7. CONCLUSION AND FUTURE PERSPECTIVES

7.1. Conclusion

Vascular insulin resistance manifests in decreased production of nitric oxide subsequently leading to vasoconstriction and atherosclerosis. Inflammatory molecules such as IL-6 is known to induce insulin resistance in vascular endothelial cells during pathological conditions such as type 2 diabetes. Epigenetic mechanisms including promoter DNA methylation has been demonstrated in the development and progression of metabolic disorders and atherosclerosis. However, precise and underlying epigenetic mechanisms governing the vascular insulin resistance are not known. The present study was designed to functionally link inflammatory mediators (IL-6), epigenetic mechanisms (DNA methylation) and vascular function (insulin resistance). We demonstrated (i) cross-talk between IL-6 and insulin signaling pathways and its concomitant effects on angiogenesis, (ii) influence of IL-6 on the regulation of DNA methyltransferases and its consequences on DNA methylation and (iii) IL-6 induced differential CpG methylation of specific genes.

The first objective of this study was to investigate the role of IL-6 on insulin resistance in human endothelial cells. First, we established an in vitro model to culture endothelial cells by isolating and characterizing them from umbilical cord and foreskin. Further, experiments were performed to understand the responses of signaling intermediates as a consequence of interplay between IL-6 and insulin. Although both IL-6 and insulin activated STAT3, Akt and eNOS phosphorylation, their temporal responses and magnitude varied. Interestingly, these effects were abrogated when the cells were treated with insulin and IL-6. This indicated the insulin and IL-6 together possess an antagonistic role. IL-6 pretreatment led to decreased insulin responsiveness which was marked by reduced eNOS phosphorylation. Similarly, chronic insulin conditions also induced a state of insulin resistance and abrogated IL-6 effects. Further IL-6 induced serine phosphorylation of IRS1 confirmed that IL-6 as one of the potential inducers of vascular insulin resistance. As a functional consequence, insulin and IL-6 effects were measured on the ability of endothelial cells to form sprouts in 3D cultures. Both collagen and Matrigel assays showed reduced angiogenesis in the presence of IL-6 and insulin together, although both molecules showed facilitated tube formation and outgrowth of sprouts when treated alone.

Our next objective was focused on elucidating the influence of IL-6 on DNA methylation levels of the endothelial genome and its underlying mechanisms. HPLC analysis of global methylcytosine measurement revealed that IL-6 induced global DNA hypomethylation in endothelial cells. As DNA methylation is mediated by DNA methyltransferases such as DNMT1, DNMT3A and DNMT3B, we investigated effects of IL-6 on these enzymes. Time
and dose-dependent experiments revealed that IL-6 reduced the levels of DNMT1 and DNMT3B but not DNMT3A levels. Further experiments showed that IL-6 induced reduction in DNMT1 levels was through proteasomal degradation.

Additionally, our cell cycle analysis indicated that IL-6 increased the population of S-phase cells, which was kinetically associated with DNA hypomethylation, suggesting IL-6 reduced replication-associated DNMT1 levels may be due to activation of specific genes by DNA hypomethylation. Interestingly, functional blocking of DNA methyltransferases also resulted in a state of insulin resistance in endothelial cells. This suggested endothelial insulin resistance is associated with epigenetic reprogramming.

Next, we explored CpG island microarray to identify IL-6 induced differentially methylated CpG islands in the endothelial genome. We observed significant changes in methylation patterns across the genome. Probe annotation based on genomic location revealed significant number of differentially methylated CpGs were located at both gene body and promoter regions. Further analysis showed 2438 hypomethylated probes corresponding to 199 genes promoter and 1892 hypermethylated probes associated with 98 genes promoter. Based on the pathway analysis and disease association, we selected eleven genes for further validation. Among hypomethylated genes RPS6KA2, MAP3K8, PIK3R2, FOXD3 and EXOC7 were considered. On the other hand, hypermethylated genes including FOXC2, IGF-1R, ITPKB and EPHA6 were validated.

Taken together our data suggested that IL-6 plays a crucial role in inducing vascular insulin resistance through modulating DNMT1 levels and thereby regulating genes involved in insulin signaling, inflammation and angiogenesis. Our investigations showed a connecting and functional link between inflammation, epigenetic mechanisms and insulin resistance in endothelial cells.

### 7.2. Future Perspectives

Insulin resistance plays a vital role in the development and progression of type 2 diabetes that leads to cluster of cardiovascular risk factors known as ‘metabolic syndrome’. Several studies have shown the potential role of chronic inflammation in the development of cardiovascular diseases during diabetes. In agreement with the earlier studies, our present study also demonstrated that IL-6, an inflammatory molecule alters the insulin signaling pathway thereby promoting endothelial dysfunction. We also demonstrated the effects of IL-6 on DNMTs that led to differential methylation of several genes which might affect insulin signaling pathway recurrently.

As development of metabolic syndrome is strongly influenced by environmental factors such as lifestyle and dietary habits and, epigenetic changes serve as a “translator” between
environmental changes and gene expression patterns. Further understanding of the factors influencing epigenetic changes and its molecular mechanism would be beneficial for the development of new therapeutic targets. Clinical and experimental studies have shown that despite glycemic control by pharmacological intervention, diabetic individuals’ show overt vascular complications, indicating epigenetic changes during acute phases of diabetes which might be permanent. These epigenetic alterations during early hyperglycemic condition are referred as ‘metabolic memory’ or ‘legacy effect’. Several factors such as environment, lifestyle and excessive caloric intake might lead to abrogation of normal epigenetic profile that results in overexpression several genes involved in inflammation, oxidative stress and cellular homeostasis. Epigenome can mitotically inherit gene expression patterns during proliferation of the cells. These may confer the ability to memorize changes in gene expression patterns in response to external stimuli and fail to regain normal patterns once the stimulus is removed. Hence, better understanding of these permanent epigenetic alterations and its inducers during diabetic hastened cardiovascular complication may provide better therapeutic targets.