are found in circulation at physiologically relevant levels in healthy individuals (~200 pM) and are greatly reduced in patients with Jo-1 Abs, leading us to investigate the source of Resokine and explore the possibility that insufficiency plays a role in the pathology of inflammatory myopathies and ILD associated with Jo-1 Abs.

Evolution-elaborated Physiological Systems for Homeostasis

Physiocrine Hypothesis: Physiological Modulation From Primordial Tissue Homeostasis

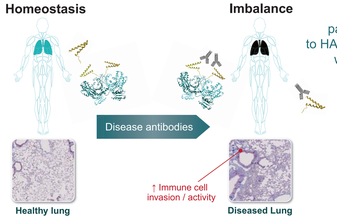


Newly Evolved Histidyl-tRNA Synthetase iMod Domain and the Resokine Pathway



Disrupting the Resokine Pathway Promotes Lung Damage

Evidence for Homeostatic Role of Resokine in Humans



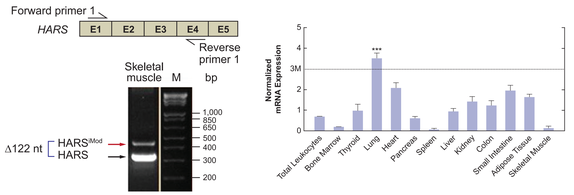
100% (18 of 18) anti-Jo-1 patients tested positive for antibodies to HARS (known extracellularly as Resokine) with high titers to the iMod domain

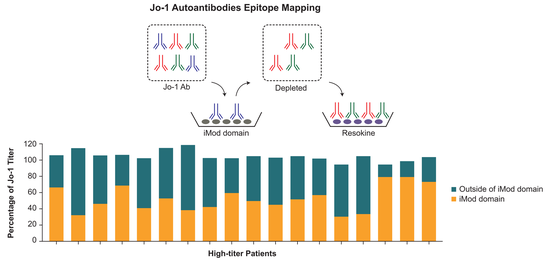
Characteristics of Jo-1 Associated ILD

- Increased T-cells in BALF
- Decreased CD4/CD8 ratio
- Histology ranges from NSIP to DAD as severity of disease increases

BALF, bronchoalveolar lavage fluid; DAD, diffuse alveolar damage; ILD, interstitial lung disease; NSIP, nonspecific interstitial pneumonia.

Resokine Splice Variant (SV9) Represents the iMod Domain, the Target of a High Percentage of Disease-linked Jo-1 Antibodies

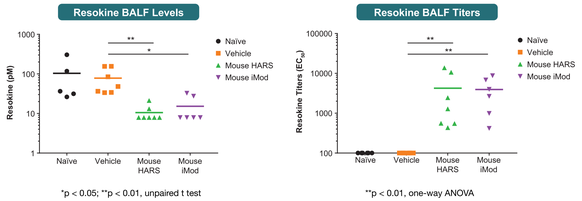




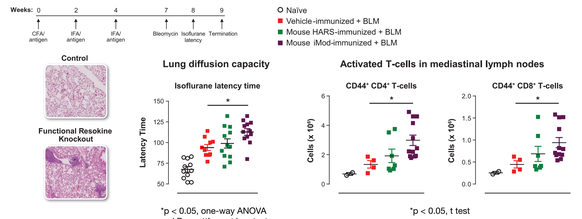
- The predominant epitopes are in the N-terminal iMod domain
- Additional epitopes are present throughout HARS, indicating epitope spreading has occurred

Pathological Remodeling of the Lung in a Model of Resokine Pathway Disruption

Vaccination of Mice With HARS or iMod Domain Alone (+ Adjuvant) Generates Robust Antibody Response in BALF That Depletes Resokine



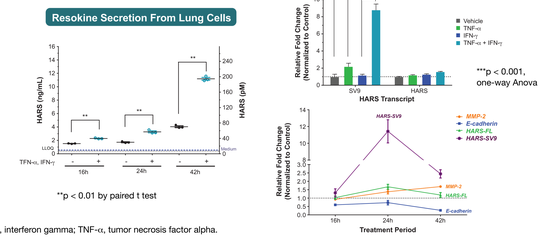
Exacerbated Response to Bleomycin-induced Injury in Mice With Resokine-antibody Blockade



BALF, bronchoalveolar lavage fluid; BLM, bleomycin; CFA, Complete Freund's adjuvant; IFA, Incomplete Freund's adjuvant.

Regulation of Resokine Splice Variant in Inflammation

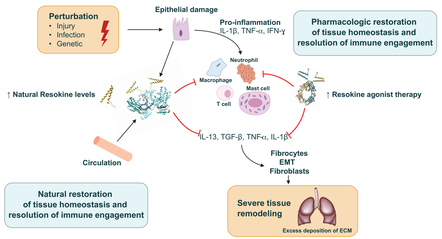
Resokine iMod-SV Is Upregulated in Airway Epithelial Cells and Secretion Is Increased in Response to Inflammation



IFN-γ, interferon gamma; TNF-α, tumor necrosis factor alpha.

Regulation of the Resokine Pathway in Lung Inflammation

Resokine Pathway Promotes Lung Homeostasis



Results

- We characterized serum from a number of Jo-1–positive patients and found the majority exhibited strong cross-reactivity with the N-terminal portion of Resokine and that a splice variant (SV9), which encodes only the N-terminal domain of the protein, is enriched in human lung tissue. Here, we demonstrate that in the lung-derived adenocarcinoma cell line A549, Resokine is actively secreted following stimulation with the inflammatory cytokines IFN-γ and TNF-α, two key players in the initiation of lung inflammation and fibrosis. Secretion is dose dependent and synergistic. SV9, but not the full-length mRNA, is increased following inflammatory cytokine stimulation and its peak expression precedes that of the secreted protein.
- Repeated vaccination of mice with murine Resokine in the presence of adjuvant can break tolerance and generate auto-antibodies. Resokine was present in bronchoalveolar lavage fluid of controls and undetectable in vaccinated animals. In animals with a high titer to Resokine, infiltration of immune cells (including T-cells) into skeletal muscle and lung was detected. Furthermore, in vaccinated animals receiving bleomycin intratracheally, lung function was more severely impacted and both CD4+ and CD8+ T-cells were increased in the mediastinal nodes. Interestingly, these pathologies were observed only in mice that carry a genetic mutation in dysferlin (and other loci) but not in wild-type mouse strains (e.g., C57bl/6).

Conclusion

- Immune invasion pathologies observed in mice forced to break tolerance to Resokine in the background of another tissue-damaging genetic mutation are similar to activity observed in patients with Jo-1 autoantibodies. This suggests that induction of Resokine insufficiency may disrupt immune homeostasis in a manner that manifests as pathological in lung and muscle. The findings that Resokine circulates at pM levels and the splice variant SV9 is synergistically induced and secreted by lung-derived cells in an inflammatory environment provide evidence for an extracellular niche in which autoantibodies could mediate neutralization of these proteins to induce pathology.

References

Zhou JJ et al. Secreted histidyl-tRNA synthetose splice variants elaborate major epitopes for autoantibodies in inflammatory myositis. *J Biol Chem*, 2014.

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Disclosure

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