SYNOPSIS

Synopsis

Introduction:

The pancreas, specifically the endo-pancreatic beta-cells that are only present and constitute the majority of the islets of Langerhans, are the only source of circulating insulin, which is essential for maintaining glucose homeostasis (Fleming and Rosenberg 2007). Insulin producing beta cell death and malfunctioning is the hallmark of both Type 1 and Type 2 diabetes. Hence, in order to ameliorate the symptoms of diabetes mellitus replenishment of beta cells within the islets is mandatory. Many distinct mechanisms have been discussed to account for beta-cell regeneration viz. (i) trans-differentiation of cells into endocrine beta-cells; (ii) emergence of new beta-cells from pancreatic ductal epithelium; and (iii) replication of pre-existing beta-cells. However, there are also numerous reports that provide evidence for existence of specific pancreatic endocrine precursor cells which have the potential to differentiate into new islet cells but till date these progenitors have not been completely defined in their characteristics. Hence, in this study we have isolated Pancreatic resident progenitors (PRPs) and have completely characterized their functional potential which along with novel bioactive, Swertisin be an effective therapeutic intervention for diabetes mellitus (Dadheech, Soni et al. 2013, Dadheech, Srivastava et al. 2015).

Beta cell death so far has been widely explored and is known to be associated with a nuclear (DNA repair) enzyme called Poly (ADP ribose) Polymerase-1 (PARP-1). This is an important molecule which plays direct role in beta cell necrosis in response to type-1 diabetes and also credits in endothelial injury that underline the etiology of various diabetic complications resulted in type-2 diabetes (Szabo 2005). Role of PARP in diabetes is being explored since last thirty years with early efforts employing PARP-inhibitors (Yamamoto, Uchigata et al. 1981) and

later studies using PARP-$1^{-/-}$ mice or cells (Heller, Wang et al. 1995, Burkart, Wang et al. 1999, Masutani, Suzuki et al. 1999, Pieper, Brat et al. 1999). The best established ones are the role of PARP in the destruction of pancreatic islet cells and role of PARP in hyperglycemia-induced diabetic complications (Garcia-Soriano, Virag et al. 2001, Brownlee 2005) Major emphasis by the scientific community in exploring the role of PARP-1 is centered over beta cell death, survival, physiology and insulin secretion, however least attempts were made to understand the possible mechanism of this enzyme in beta cell regeneration or differentiation. PARP's are now known to be involved not only in cell death and apoptosis, but to have multiple functions over cell differentiation, proliferation and gene expression control. Preliminary reports from our lab demonstrated that stable knockdown of PARP-1 protein completely abolishes islet cell differentiation from HumanPANC-1 Cells even in presence of suitable growth factor for differentiation (Dr. Nidheesh Dadheech Thesis; 2013). Thus understanding the role of PARP-1 and its DNA-Protein interaction with key transcription factors in PARP-1 Knockdown (KD) Human/ Mice primary cultured pancreatic progenitor cell will highlight PARP-1's significance in beta cell differentiation.

Hypothesis:

We hypothesize that PARP-1 has a role in islet differentiation and governs the fate of PRPs by interacting with specific and crucial transcription factors governing beta cell fate.

Significance:

Systematic study of PARP-1 in islet cell formation will help in answering many questions regarding; (1) pancreatic precursor proliferation and differentiation. (2) regulation of new beta cell formation in diabetic pancreatic tissue. The information gained by this project will helps in

better understanding of neoislet formation, regulation and developing newer PARP modulated stem cell therapeutic approach for diabetic patients for efficient islet cell differentiation.

Specific Objectives: Major objectives of the present study are –

1. Establishment of Human/Mice Pancreatic Stem/Progenitors cells for PARP-1+/+ and PARP-1 Knockdown(KD) cells.
2. Transcriptome analysis of Islet neogenic pathway genes during islet differentiation from PARP-$1^{+/+}$ and PARP-1 KD Stem/Progenitors cells.
3. Transcription control of PARP-1 in Islet Neogenesis.
4. In-vivo assessment of role of PARP-1 in pancreatic regeneration in mice.

Objective 1: Establishment of Human/Mice Pancreatic Stem/Progenitors cells for PARP-1+/+ and PARP-1 Knockdown(KD) cells.

