SUMMARY AND CONCLUSION

CHAPTER 6

Summary

The thesis highlights the regulatory role of PARP-1 in islet differentiation from pancreatic endocrine resident progenitors (PREPs). Objective 1 (Chapter 3) of the thesis focuses on the identification, characterization, and islet differentiating potential of PREPs. PREPs were isolated from purified islets from the pancreas of BALB/c mice, and were further characterized extensively for candidate markers. PREPs were positive for stem cell markers like Nestin and CD133 and also demonstrated mesenchymal stem cell markers such as CD90, CD44 and negative for CD45. After marker validation, multipotency of PREPs was functionally assessed by trilineage differentiation where they successfully differentiated into adipocytes, osteocytes and chondrocytes. Since the source was pancreas, we further analysed for unique pancreatic progenitors characteristics. They were positive for Glut2 which has been also previously reported for pancreatic progenitors as well as for CD34 which is now perceived to be a tissue specific progenitor marker. Thus, we believe that these two markers can be used to identify and enrich the PREP population from adult pancreas. Further, these progenitors were positive for key transcription factors essential for islet neogenesis viz. Pdx-1, Neurog3, NeuroD, Pax4, Nkx6.1, and activated pSmad3. Presence of the above markers suggested that PREPs have inherent potential to generate functional islets. Validation of PREPs for its islet differentiation were monitored by temporal molecular characterization during islet differentiation using two separate differentiating agents -Activin A and Swertisin into mature islet clusters in a span of 4 days. Thus, confirming that they take the shortest route to form functional islet clusters with DMEM KO media against DMEM Ham's F-12 media which took eight days. The functional characterization of these clusters specified Swertisin to be more potent than Activin A in forming islet clusters.

After defining PREPs and their islet differentiation potential, we explored the role of PARP-1 in the paradigm of islet differentiation from PREPs. In chapter 4, PARP-1 was silenced in PREPs. PARP1 knockdown altered the morphology, decreased proliferative potential and significantly deviated marker profile of these cells. CD34 and Nestin were downregulated along with all the key transcription factors essential for islet neogenesis. These cells also lost their trilineage differentiation potential, thus stating that PARP1 is important for maintenance of multipotency. The down regulation of pancreatic progenitor markers, and loss of differentiation potential suggested loss of their pancreatic progenitor phenotype which was confirmed by their inability to form islet clusters with either Activin A or Swertisin. Further, to distinguish whether the PARP-1 enzyme activity or the PARP-1 protein is essential for islet differentiation, PARP-1/2/3 inhibitor, ABT-888 was used. The results confirmed that inhibition of PARP enzyme activity showed no effect on PREPs ability to generate functional islets suggesting that PARP-1 protein has crucial role in islet differentiation independent of its enzyme activity. Temporal comparative molecular characterization between the normal and the PARP-1 KD PREPs demonstrated persistent downregulation of the key transcription factors during islet differentiation. We demonstrated similar results with the transcriptome analysis in PANC-1 cell line. Further, we confirmed that expression of PARP-1 was vital to islet differentiation by recovering the PARP-1 expression in the knockdown cells which resulted in complete recovery of PREPs to generate functional islets. The kinetics of SMADs during islet differentiation from normal PREPs was observed to be similar to that reported during pancreatic development, where phosphorylated SMAD2/3 form a complex with SMAD4 and translocate to the nucleus to regulate specific genes. Also, interestingly phosphorylated Smad3 expression decreased with depletion of PARP-1. Thus, further our experiment of co-immunoprecipitation data clearly confirmed that PARP-1 directly interacts with pSMAD3 and may act as its positive coregulator during islet differentiation. Finally, to

screen key transcription factors essential for islet differentiation under possible control of PARP-1, Chip was performed. We confirmed that PARP-1 bound to the promoter sequences of six important genes viz. Reg3a, Pdx-1, Neurog3, NeuroD, Pax4 and Nkx6.1 respectively. This is the first report to map PARP-1's mechanistic role in islet differentiation from stem/progenitors.

After clearly defining the role of PARP-1 in islet differentiation with various strategies under *in vitro* condition (chapter4), we extended the study at *in vivo* level (chapter 5). Both PARP-1 KD PREPs and normal PREPs were transplanted with and without Swertisin in STZ induced diabetic mice. We observed that almost all the PREPs homed in to the damaged pancreas and did not get trapped into any other major organ. However, we couldn't locate PARP-1 KD cells in any of the organs and there were no cells localized into the pancreas. Also, there was no difference in homing between the PREPs transplanted with and without Swertisin. The groups in which PREPs migrated to pancreas ameliorated their diabetic condition by normalizing blood glucose and insulin levels. Although in PARP-1 KD group no homing was observed, Swertisin treatment demonstrated reduction in fasting blood glucose. While screening the transplanted PREPs, in pancreatic tissue, it was noted that the transplanted PREPs localized within the pancreas had differentiated into small islet clusters, which were non-existent in the PARP-1 KD groups. It was interesting to notice that similar transcription factors were upregulated in the normal PREPs transplanted groups both with and without Swertisin as observed under in vitro differentiation, providing strong evidence for pancreatic endocrine regeneration. Present data also proves that PARP-1 is not only essential for islet differentiation/neogenesis but also for homing of stem/progenitors. As we observed high expression of transcriptional factors of islet neogenesis and amelioration of blood glucose in Swertisin treated PARP-1 Knockdown PREP transplanted group, another experiment with an extended period of Swertisin administration was carried out in STZ

diabetic mice. It was observed that Swertisin treatment reversed hyperglycaemia to normoglycemia by tenth day of its treatment with significant increase in fasting insulin levels indicating amelioration in pancreatic endocrine function. Further, transcription factors essential for islet neogenesis within the pancreas of the Swertisin treated mice were significantly upregulated along with pancreatic progenitor markers suggesting that Swertisin could trigger pancreatic progenitors to form new functional islet clusters and thus ameliorate diabetic condition.

