Conclusion

Endometriosis is an inflammatory gynecological disorder among reproductive-aged women caused by the growth of endometrial cells outside the uterus, most commonly in the pelvis. The ectopic growth depends on hormonal environment, inflammatory response and dysregulation of ECM remodelling. Almost 10-15% of reproductive women suffer from endometriosis worldwide and the current therapeutics available are not sufficient to treat the disease. Thus, understanding the molecular pathology for development of proper therapeutics against endometriosis is a necessity. In this regard, the present thesis work focuses into the importance of MMP-7 for the pathopahysiology of endometriosis.

The upregulation of MMP-7 activity in the uterine tissues of endometriosis effected patients as compared with the control eutopic endometrium was seen. Interestingly, the overall expression for MMP-7 was found significantly higher in control endometrium, while the MMP-7 activities was higher in uterus of the endometriosis affected women patients. The discrepancies can be explained with substrate zymography, where the women without endometriosis had only proform of MMP -7, wheras the endometriosis patients had mainly active MMP-7 in the eutopic tissues. In addition, MMP-7 activities were elevated with severity of ovarian endometriosis both in serum and ectopic tissues. MMP-7 is mainly reported to be secreted by cancer cells and principally involved in metastasis. MMP-7 knockout mice demonstrates reduced prostate tumorigenicity by means of suppression of invasiveness. Thus, presence of MMP-7 in a benign disease like endometriosis indicates its increased invasive nature. Herein, we found that the major source for MMP-7 in ovarian endometriosis is glandular epithelium. Our study hypothesizes that endometriosis with increased MMP-7 may be involved with EMT phenomenon and a special metastatic-like event in endometriosis may exist, which can be explained with field cancerization.

We report that EMT is also associated with ovarian endometrioma, especially during the late stages of disease. Interestingly, significantly increased slug/snail2 expressions were observed in late stages of ovarian endometriosis, but no measurable increased for snail1 was observed. The selectivity over slug may result from (i) the benign nature of the disease, or (ii) selection of the specific subtype (i.e. ovarian endometrioma). Although, a few studies showed elevated sail1 expression in deep infiltrating endometriosis. In addition, we found elevated levels of total collagen with progression of ovarian

endometriosis. The abnormal deposition of the collagen in cancer tissues are reported to increase cellular invasiveness via mechano-transduction, and can act as a promoting factor for EMT in endometrial cells. Since, endometrium is developmentally derived from intermediate mesoderm via mesenchymal to epithelial transition. Thus, by retaining some imprint of their mesenchymal origin, endometrial epithelial cells are prone to return to mesenchymal states via EMT. Therefore, under inflammatory milieu of ectopic ovarian endometriosis, growth factor–mediated type-II EMT is expected in endometriosis. Apart from EMT in endometriosis, fibroblast to myofibroblast trans-differentiation is also recently reported in baboon model of endometriosis.

Several EMT promoting factor, i.e. EGF, HBEGF and TGF-β showed elevation with severity of endometriosis. TGF-β was found to upregulate MMP-3, while EGF upregulated mainly MMP-7 to induce the EMT in the disease specific endometriosis cells. The EGF mediated activation of MMP-7 was confirmed by treatment of EGFR inhibitor, as well as by MMP inhibitor and silencing of MMP-7. We found that EGF mediated phosphorylation of EGFR promoted pERK-1 and cFOS and pcJUN. CHIP analysis reconfirmed the MAPK-mediated upregulation of binding for cFOS at the AP-1 binding site of MMP-7 promoter region suggesting increased transcriptional activation of MMP-7 as an early response gene. Apart from MAPK, other signaling pathways including WNT-TCF4, play roles in transcriptional activation of MMP-7 in endometriosis, which needs further study. Interestingly, we found that inhibition of MMP-7 suppressed EMT, but inhibition of EMT by silencing vimentin did not suppress MMP-7, which suggested that MMP-7 is not a consequence of EMT phenomenon, rather one of the triggering factor for EMT. Moreover, MMP-7 knockout mice were reported to exhibit decreased EMT and metastasis in mouse model of prostate carcinoma. In addition, cleavage of E-cadherin into soluble E-cadherin through addition of exogenous active MMP-7 is sufficient to facilitate EMT in endometriotic epithelial cells. We demonstrated that the elevated MMP-7 act as a triggering factor for EMT by cleaving E-cadherin and subsequent loss of cell adherence, and leading to the slug-mediated transcriptional activation of mesenchymal proteins, including vimentin, N-cadherin etc.

Finally, we have looked into -181A>G SNP of MMP7 gene in a case-control cohert study in East Indian population of a total of 260 women, among which 130 is control and 130 is endometriosis patients. However, our study failed to detect any significant

association of the MMP7 polymorphism with the risk of endometriosis development. Interestingly, the AG genotype showed higher expression levels of MMP7 than AA genotype. Moreover, the AG and AG+GG genotypes showed significantly higher association for severe stages of endometriosis, as compared to the AA genotype. Herein, elevated MMP-7 responses in G allele individual indicated increased disease progression rate. This also suggested that the G genotype populations are prone to develop severe endometriosis compared to only AA genotype. Furthermore, we found that development of severe endometriosis is associated with G alleles due to the fast progression rate of the disease, as compared to the AA allele.

In summary, the thesis work highlights the role of MMP-7 in the pathogenesis of endometriosis through both genotypical and functional responses. We document that increased MMP-7 in glandular epithelial cells of late stages of ovarian endometriosis augment cellular invasion and EMT responses. EGF mediated activation of MMP-7 through MAPK-AP-1 signaling is involved in the cleavage of E-cadherin to sluble E-cadherin in endometriotic epithelial cells and thus, resulting in loss of cell adherence and polarity, leading to the slug-mediated transcriptional activation of mesenchymal proteins, and EMT process. Lastly, the MMP7 -181A>G SNP is found not to be associated with endometriosis development in Eastern India population. However, the G genotype endometriosis-affected women are found to have higher MMP-7 expressions and has higher risk to develop severe stages of endometriosis in relatively lower time span as compared to the AA genotype women in the Eastern Indian population.