Isolation and characterization of PRPs:

Islets were isolated and purified from young BALB/c male mice pancreas using collagenase Type-V digestion. These islets were than cultured in DMEM high glucose complete medium for isolating PRPs. PRPs were slowly purified with sequential passaging in DMEM high glucose media. The purified fibroblastic cells were than characterized by immunocytochemistry and flow cytometry for stem/progenitor markers. Immunocytochemistry confirmed the presence of Nestin and E-Cadherin, which indicated that the purified cells were positive for stem/progenitor phenotype. Pluripotency marker like Oct-4 was found to be negative. Vimentin, SMA and Fibronectin provided evidence for their mesenchymal nature and PDX-1 and Neurog3 confirmed their pancreatic endocrine lineage. Also, Ck-19 indicated their ductal origin within pancreas. These cells were negative for C-peptide and Glucagon indicating their progenitor nature. Flow Cytometry data showed positive staining for stem/progenitor cell marker Nestin,

Vimentin along with mesenchymal stem cell (MSC) surface markers like CD133, CD34, CD44 & CD90 and negative for CD45 & CD117. PDX-1 was observed to be positive, reconfirming their pancreatic origin. Further, a trilineage differentiation viz. Adipogenesis, Chondrogenesis and Osteogenesis with these cells was performed, another confirmatory step for characterizing MSCs. Since, these cells were adult MSCs of the pancreatic endocrine lineage, they were called Pancreatic resident progenitors.

Silencing of PARP-1 gene in PRPs:

Beta cell formation is controlled by timely and sequential expression of transcription factors. It has been reported previously that PARP-1 can regulate Reg1, a pancreatic regeneration controlling transcription factor. Hence, in order to understand the role of PARP-1 in Islet differentiation from stem/progenitors we decided to silence PARP-1. RNA interference technology was used to design a shRNA construct to knockdown PARP-1 in PRPs. The construct was made using pBSU6 plasmid as the backbone vector along with PARP-1 siRNA sequence that targeted the PARP-1 catalytic domain specifically. The PARP-1 shRNA construct was named pSIP912. We received both the PARP-1 shRNA vector and the empty pBSU6 vectors as gifts from Dr. Girish Shah, collaborator at Laval University, Quebec, Canada. Since, the vector did not have either a reporter or a selection gene we had to co-transfect these vectors with pGFPN1. Transfection in PRPs was performed using Neon, electroporation system. G418 antibiotic was used for selection. Clones that survived were picked, scaled up and screened for PARP-1 knockdown by western blotting. Clone 4 and clone11 among all the clones were found negative for PARP-1 expression, where clone 4 was a complete, 11 was a partial knockdown. These knockdowns were stable. Hence, all our studies were continued with clone 4. We in parallel also used PARP-1 siRNA to reconfirm our findings.

Characterization of PRPs after PARP-1 knock down:

The PRP PARP-1 KD (clone 4) cells had different morphology from their parent cells. These cells had significantly higher doubling time (from 21.94±2.5 to 34.46±2.8 hr) due to their slower growth rate. On further characterization of these cells we didn't observe any significant changes in stem cell surface markers viz. CD34, CD44, CD45 and CD90 in the PARP-1KD cells. We also observed a significant reduction in the expression of all the key pancreatic transcription factors involved in islet neogenesis viz. PDX1, Neurog3, NeuroD, MafA and Nkx6.1. Further, in order to confirm that PARP-1 knockdown has abated the ability of PRPs to differentiate, we performed a trilineage differentiation study along with the PARP-1positive cells. The results were in line with our above data where we observed that the PARP-1KD cells were unable to differentiate into adipocytes, osteoblasts and chondroblasts. Thus, PARP-1 knockdown mitigated the differentiation potential of PRPs indicating role of PARP-1 in differentiation of stem/progenitors.

Objective 2: Transcriptome analysis of Islet neogenic pathway genes during islet differentiation from PARP-1+/+ and PARP-1 KD Stem/Progenitors cells.

