Introduction

Systemic lupus erythematosus (SLE) is an autoimmune inflammatory disease with enormous heterogeneity in clinical manifestations and autoantibody repertoires. The classical manifestations of SLE include nephritis, pericarditis, arthritis, rashes, etc., and are supposed to result from exuberant autoantibody responses against nuclear and cytoplasmic components. Two major groups of autoantibodies present in SLE patients are predominantly directed against nuclear antigens specifically double stranded DNA (dsDNA) and extractable nuclear antigens (ENA) though, the mechanisms underlying this distinct autoantibody profile is poorly understood (Sherer et al., 2004; To et al., 2005). The etiology of SLE is multifactorial involving genetic, environmental and epigenetic factors (Hocheberg., 1997; Danchenko et al., 2006). However, there is no general unifying theory which could explain the basis of heterogeneity in autoantibody repertoire.

The complexity prevailing in SLE patients due to autoantibody diversity has been addressed in this study by characterizing them into different subsets based on their autoantibody profiles i.e. anti-dsDNA+ or anti-ENA+ or anti-dsDNA+/anti-ENA+. Earlier studies, in our lab evaluating each subset independently has suggested distinct autoantibody profiles in SLE patients are associated with differential and specific Toll like receptor (TLR) expression patterns (Chauhan et al., 2013). This could be the basis for the heterogeneity in autoantibody repertoires. Furthermore, identification of different sets of microRNA (miRNAs) dysregulated and its association with unique biological pathways in SLE patient with distinct autoantibody specificities have suggested that differential immunoregulatory mechanisms are operative in each subset of SLE patients (Chauhan et al., 2014). These distinct disease specific events are often missed due to great heterogeneity of autoantibody repertoires (Chauhan et al., 2013; 2014). Therefore, studying each subset independently can help develop a better understanding of unique mechanisms underlying autoantibody diversity in SLE.
Nevertheless, much more remains to be elucidated at the gene expression level and their regulatory mechanisms in SLE.

Genome wide expression profiling with the emergence of microarray technology has provided broader perspective in the identification of several new genes and pathways associated with SLE pathogenesis. These include the of type I Interferon (IFN) signature genes, cytokines and chemokine receptors, cell cycle related genes in SLE (Rus et al., 2002; Han et al., 2003; Rus et al., 2004). Recently, the advent of the next generation sequencing technology (NGS) has provided useful information on differential splice variants, different splicing events and identification of novel transcripts associated with the SLE (Shi et al., 2014).

Apart from the differential gene expression levels other factors like autoantibodies has been shown to contribute to the SLE pathogenesis in the form of specific organ manifestations. Interestingly, anti-dsDNA autoantibodies have been reported to strongly associate with renal involvement in SLE patients (Linnik et al., 2005; Mosca et al., 2006). The anti-ENA autoantibodies were found to be associated with several clinical manifestations such as Raynaud’s phenomenon and pericarditis (Hoffman et al., 2003), less incidence of renal disease (Clotet et al., 1984). This fact could further be utilized to identify the unique biomarkers associated with specific clinical manifestations in SLE patients.

It is of great interest to identify the factors associated with dysregulated apoptosis. As generation of autoantigens of nuclear origin, like dsDNA and ENA have largely been associated with dysregulated apoptosis and defective clearance of apoptotic debris in SLE. Heat shock proteins (HSPs) are widely known for its anti-apoptotic properties (Garrido et al., 1999; Shreedhar et al., 2004; Lanneau et al., 2008). Specifically, increased expression of HSP27 has been implicated in several inflammatory pathologies like rheumatoid arthritis (Sedlackova et al., 2011) and multiple sclerosis (Aquino et al., 1997). Due to the involvement
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of HSP27 in apoptosis regulation and its implication in inflammatory diseases it was of
interest to study its role in SLE and their regulatory mechanism.

In the present investigation we employed same sub-grouping approach (Chauhan et al., 2013; 2014) as previously done in our laboratory to identify the unique genetic expression patterns in SLE patients with distinct autoantibodies specificities using genomic and proteomic approaches. The SLE patients were grouped into three subsets on the basis of autoantibodies present. These include subset I- SLE patients with anti-dsDNA autoantibody; subset II- SLE patients with anti-ENA autoantibody and subset III patients having both anti-dsDNA/ anti-ENA autoantibodies. The whole study was divided into following objectives to delineate the subset specific events in SLE patients:

1. To identify the differential gene/ isoforms expression and associated pathways in different subsets of SLE patients by next generation sequencing technology.
2. To identify the differentially expressed proteins in sera of SLE patient subsets using proteomic approach.
3. To investigate the expression of pattern of HSPs in different subsets of SLE patients and their association with apoptosis.
4. To study the immunomodulatory role of heat shock protein 27.

In this study we identified unique genetic patterns in SLE patients with distinct autoantibody specificities using NGS technology (RNA-sequencing). The pathway analysis of differentially expressed genes/ isoforms in each subset revealed specific canonical pathways associated with distinct SLE subsets. Further, we investigated differentially expressed proteins in distinct SLE patients’ sera using proteomic approach. The differentially expressed proteins characterized were observed to be implicated in specific clinical manifestations. In addition, significant downregulation of HSP27 expression was observed specifically in anti-ENA+ SLE patients. Anti-dsDNA+ and anti-ENA+ subgroup showed increased expression of
apoptotic markers; caspase3 and poly (ADP-ribose) polymerase (PARP). Negative correlation of HSP27 expression with caspase3 and PARP expression is suggestive of its association with apoptosis in SLE.

Further, studying the immunomodulatory role of HSP27 in THP-1 (Human monocytic cell line) we identified HSP27 utilizes TRIF related adapter molecule (TRAM) for downstream signalling via TLR4.

Taken together, the results suggest that employing the ‘sub-grouping’ approach can help in the identification of subset specific events operative in SLE patients which could have been masked if the SLE patients were studied as single group. The observations made from this study suggest that the patients with autoantibodies against dsDNA are distinct from the patients with anti-ENA autoantibodies at various levels. Identifying the subset specific defective processes associated with varying disease manifestations in SLE could further be employed for designing target specific therapy in SLE.