6. Summary

- RT-PCR and RT-qPCR analyses showed S100b specifically increased IP-10 mRNA level in THP-1 cells and PBMC.

- Monocytes from Diabetes patients also revealed increased IP-10 mRNA levels.

- Act-D (general transcription inhibitor) treatment did not inhibit IP-10 mRNA accumulation suggesting S100b induced IP-10 mRNA is not due to increased transcription. Also, cycloheximide treatment showed inhibition of IP-10 mRNA expression suggesting the involvement of new protein.

- IP-10 mRNA half-life experiment confirmed increased IP-10 mRNA by S100b is due to increased mRNA stability.

- The Luc-mRNA stability assay and luciferase activity suggested that IP-10 3’-UTR is essential for S100b-mediated stabilization of IP-10 mRNA and has positive control cis-element.

- RNA-affinity capture method identified the presence of hnRNPK and HuR in the IP-10 3’UTR.

- RT-PCR analyses extrapolated that S100b could induce hnRNPK mRNA levels in THP-1 cells.

- RNA immuno-precipitation confirmed the presence of hnRNPK in IP-10 3’UTR.

- hnRNPK knock-down leads to the destabilization of IP-10 mRNA in S100b treated THP-1 cells. Also, overexpression of hnRNPK promoted IP-10 mRNA accumulation. These loss-off and gain-on function approaches confirmed that hnRNPK mediates S100b induced IP-10 mRNA Stability.
S100b induced hnRNPK translocation from nucleus to cytoplasm was analyzed by GFP-tagged hnRNPK using fluorescent microscope.

S100b treatment constrained non-phosphatable hnRNPK (hnRNPK$^{S\rightarrow A}$ mutant) within nucleus and did not stabilize IP-10 mRNA suggesting translocation of hnRNPK from nucleus to cytoplasm was necessary for the stabilization of IP-10 mRNA.

Mutating serine moieties at 284 and 353 to Aspartic acids of hnRNPK restored S100b induced translocation of hnRNPK from nucleus to cytoplasm and increased IP-10 mRNA stability. This confirmed that translocation of hnRNPK from nucleus to cytoplasm was necessary for the stabilization of IP-10 mRNA.

Western blot analyses showed that S100b treatment demethylates lysine moiety 219 of hnRNPK. Also, mutating this lysine moiety into isoleucine increased IP-10 mRNA stability.

Both methylation defective plus phosphomimetic hnRNPK increased IP-10 mRNA stability even in normal grown THP-1 cells.

RNA-IP analyses using anti-GFP specific antibody, showed enhanced binding of non-methylable hnRNPK$^{K\rightarrow I}$ on IP-10 mRNA, than the hnRNPK$^{WT}$ in S100b treated cells suggest, among the total hnRNPK pool, demethylated hnRNPK specifically binds on IP-10 mRNA and stabilizes it.

S100b induces increased LSD-1 mRNA level in THP-1 cells.

LSD-1 demethylates lysine-219 of hnRNPK and this was confirmed by LSD-1 knock down experiment.

Depletion of LSD-1 leads to destabilization of IP-10 mRNA in THP-1 cells supports the involvement of LSD-1 in S100b induced IP-10 mRNA stability.
Figure 38: Portrays the overall findings of molecular mechanisms of S100b induced IP-10 mRNA stabilization in THP-1 cells.