Islet differentiation from PRPs:

PRPs have all the transcription machinery required for islet differentiation. This can be confirmed by the inherent presence of key transcription factors like Neurog3, MafA, NeuroD and Nkx6.1. Also, phosphorylated Smad3 in the cytoplasm indicated the same. Hence, Swertisin(15µg/ml) a novel islet differentiating bioactive and Activin A(5ng/ml) as positive control were used for inducing islet differentiation. We observed cluster formation within five hr which very rapidly mature to form insulin producing clusters (IPCs) within 3 days of induction. This is the fastest differentiation of any stem/progenitor population reported till date. Also, the

insulin transcript levels by qPCR and Glucose stimulated insulin response (GSIS) of these IPCs were measured by using C-peptide ELISA, where we observed that Swertisin induced IPCs were significantly better than Activin A induced. We further performed a temporal gene and protein expression analysis, which confirmed that sequential expression of key transcription factors viz. Pdx1, Nerog3, MafA, NeuroD, Pax4 and Nkx6.1 is necessary for the formation of insulin producing islet clusters.

Assessment of islet differentiation in PARP-1 positive, PARP-1 KD and PARP-1 inhibited (ABT888) PRPs:

PARP-1 positive PRPs, PARP-1KD and PRPs treated with ABT-888(PARP-1/2 inhibitor 5µM) were differentiated into islet like cell clusters and were further functionally characterized. Time dependent microscopic profiling was performed to observe morphological changes during the Islet differentiation process and assess the rate and extent of differentiation. Islet differentiation for the PARP-1KD cells was observed to have significantly subsided. Also, DTZ staining of the IPCs were done to confirm presence of Insulin within the clusters which was negative in the PARP-1KD cells. There was no significant change in the ABT-888 treated group from the control group, which suggested that it was the PARP-1 protein that was essential for islet differentiation and not its catalytic activity. The differentiated IPCs were further functionally characterized by immunocytochemistry for PARP-1, C-Peptide and Glucagon. No expression of C-peptide or glucagon was observed in the PARP-1KD IPCs, whereas there was no change observed in the ABT-888 treated group from the control group of PARP-1positive PRPs where c-peptide and glucagon were presence in abundance. Further, Time dependent gene and protein expression demonstrated down regulation of pancreatic developmental markers like Pdx1 and Neurog3 which are paramount for islet differentiation. There was no compensatory expression

of PARP-2 indicating that PARP-2 was not involved in islet differentiation. Also, Nestin persisted throughout PARP-1 knock down cells confirming their inability to form islets. We also observed significant downregulation of various key transcription factors viz. Pdx1, Neurog3 and Nkx6.1, which is the prime reason for mitigation of islet differentiation from PARP-1KD PRPs.

Recovery of PARP-1 in PARP-1KD cells:

We wanted to confirm PARP-1's role in the paradigm of islet differentiation from Stem/progenitors and for this we recovered PARP-1 expression in the PARP-1 KD PRP's. We used p3xFLAG_hPARP-1_WT_RSiP, which is a recovery vector having a silent mutation where the siRNA acts and does not allow the siRNA to recognize the sequence. Thereby recovering PARP-1 expression. Here, we transfected the PARP-1 KD cells and confirmed PARP-1 recovery by immunoblotting. These cells were further induced for islet differentiation. The IPCs formed were mature and functionally active as confirmed by positive DTZ staining and insulin & glucagon staining by Immunocytochemistry. The PARP-1 recovered IPCs scored similar on GSIS assay as control PRPs with C-peptide release assay. This confirmed our hypothesis of PARP-1 being absolutely essential for islet differentiation from stem/progenitors.

Confirming the hypothesis in PANC-1 cells, which serves as the human pancreatic progenitor model system**:**

PARP-1 knockdown was created in this system in the similar manner as PRPs. Our previous lab studies on PANC-1 have shown day 5 of PANC-1 differentiation in a ten-day protocol to be vital as there is a shift in the expression of PARP-1 and other pancreatic key transcription factors during this time. Hence, PANC-1 and PARP-1 knock down cells at day 5 of islet differentiation were harvested and proceeded for qPCR taqman gene array, where the entire Transcriptome relevant to islet differentiation was screened and compared for their differential

relative expression. We observed all the genes involved in islet differentiation viz. Isl1, MafA, MafB, NeuroD, Neurog3, Pdx1 among others to have downregulated indicating abatement of islet differentiation as observed phenotypically. However, we observed that markers like Gata6, Hnf1b, Prom1 and FoxA2 responsible to maintain progenitor stemness persisted. Also, we did not find any significant increase in the expression of PARP-2 as observed with PRPs indicating that it does not play any important role in islet differentiation from stem/progenitors. Protein expression analysis gave us similar results as gene expression, which confirmed the mandatory requirement of PARP-1 in islet differentiation.