This study allowed us an insight into the definite role of PARP-1 in islet differentiation from pancreatic progenitors. We now clearly comprehend that PARP-1 is uniquely poised to interact with specific signaling molecules and regulate timely expression of essential transcription factors that drive the islet differentiation for producing mature functional islets. However, whether the same is true with other stem cell sources for generation of islets is still questionable as the kinetics followed by transcription factors with other stem cell sources may vary significantly, which can be further explored. This study focused on transcription factors that are involved in overall beta cell homeostasis hence it will not be surprising if PARP-1 is also involved in functional aspects of mature islets viz. insulin biosynthesis and secretion. Also, it would be very interesting to explore its probable involvement in beta cell replication. All these questions are extremely relevant clinically and can help in developing tools for future diabetic therapeutics.

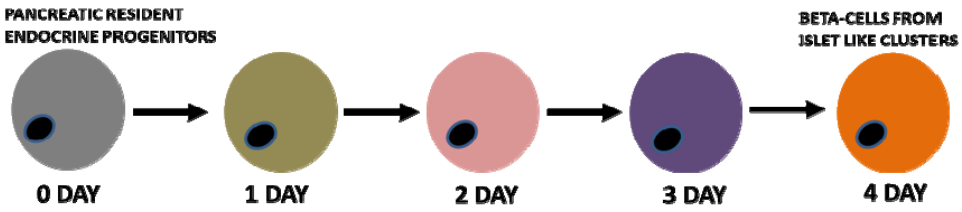
Conclusion

The conclusions from the present study in this thesis are as follows:

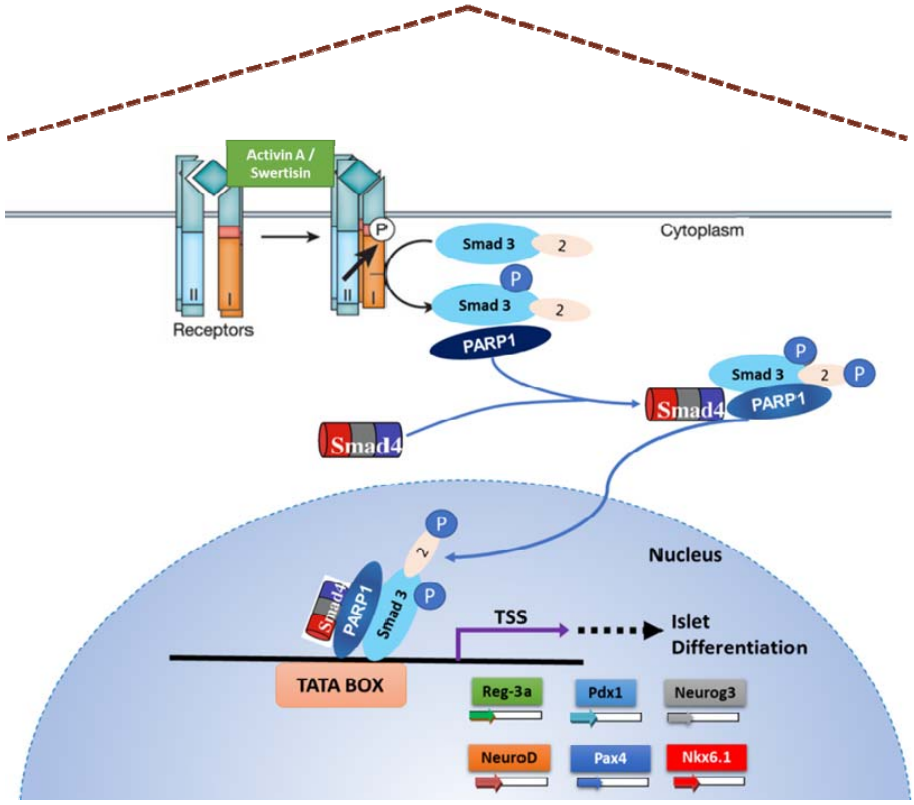
- In the present study, we were able to identify and define a pancreatic progenitor population with unique markers, with inherent potential to take the shortest route for producing functional islet clusters.
- We establish that PARP-1 expression is imperative for successful islet differentiation from stem/progenitor populations.
- PARP-1 interacts directly with phosphorylated SMAD3 and positively regulates its function during islet differentiation from pancreatic progenitors.
- PARP-1' controls the transcription of key transcription factors essential for islet differentiation by binding to their respective promoter regions viz. Reg3a, Pdx1, Neurog3, NeuroD, Pax4 and Nkx6.1.
- PREPs can completely home in to the damaged pancreas without trapping in any other major organ.
- PARP-1 expression is essential for homing of stem cells to the damaged tissue.
- Swertisin, a potent bioactive can stimulate islet neogenesis within diabetic pancreas by triggering pancreatic progenitors to ameliorate the diabetic condition. Thus, acting as an effective therapeutic intervention in treating the diabetic condition.

The present study thus clearly proves role of PARP -1 and Swertisin in islet neogenesis and shall be used in designing better therapeutic strategies for diabetes treatment.

Summary



In Vitro Study



In Vivo Study