Conclusions

Chapter 7

1. Identification of unique genetic patterns in SLE patients with distinct autoantibody specificities by next generation sequencing

- In whole transcriptome sequencing of distinct SLE subset we identified a total of 1283 isoforms dysregulated in anti-dsDNA$^+$ patients; anti-ENA$^+$ patients had 1094 dysregulated isoforms whereas anti-dsDNA$^-$ENA$^+$ showed 819 isoforms.

- The uniquely expressed isoforms in each SLE subset were observed to be associated with specific disease events. Glucocorticoid receptor signaling and multiple cytokine signaling pathways were observed to be dysregulated in anti-dsDNA$^+$ patients whereas IFN signaling was dysregulated in anti-ENA$^+$ patients. SLE subset with both anti-dsDNA and anti-ENA autoantibodies shows actin based motility by Rho signaling pathway to be affected.

- The different isoforms of a genes were observed to be expressed differentially in SLE patients’ subsets. Among those isoforms of the IFN signature genes express predominantly in anti-ENA$^+$ SLE subsets. This together with dysregulation of IFN signaling in anti-ENA$^+$ subsets clearly distinguish it from other two SLE subsets.

- Isoforms of the granulocyte signature genes express in all SLE subsets but they have predominant expression in anti-dsDNA$^+$ patients as compared to other SLE subsets.

- Differentially expressed genes in each subsets revealed a total of 258 genes in dysregulated in anti-dsDNA$^+$ patients; anti-ENA$^+$ patients had 128 dysregulated genes whereas anti-dsDNA$^+$ENA$^+$ showed 89 differentially expressed genes.

- Chemokine, cytokine, cell cycle related genes were uniquely dysregulated in anti-dsDNA$^+$ SLE patients; metabolism related enzymes were differentially expressed in anti-ENA$^+$ patients and endocytosis related genes expressed in anti-dsDNA$^+$ENA$^+$ SLE patient.
Identification of distinct pathway associated with specific group of SLE patients clearly distinguish the patients with anti-dsDNA autoantibody, patients with autoantibodies against ENA and patient positive for both (anti-dsDNA and anti-ENA) autoantibodies.

2. Identification of differentially expressed proteins in distinct subset of SLE patients sera by MALDI-TOF

- Increased expression of hemopexin and Apo-AIV was observed specifically in anti-dsDNA\(^+\) SLE sera whereas downregulation of serotransferrin and Apo-AI was observed in anti-ENA\(^+\) patients sera in particular.
- Haptoglobin was over expressed in all SLE subset while reduced Apo O expression was found in anti-dsDNA\(^+\) and anti-ENA\(^+\) subsets.

3. Heat shock protein 27 and its regulatory molecules express differentially in SLE patients with distinct autoantibody profiles

- Reduced expression of HSP27 was observed specifically in anti-ENA\(^+\) SLE patients as compared to controls.
- Specific downregulation of Brn3a in anti-ENA\(^+\) subgroup and positive correlation with the HSP27 expression suggests its role in HSP27 regulation.
- Overexpression of hsa-miR-939 in specific subset of SLE patient could contribute to the decreased Brn3a expression.
- Increased expression of apoptotic markers, caspase 3 and PARP in ungrouped SLE patients suggests high incidence of apoptosis in SLE patients. Expression of caspase 3 and PARP was upregulated in anti-dsDNA\(^+\) and anti-ENA\(^+\) SLE subsets.
- Negative correlation was observed between HSP27 and Caspase; HSP27 and PARP
- This suggests that decreased HSP27 may have association with increased rate of apoptosis in anti-ENA\(^+\) patients.
SLE patients with distinct autoantibody profile have differential immunoregulatory mechanisms.

4. **Heat shock protein 27 induces proinflammatory cytokine via TRAM mediated signaling of TLR4**

- HSP27 has an immunomodulatory role. It triggers inflammatory response by inducing IL-1β and TNF-α expression.
- Stimulation of TLR4 expression suggests that HSP27 may be mediating its effect through TLR4.
- HSP27 may be utilizing MYD88 independent pathway (TRAM) unlike LPS.
- Identifying the intermediate signaling molecule utilized by HSP27 could be targeted for therapeutics in inflammatory disease.

Unique genetic expression patterns were observed in SLE patients with distinct autoantibody profile to specific nuclear autoantigens. Identification of differential immuno-regulatory mechanisms operative in distinct SLE patients’ subset based on autoantibody profiles that sets them apart. It clearly differentiates SLE patients with autoantibodies against dsDNA from patients with anti-ENA autoantibodies at various levels. Altogether, this study suggests that these subset specific events could be autoantibody driven in SLE patients. The ‘subgrouping’ approach employed in this study proved useful for delineating the diverse disease pathways and developing greater in-depth understanding of SLE. It may also address the challenges faced in diagnosis and treatments due to tremendous heterogeneity in SLE. Indeed several immunosuppressive drugs are available for treatment of SLE but when specific clinical manifestation is considered these have low efficacy and many side effects. Identifying the subset specific defective processes associated with varying disease manifestations in SLE could further be considered for designing target specific therapy for different SLE patients’ subsets.