CHAPTER 5

CONCLUSIONS AND FUTURE TRENDS
5.1 Conclusions

Endometrium, a heterogeneous tissue lining the uterus, is highly dynamic and exhibits marked cyclical changes during the menstrual cycle. Any aberration in endometrial physiology would lead to diseases like endometriosis, endometrial polyps, hyperplasia and endometrial cancer. Therefore, there is a need to understand the molecular basis of function of the endometrium per se. Further, the identification of molecular differences in the endometrium of women with endometriosis is an important step towards understanding the pathogenesis of this condition and for developing novel strategies for the treatment of associated infertility and pain. It also sets a framework for further investigations on mechanisms underlying the pathogenesis of endometriosis.

Earlier studies using the conventional approach of purifying and characterizing the proteins from the endometrium in different physiological states have led to the identification of a number of endometrium-specific proteins and also the functions of some of these proteins like, sialic acid binding protein (54 kDa glycoprotein) (Sen et al., 2001), glycodelin (Vigne et al., 2001), IGF-binding proteins, PP14 (Seppala et al., 1992), and CYP3A7, a cytochrome P450 isoform, which metabolizes estrogens (Sarkar et al., 2003) in human proliferative as well as secretory phase endometrium. These earlier studies were limited with respect to the identification of only a few proteins. However, the advent of genomics and proteomics has led, not only to the identification of several genes and proteins that are characteristic of the different phases of the menstrual cycle (Kao et al., 2002; Talbi et al., 2006; DeSouza et al., 2005; Chen et al., 2009), but have also provided clues for a better understanding of the diseases like endometriosis (Eyster et al., 2002; Matsuzaki et al., 2005; Wu et al., 2006; Burney et al., 2007; Zhang et al., 2006; Fowler et al., 2007; Have et al., 2007;). However, studies carried out so far did not provide a consistent profile of the proteome of the human endometrium and not many differentially expressing proteins in endometriosis are identified.
In the present thesis, using two-dimensional gel electrophoresis (2D-PAGE) combined with semiquantitative computerized analysis and immunoblotting, we have established the proteome of human endometrium in the molecular weight range of 10-110 kDa and pl range of 4-7 and investigated the differential expression of proteins in human endometrium in the proliferative and secretory phase of the menstrual cycle. In the present work, 2D-PAGE of human endometrium protein led to the resolution of over 200 spots. Subsequent mass spectrometry analysis of 215 spots allowed the identification of 194 proteins. 57 of the 215 spots were found to be differentially expressed, out of which 49 could be identified using MALDI MS and/or MS/MS. These differentially expressed proteins included structural proteins, molecular chaperones, signaling proteins, metabolic proteins, proteins related to immunity, RNA biogenesis, protein biosynthesis, and others. The differential expression of seven representative proteins in secretory and proliferative phase endometrium tissue was confirmed by immunoblot analysis.

Further attempts were made to get a more consistent profile of aberrant protein expression in proliferative and secretory phase of eutopic endometrium from women having endometriosis. More than 70 proteins were found to be differentially expressed in the proliferative phase of eutopic endometrium in stage IV and secretory phase of stage II, III and IV endometriosis. Among these, 48 protein spots which were consistently differentially expressed from stage II to IV endometriosis were successfully identified by MALDI MS and/or MS/MS. Thus, the present study identifies many more differentially expressed proteins in the proliferative and secretory phase of eutopic endometrium compared to the earlier studies (Zhang et al., 2006; Fowler et al., 2007; Have et al., 2007). The differentially expressed proteins include structural proteins, proteins involved in stress response, protein-folding and protein-turnover, immunity, energy production, signal transduction, RNA biogenesis, protein biosynthesis and nuclear proteins. Immunoblot and immunohistochemical analyses confirmed the observed changes in eight representative proteins.
Subsequently, attempts were made to assign a function to one of the differentially expressed proteins to endometriosis. For this purpose, DJ-1 was chosen for detailed studies. DJ-1, originally identified as an oncogene product, is a protein with various functions in cellular transformation (Nagakubo et al., 1997), oxidative stress response (Taira et al., 2004; Zhou and Freed, 2005), and transcriptional regulation (Fan et al., 2008). More recently, high DJ-1 levels have been reported in tumors of the lung, ovary, esophagus, thyroid, pancreas, and brain, and in leukemia, suggesting that enhanced expression of DJ-1 may play a role in cancer initiation and/or progression under certain circumstances (MacKeigen et al., 2003; Alsoe et al., 2008; Davidson et al., 2008; Liu et al., 2008). In the present study, we determined the role for DJ-1 in normal endometrial as well as in endometriotic cell survival, attachment, proliferation, migration and invasion either by overexpressing DJ-1 in normal endometrial cells or by knocking down DJ-1 expression using siRNA in endometriotic cells. Our studies indicated that DJ-1 protects endometrial cells from oxidative stress mediated apoptosis and overexpression of DJ-1 in normal endometrial epithelial cells increases the adhesion on collagen type IV. However, no significant difference was observed in case of stromal cells. We further demonstrated that DJ-1 regulates cell proliferation, migration, and invasion in normal endometrial and endometriotic epithelial cells whereas in case of normal endometrial and endometriotic stromal cells, it regulates cell proliferation and invasion but not migration. Furthermore, the present study also indicated that DJ-1 regulates these cellular processes by modulating PI3K/Akt pathway by interacting and negatively regulating PTEN.

5.2 Salient findings of the thesis

1. Proteome of human endometrium comprising of 194 protein spots has been established in the molecular weight range of 10 to 110 kDa and pI range of 4 to 7.

2. Comparative study between proliferative and secretory phase endometrium showed that 57 protein spots were differentially expressed out of which 49 protein spots were
identified using MALDI MS and/or MS/MS. The differential expression of seven representative proteins in secretory and proliferative phase endometrium tissue was confirmed by immunoblot analysis.

3. Differential proteome profiling both in proliferative and secretory phase of eutopic endometrium from women having endometriosis using 2D-PAGE and mass spectrometry revealed that more than 70 proteins were found to be differentially expressed in the proliferative phase of eutopic endometrium in stage IV and secretory phase of stage II, III and IV endometriosis.

4. Among these, 48 protein spots which were consistently differentially expressed from stage II to IV endometriosis were successfully identified by MALDI MS and/or MS/MS.

5. Immunoblot and immunohistochemical analyses confirmed the observed changes in the eight representative proteins.

6. Functional characterization of one of the candidate proteins, DJ-1 revealed that this protein might be involved in pathogenesis of endometriosis by regulating endometrial cell survival, proliferation, migration, and invasion at ectopic sites.

This study for the first time establishes proteome of human endometrium and implicates DJ-1 in the pathogenesis of endometriosis.

5.3 Future trends

Since the cellular and molecular mechanisms involved in the development and progression of endometriosis are still unclear, there is a need to identify proteins that are unique to endometriosis and also study the functional role of other differentially expressed proteins in endometriosis.
There are certain questions that remain to be answered:

1. What is the molecular basis for the selective effects of DJ-1 on epithelial and stromal cells?
2. How does DJ-1 regulate processes of cell proliferation, migration, and invasion?
3. What is the role of other differentially expressed proteins in endometriosis?