Objective 3: Transcription control of PARP-1 in Islet Neogenesis.

Monitoring the signaling Pathway:

TGF-b pathway is an essential pathway that mediates islet differentiation. In this pathway, upon induction Regulatory Smads i.e. Smad2 and Smad3 get phosphorylated and form a complex with Smad4. This complex regulates the expression of key transcription factors. On the contrary, inhibitory Smad7 expression decreases allowing Smad2 and 3 activation.

We observe that Smad3 in PRPs inherently phosphorylated, which along with the presence of other transcription factors provide explanation for their rapid islet differentiation. During islet differentiation from PRPs, Smad3 get further phosphorylated and expression of Smad4 significantly increases along with downregulation of Smad7 propelling the islet differentiation forward. Elevated levels of phosphop38MapK was also observed suggesting PRPs follow ACT-MEPK-TKK signaling pathways on induction with both Activin A and Swertisin. Higher levels of these signaling molecules were observed in the Swertisin treated group in comparison with Activin A treated group. This is reflected upon increased expression of Transcription factors in the Swertisin group.

Comparative expression between PARP-1 positive and PARP-1 KD PRPs demonstrated a marked decrease in the phosphorylation of Smad3 both in the undifferentiated cells and during the islet differentiation from both Activin A and Swertisin, which lead to a decreased expression of all the downstream transcription factors mitigating islet differentiation. We further observed similar results with PANC-1 cells where upon PARP-1 knockdown, phosphorylation of Smad3 and Smad4 expression was significantly downregulated.

Our above results have established a relation between PARP-1 and activation of Smads. Literature already states the importance of Smad signaling in islet differentiation but in order to establish this in our model system we used SIS3(10μM), a specific Smad3 inhibitor that inhibits its phosphorylation during islet differentiation from PRPs. We observed complete abatement of islet formation confirming that Smad activation is a necessary step in IPC formation. Further, in order to understand the signaling mechanism with respect to PARP-1 in islet differentiation and based on the gene and protein profile data we performed Co-Immunoprecipitation of PARP-1 on day 4 of the PRPs islet differentiation. We observed a robust interaction of PARP-1 with the TGF-b signaling molecules of Smad3 and pSmad3. We observed that as differentiation progresses, the interaction of PARP-1:SMAD3 goes down whereas the interaction of PARP-1:pSMAD3 increases thus propelling islet differentiation ahead. We then confirmed the same in PANC-1 cells on the 5^{th} day of islet differentiation. Thus confirming that PARP-1 is needed to activate Smad3 during islet differentiation from stem/progenitors.

Transcription control of PARP-1 in Islet Neogenesis:

In order to completely map the role of PARP-1 in islet differentiation it becomes mandatory to understand its transcription control activity. The transcriptome profile from objective 2 has given us a fair hint about few key transcription factor targets. To confirm PARP-1:DNA

interaction we need perform Chromatin Immunoprecipitation (CHIP) assay or CHIP Sequencing which is under progress.

Objective 4: *In vivo* assessment of role of PARP-1 in pancreatic regeneration in mice

We were able to effectively confirm the role of PARP-1 in islet differentiation from stem/progenitors *in vitro*. Hence, we wanted to explore further by performing an in vivo study while raising two vital questions, first if PARP-1 knockdown cells can differentiate *in vivo* and secondly whether PARP-1 KD has any effect on the homing of stem cells on damaged tissue. We created STZ induced Type I diabetic model for this study where 65mg/kg. b.wt. dose was given to young (6-8 weeks) female mice for 5 days. One million GFP and PARP-1 positive PRPs and GFP positive and PARP-1 KD PRPs were transplanted at tenth day of the STZ induction along with and without Swertisin.

