Presentation # F2-858

SQSTM-1/p62: A Novel Therapeutic Target in Infectious and Inflammatory Disease

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Background

IDR-1 (KSRIVPAIPVSLL-NH2) is a synthetic peptide with no antimicrobial activity that enhances infection control while suppressing inflammation. Treatment of mice with IDR-1 at the time of infectious challenge provides protection from otherwise lethal bacterial infection, and modulates cytokine and chemokine expression downstream of TLR stimulation. Previously, the effects of IDR-1 were postulated to impact several transcriptional pathways, including MAPK p38 and C/EBP, but the preceding molecular events remained unknown.

In this study, the cytoplasmic protein p62 has been identified as a molecular target of IDR-1. p62 is a multi-domain scaffold (adaptor) protein, with many known interacting partners, including PKCζ, p38, RIP1, and TRAF6. p62 comprises an N-terminal PB1 domain that is primarily important for aPKC binding, a ZZ domain which interacts with RIP1, and a TBS sequence domain recognized by TRAF6. Additionally, a C-terminal UBA domain binds to polyubiquitin – a function considered to be the basis of the association between p62 and protein trafficking to the proteosome. Variation in p62 expression levels has been implicated in various disease states but its function in antimicrobial immunity has not yet been investigated.

p62 has recently been recognized as a nodal point in cellular signaling pathways, in particular implicated in regulation of NF-κB. In addition, recent studies demonstrate that p62 expression contributes to regulating macrophage mediated and cancer-associated inflammation, raising the question as to whether IDR-1 might affect inflammatory responses in the absence of pathogen stimulation.

Results

IDR-1 binds to p62

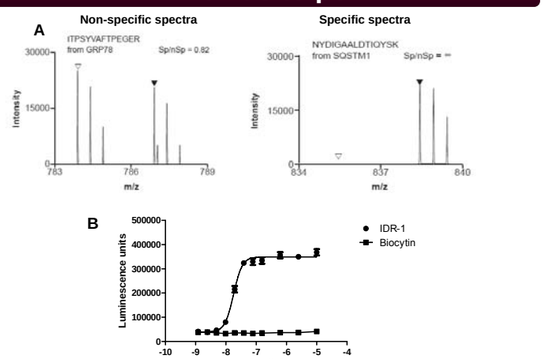

Figure 1. A SILAC; desthiobiotin-IDR-1 pull-down. Mass spectra from representative tryptic peptides of p62 are observed only in the labeled condition (▼, right) indicating specific binding of p62 to IDR-1 **B** IDR-1 binds to recombinant p62 *in vitro.*

IDR-1 binds to the ZZ domain of p62

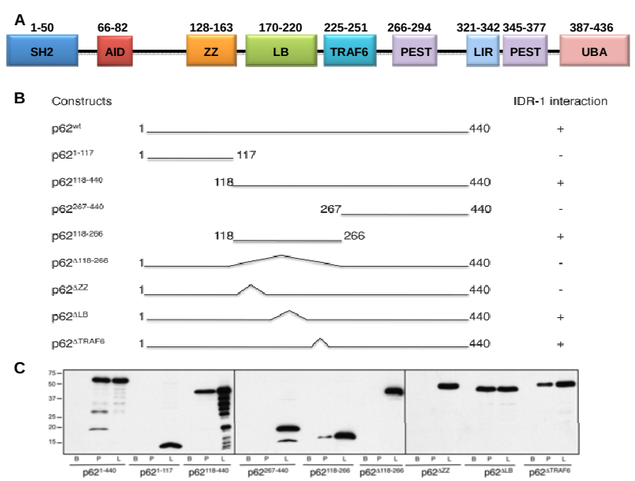

Figure 2. A Schematic representation of the p62 domains. **B** Constructs used for mapping of p62 domains that interact with IDR-1 **C** Lysates from HEK293T cells overexpressing FLAG-tagged deletion mutants of human p62 were pulled down against biocytin (B) or biotinylated IDR-1 (P). Cell lysates (L) were used as control. Proteins pulled down were immunoblotted with anti-FLAG antibody.

IDR-1 affects intracellular p62 complexes

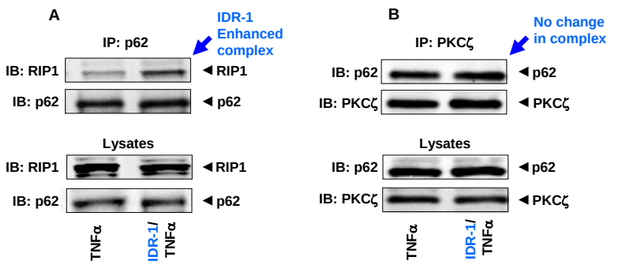

Figure 3. Co-immunoprecipitation analysis of p62 molecular complexes in HEK293T cells**. A** IDR-1 enhances p62-RIP complex formation in the presence of TNFα stimulation. **B** IDR-1 has no effect on p62-PKCζ complex formation in the presence of TNFα stimulation.

