Sequestosome-1/p62 is the key intracellular target of innate defense regulator peptide

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Background

Inimex’ Innate Defense Regulators (IDRs) are synthetic peptides with no antimicrobial activity that enhance infection control while suppressing inflammation. Treatment of mice with IDRs 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 signaling 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 Inimex’ IDRs. 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 proteasome. 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 IDRs might affect inflammatory responses in the absence of pathogen stimulation.

Results

IDR-1 binds to p62

Figure 2. 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. Similar results were obtained with other IDRs in Inimex’ portfolio.

IDR-1 affects intracellular p62 complexes

Figure 4. 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. Similar results were obtained with other IDRs in Inimex’ portfolio.

IDR induces Ubiquitylation of RIP1

Figure 6. HEK293T cells were pre-treated with IDR and proteasomal inhibitor MG132, followed by 5 min TNFα stimulation. RIP1-Ub levels were determined by ELISA in cell lysates using an anti-RIP1 capture antibody and an anti-Ubiquitin detection antibody.

IDR-1 modulates p62 mediated signaling

Figure 7. 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

Conclusions

- Inimex’ IDRs bind to the ZZ domain of p62
- IDR binding to p62 results in selectively enhanced complex formation, thereby affecting the balance of pro-inflammatory and anti-inflammatory signaling
- p62 plays a key role in infection control (See Poster # 330)