Homing of GFP positive PRPs in the pancreas of STZ treated Type 1 Diabetic mice

The Liver and Pancreas of the diabetic mouse model were harvested after transplantation and their single cell suspension was screened for GFP positive PARP-1 positive and PARP-1 knock down PRPs through Flow Cytometry. The localization of GPF positive cells was seen only in the diabetic groups transplanted with PARP-1 positive PRPs (~30%). There was no localization seen in the groups transplanted with PARP-1 KD PRPs. These results suggest that PARP-1 plays a role in homing of PRPs to the damaged tissue i.e. pancreas. Swertisin had no augmenting effect on homing. Liver did not show any homing across the groups. Kidney, Lungs and Spleen were also screened for localization of GFP positive PRPs by Fluorescence microscopy, which did not show GFP positive population in the histological sections of Kidney, Lungs and Spleen across all the 5 experimental groups.

Assessment of amelioration of Endocrine pancreatic function with respect to islet neogenesis after transplantation of GFP +ve PARP-1 positive and PARP-1 KD PRPs alone and along with Swertisin into STZ treated Type 1 Diabetic mice

Fasting Blood Glucose levels were monitored at 4 time points i.e. Before STZ treatment, After STZ treatment, Before PRPs transplantation and after PRPs transplantation. The fasting blood glucose levels indicated the status of the severity of diabetic condition induced in the Balb/c mice. A 2.5 times increase in the blood glucose was seen in the experimental groups showing that they had become diabetic. The fasting blood glucose levels range in from 80-120 mg/dL and >200mg/dL was termed as hyperglycemic. These diabetic mice were then treated for diabetes by transplanting PRPs alone and with Swertisin treatment. After transplantation at fourth day it was observed that the group transplanted with PARP-1 positive PRPs reverted back to normoglycemic condition (100±20 mg/dL) whereas there was an increase in the blood glucose levels in the group transplanted with PARP-1 KD (499±117 mg/dL) PRPs. The groups transplanted with PARP-1 positive and PARP-1 KD PRPs along with Swertisin treatment did not show a complete normoglycemic condition but their blood glucose levels were lowered (162±79 mg/dL) as compared to the levels before transplantation (209±45 mg/dL).

Flow cytometry was done to verify for a dual positive GPF and Insulin population of cells in the mice pancreas. This population gave an impression of the cells which after transplantation got differentiated to β cells. The population of cells dual positive for GFP and Insulin (~30%) confirmed the differentiation of transplanted GFP positive PARP-1 positive PRPs into functional β cells. The groups transplanted with PARP-1 KD PRPs didn't localize in the pancreas hence there was no dual positive population observed. The groups treated with Swertisin along with transplantation of PARP-1 +ve and PARP-1 KD PRPs depicted similar results as compared to

the groups transplanted with PRPs alone. Thus, suggesting that there was no synergistic effect of Swertisin in *in vivo* differentiation of PRPs after transplantation.

To further confirm the differentiation of transplanted PRPs to from Neoislets confocal imaging was performed. Co-localization of GFP with Nkx 6.1, which is a specific β cell marker was observed. Also, co-localization of GFP with C-peptide was observed to confirm functionality of transplanted GFP+ve PRPs that have homed into pancreas and have differentiated into insulin producing β cell clusters.

The level of expression of the transcription factors of the Islet Neogeneic pathway were assessed by Western blotting. All the key transcription factors involved in islet neogenesis viz. Pdx1, Neurog3, MafA and Nkx 6.1 along with phosphorylated Smad3 and Smad4 were found to be elevated in the PARP-1+ve PRPs group but the PARP-1KD group didn't show any upregulation of these transcription factors. This suggested that the Transplanted PARP-1 +ve PRPs homed inside the pancreas and successfully differentiated in new IPCs. This was also confirmed by analyzing serum C-Peptide levels of the transplanted mice. Swertisin group also showed upregulation of pancreatic islet differentiating transcription factors but this is due to its potent islet neogeneic property. Hence, with this study we confirmed further the indispensable need of PARP-1 in stem/progenitors for islet differentiation.