IDR-1 modulates p62 mediated signaling

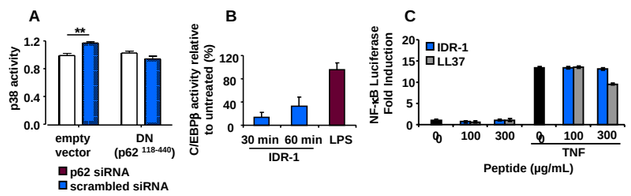

Figure 4. A IDR-1 treatment activates the p38 signaling pathway in a p62 dependent manner as determined by a p38 activity driven luciferase assay in A549 cells **B** Treatment of A549 cells with IDR-1 induces C/EBPβ activity **C** IDR-1 treatment of NF-κB-luciferase-A549 cells does not affect NF-κB activity in the presence or absence of TNFα stimulation. ** p<0.01

IDR-1 and p62 modulate bacterial replication *in vitro* and *in vivo*

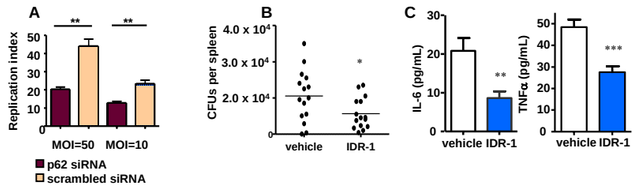

Figure 5. A p62 restricts intracellular *S. Typhimurium* infection in HeLa cells. **B** IDR-1 pre-treatment of mice reduces *S. Typhimurium* infection *in vivo.* **C** IDR-1 modulates the cytokine response to S. *Typhimurium* in vivo. * p< 0.05; ** p<0.01; *** p<0.001

IDR-1 modulates inflammation *in vivo*

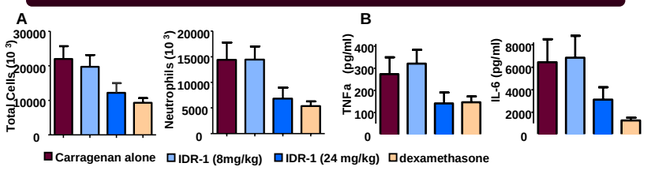

Figure 6. IDR-1 reduces the inflammatory response in a mouse air pouch model of acute inflammation. Animals were injected with carrageenan solution to induce inflammation. IDR-1 was administered 12 hr prior to carrageenan treatment. Control mice were either left untreated (carrageenan alone) or pre-treated with dexamethasone (5 mg/kg). **A** Number of total leukocytes and neutrophils in the air pouch. **B** TNFα and IL-6 levels in the air pouch lavage fluid determined by ELISA.

Proposed IDR-1/p62 mechanism of action

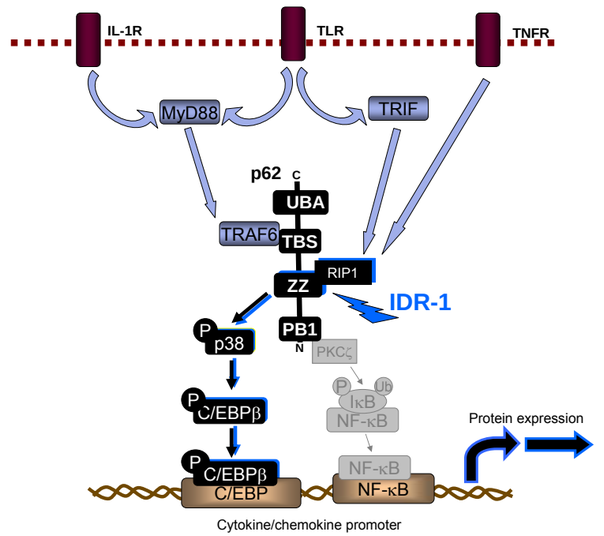

Figure 7. IDR-1 binds to the ZZ domain on the p62 protein. This event results in stabilization of the intracellular complex with RIP1. IDR-1 interaction with p62 activates the p38 MAPK pathway, which results in phosphorylation and activation of the transcription factor C/EBPβ. In the presence of TNFα or TLR stimulation, the NF-κB pathway is activated, although IDR-1 does not change complex formation with PKCζ nor, consequently, modulate NF-κB activity. Activation of transcriptional complexes results in modulation of cytokine/chemokine production in anti-infectious and inflammatory immune responses.

Conclusions

- IDR-1 binds to the ZZ domain of p62 to modulate specific protein-protein interactions.
- p62 plays a key role in infection control.
- Therapeutic intervention targeting p62 is capable of both enhancing clearance of infection and suppressing the attendant inflammation, as well as suppressing inflammation in the absence of any infectious challenge.

Inimex Pharmaceuticals Incorporated