Conclusion
Endometriosis is a gynecological disorder that affects ~10% of reproductive women worldwide. It’s an enigma to modern society, with etiology unidentified, therapeutics inadequate and the percentage of affected women are increasing with time. The current therapeutics relies on GnRH-based, SERM and aromatase inhibitor-based drugs, which are able to manage pain responses, but are not adequate to regress endometriosis. To understand the molecular pathology for development of proper therapeutics against endometriosis is a necessity. In this regard, the present thesis looked into a group of proteases, MMPs that are involved in ECM degradation and play important roles for pathogenesis of endometriosis.

The present study reports elevated responses of MMP-2 activity with progression of ovarian endometriosis, which is also associated with decreasing TIMP-2 and elevated MT1MMP expressions. Endometriosis, being an angiogenesis-dependent growth, can be regulated through inhibiting blood vessel formation; moreover, few in vivo studies have already reported to regress endometriosis by multi-kinase inhibitors. The approach for inhibiting angiogenesis was different in our study, as we inhibited specific MMP-2 activity. Targeting MMP in endometriosis is not completely novel; inhibition of total MMP can regress endometriosis in nude mice model. However, effect of inhibition of specific MMP in relation to angiogenesis in endometriosis is reported for the first time. We found that inhibition of MMP-2 not only reduced tube formation of endothelial cells, but also regressed angiogenesis in CAM assay. It was obvious, that inhibition of total MMP should have profound effect against angiogenesis, as different MMPs can influence different growth factors, associated with angiogenesis. In this regard, MMP-9 can be an important factor, as MMP-9 increase soluble VEGF level by cleaving bound VEGF. Our study report elevated MMP-2 activity alongwith MMP-9 activity in PGE2-induced tube formation of endothelial cells. However, decrease in cellular invasiveness and endothelial tube formation upon inhibition of only MMP-2 proves relevance of MMP-2 for the process of angiogenesis. The regulation of PGE2 over MMP-2 activity through pAKT mediated responses is another important information that came from the first chapter.

The next chapter deals with MMP-1/collagenase-1 and its involvement in the pathogenesis of ovarian endometriosis. Although earlier few studies have identified presence of MMP-1 in endometriosis, we report elevated expressions of MMP-1 in serum and ectopic tissues with disease severity. We also identified reduced TIMP-3
expression in late stages of ovarian endometriosis. The reduced TIMP-3 expression with progression of endometriosis was previously reported by our lab in animal model. Moreover, TNF-α-mediated elevated MMP-1 responses were already reported in endometriotic stromal cells. Interestingly, we found not only inflammatory factor like IL1β, but also pro-angiogenic factors like PGE2 can elevate MMP-1 expression in endometriotic cell line. Moreover, PGE2 and IL1β also elevate MMP-2 and MMP-9 activity respectively and together upregulate both the gelatinases. PGE2 and IL1β show a synergistic effect for MMP-1 expression and cellular invasion, indicating that presence of an angiogenic factor with inflammatory responses aggravate disease progression. Indeed, increased invasiveness of endometriotic cells resulted from elevated MMP responses; where alongwith MMP-1, we should also consider increased gelatinases activities. Furthermore, knockdown of MMP-1 through siRNA treatments significantly reduces cellular invasiveness, proving the relevane of MMP-1 over cellular invasiveness. PGE2 also rescues endometriotic cells from inflammation-induced decreased cellular viability, which suggest protective roles PGE2 over cellular death. Another interesting fact that emerges from the present study is that even in influence of inflammatory or angiogenic or both factors JNK-cJUN-mediated responses remain the principle regulator of MMP-1 transcription in endometriosis. The expression status for MMP-1, however, might change depending upon the stimulatory factors. We also found ETS-1 involved with AP-1 mediated MMP-1 transcription, however the exact roles for ETS remain unexplored for the present study. Recent studies on endometriosis show increased cellular invasiveness and EMT-like phenomenon in the disease. In this regard, our study has found one interesting clue from collagen staining. We found endometriosis progression is associated with increased collagen levels with fragmented and irregular deposition of fibrile collagen. Similar to disease like fibrosis, dysregulated deposition of collagen in higher stages of endometriosis might increase tissue stiffness and mechanical-stress promoting cellular invasiveness and EMT-like processes.

In the final chapter, we have utilized mouse model of endometriosis, where mouse endometrial cells are inoculated into the peritoneum of another mouse to develop endometriosis. The model was used to look into disease patho-physiology and therapeutic treatments by curcumin. However, this model puts forward a number of limitations. Indeed, mouse does not menstruate, which makes the disease an ‘induced endometriosis’ model. Moreover, it is difficult to identify proper endometriosis glands
throughout the peritoneum. The numbers of lesions are inconsistent among animals due to graft rejection and give a very limited window of time to evaluate disease pathogenesis or therapeutic treatments. Researchers are also using surgical model of induced endometriosis, which differ from the described model. In surgical model, uterine horns are stitched into the peritoneum of the same mouse. Because these implants already contain functional and well organized endometrial glands and stroma during transplantation, the model is much of an ‘endometrial regression’ model rather than ‘induced endometriosis’. The present study with mouse model of endometriosis found increased MMP responses with endometriosis progression. Because MMP play important roles in cellular remodeling and ectopic establishment of the lesions, we found increased MMP-1, -3, -9 and -14 responses during endometriosis in mice. MMP-2, being more like a constitutive MMP, acts through increased activation process and both in vivo and in vitro study has found relevant roles of MMP-2 in angiogenesis. The angiogenic responses for in vivo model were depicted from elevated VEGF, COX-2 expressions. Moreover, mouse model of endometriosis showed decreased Bax/Bcl-2 ratio indicating lower apoptotic responses, which might also be a part of endometriosis auto-regression. Treatments with curcumin regressed endometriosis by inducing severe apoptotic responses through mitochondria-mediated pathway. Curcumin also elevated p53 and p21 responses in animal model of endometriosis indicating presence of cellular senescence along with mitochondrial apoptosis. For conventional treatments, curcumin does have disadvantage due to its low bioavailability, however, because curcumin is widely used as dietary component in many nations, it can act as a preventive measure against endometriosis.

In summary, the thesis work was focused on matrix based cellular signaling with major interest on MMPs and TIMPs in ovarian endometriosis. We document the pro-angiogenic role of MMP-2 in endometriosis through prostaglandinE2 mediated signaling responses. We found MMP-1 is involved with endometriosis progression by regulation of inflammatory and angiogenic responses, where JNK-cJUN pathways mediate the transcriptional regulation. Moreover, TIMP-3 play important roles in regulation of MMP-1 and subsequent cellular invasion. Mouse model of endometriosis showed elevated MMP responses with disease progression, which were significantly inhibited by curcumin treatment. Curcumin regressed endometriosis by inducing severe apoptosis in the endometriotic lesions through mitochondria-mediated pathways.