Conclusion:

- Characterization of PRPs using various stem/progenitors cell markers by immunocytochemistry, Flow Cytometry and Western Blotting proves that these are pancreatic resident progenitor cells. Trilineage differentiation of PRPs gives us substantial evidence for PRPs have mesenchymal stem cell properties and are multipotent in nature.
- Islet differentiation from PRPs take the shortest route of differentiation reported till date. Time dependent protein profiling of differentiation of PRPs into ILCCs provides significant

data on PRPs having inherent potential for islet differentiation that follows the ACT-MEPK-TKK signaling using Activin A and Swertisin.

- Activin A and Swertisin produce similar number of IPCs. However, Swertisin treated PRPs have a significantly higher insulin transcript than Activin A treated. Also, the amount of c-peptide released by Swertisin induced IPCs was significantly higher confirming higher GSIS index, which suggests that Swertisin is also a better insulin secretagogue.
- The stem cell surface markers in the PARP-1 knock down cells were not extensively hampered but the expression of pancreatic regulatory transcription factors were downregulated comprehensively, which indicates PARP-1's regulatory role during differentiation. Characterization of PARP-1 knock down PRPs provided evidence that PARP-1 is essential for proliferation and differentiation.
- Both PARP-1 KD PRPs and PANC-1 cells fail in producing IPCs. ABT888, PARP-1/2 specific inhibitor does not hamper islet differentiation, which suggests that PARP-1 protein and not its catalytic activity is essential in islet differentiation from Stem/Progenitors. Also, Recovery of Islet differentiation along with PARP-1 recovery confirms PARP-1 being essential for islet differentiation from stem/progenitors.
- PARP-1 directly interacts with Smad3 and P-Smad3 and regulates TGF-b signaling for islet differentiation.
- In the *in vivo* study, PRPs home at the injured pancreas whereas PARP-1 KD PRPs fail to do so suggesting role of PARP-1 in regulating homing of mesenchymal stem cells. Homed PRPs were able to differentiate into insulin producing clusters.
- Swertisin treated groups showed profound lowering in blood glucose and upregulation of transcription factors in islet differentiation confirming its known potent islet neogeneic potential.

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Publications

1. Anti-apoptotic and cytoprotective effect of *Enicostemma littorale* against oxidative stress in Islets of Langerhans.

   Abhay Srivastava, Niraj M. Bhatt, Nidheesh Dadheech, Tushar P. Patel, Anubha Singh and Sarita Gupta. Pharmaceutical Biology, Online 14th Mar, 2016.

2. Swertisin an Anti-diabetic Compound Facilitate Islet Neogenesis from Pancreatic Stem/Progenitor Cells via p-38 MAP Kinase-SMAD pathway: An in-vitro and in-vivo study.

   Nidheesh Dadheech, **Abhay Srivastava**, Neha Paranjape, Shivika Gupta, Arpita Dave, Girish M Shah, Ramesh R. Bhonde, and Sarita Gupta. PLoS ONE, Volume 10 Issue 6, June 5, 2015.

3. Basal Expression of Pluripotency-Associated Genes Can Contribute to Stemness Property and Differentiation Potential.

   Nidheesh Dadheech, **Abhay Srivastava**, Muskaan Belani, Sharad Gupta, Rajarshi Pal, Ramesh R. Bhonde, Anand Srivastava and Sarita Gupta. Stem Cells Dev. Volume: 22 Issue 12: May 29, 2013.

4. A Small Molecule Swertisin from *Enicostemma littorale* Differentiates NIH3T3 cells into Islet like Clusters and Restores Normoglycemia upon Transplantation in Diabetic Balb/c Mice.

   Nidheesh Dadheech, Sanket Soni, **Abhay Srivastava**, Sucheta Dadheech, Shivika Gupta, Renjitha Gopurappilly, Ramesh R. Bhonde, and Sarita Gupta. Evidence-Based Complementary and Alternative Medicine; Volume 2013, Article ID 280392, 20 pages, February 3, 2013.

5. "Pancreatic resident endocrine progenitors demonstrate high islet neogenic fidelity with significant homing towards its niche in diabetic mice" (2017); Abhay Srivastava, Nidheesh Dadheech, Mitul Vakani and Sarita Gupta. (Communicated)

6. "Swertisin ameliorates diabetes by triggering pancreatic progenitors for islet neogenesis in Streptozotocin treated BALB/c mice". (2017); Abhay Srivastava, Nidheesh Dadheech, Mitul Vakani and Sarita Gupta. (Communicated)
7. "Herbs to Stem cells: A perspective in diabetes therapeutics: Review", (2017); Abhay Srivastava, Mitul Vakani, Gurpreet Bharatwaj and Sarita Gupta. (Communicated)
8. "Regulatory role of PARP-1 in islet differentiation from pancreatic progenitors". Abhay Srivastava, Nidheesh Dadheech, Mitul Vakani and Sarita Gupta. (Manuscript under preparation).
9. "PARP-1 in islet biology: Review". Abhay Srivastava, Nidheesh Dadheech and Sarita Gupta. (Manuscript under preparation).

Academic Achievements and Awards

1. DBT-CTEP travel award for attending Stem Cell Biology Conference at Cold Spring Harbor Laboratories, New York, from 7th to 11th October, 2015.
2. Received a Stem Cell Network travel award to attend the 2015 Till & McCulloch Meetings, Toronto Canada, October 26-28, 2015; Abstract Titled "Novel Bioactive in potentiating Islet Neogenesis from mouse Intra-Islet Mesenchymal Stem Cells" selected by the Till & McCulloch Meetings Program Committee as one of the top ten entries in the SCN / inStem Abstract Competition for junior researchers from Indian institutions.
3. First prize awarded for the best poster presentation at the three day National Symposium on 'Emerging Trends in Biochemical Sciences' at the dept. of Biochemistry, Faculty of Science, The M. S. University of Baroda, Vadodara, Gujarat titled "Coalescing Stem Cell Therapy with potent Bioactives: Regenerating Islet of Langerhans in Type I Diabetic BALB/c mice"; 29th Dec. 2014 to 31st Dec. 2014.

4. First prize awarded for the best poster presentation at the 'International Conference on Diabetes and its Complications' at Changa, Gujarat, titled "Evaluation of protective effect of *Enicostemma littorale* extract against H2O2 induced apoptosis in Islet of Langerhans", January 18-20, 2013.
5. Awarded CSIR-NET-JRF- June 2010.

Posters / Abstracts Published

1. "Novel Bioactive in potentiating Islet Neogenesis from mouse Intra-Islet Mesenchymal Stem Cells", at the Till & McCulloch Meetings, The Sheraton Center, Toronto, Canada, October 26-28, 2015.
2. "PARP-1 protein regulates Islet differentiation in mouse intra-islet progenitors through Smad signaling", at the 3rd Stem Cell Biology meeting at Cold Spring Harbor Laboratory, New York, USA, 7th to 11th October 2015.
3. "Coalescing Stem Cell Therapy with potent Bioactives: Regenerating Islet of Langerhans in Type I Diabetic BALB/c mice", at the three day National Symposium on 'Emerging Trends in Biochemical Sciences' at the dept. of Biochemistry, Faculty of Science, The M. S. University of Baroda, Vadodara, Gujarat, India, 29th Dec. 2014 to 31st Dec. 2014.
4. "PARP-1 regulates adipocyte differentiation mediated by TGF-β signalling pathway", at EMBO/EMBL Symposium: 'Translating Diabetes', 30 April- 3 May 2014, Heidelberg, Germany.
5. "Amelioration of Pancreatic Endocrine Function: Enhancing Homing and Repair of mouse Bone Marrow Mesenchymal Stem Cells by using Bioactive Compound in Streptozotocin induced Diabetic balb/c mice", at ISSCR | 12th Annual Meeting Vancouver Canada, June 18-21, 2014.

6. "Evaluation of protective effect of Enicostemma littorale extract against H2O2 induced apoptosis in Islet of Langerhans", at the 'International Conference on Diabetes and its Complications' at Changa, Gujarat, January 18-20, 2013.

Patents

1. Indian Patent Filed: "Swertisin as potent and novel molecule for islet differentiation from Human bone marrow derived mesenchymal stem cells"; Patent no. 201621012988 (2016).

Signature of Candidate Signature of Guide