EFFECT OF SECRETOME OF ADIPOSE DERIVED STEM CELLS ON ISLET DIFFERENTIATION & FUNCTIONALITY

CHAPTER 5

Chapter 5: Effect of secretome of adipose derived stem cells on islet differentiation & functionality

5.1 Introduction

Loss of functional pancreatic β cells is the underlying cause of type 1 diabetes mellitus which leads to multiple long-term complications. The critical contributor to this pathology is the autoimmune attack on functional beta cells. Apart from exogenous insulin treatment, current therapies include islet transplantation from cadaveric donors. But owing to exceeding demands placed on organ transplantation, there is still considerable uncertainty of the availability of donors, rejection post transplantation, and the need for immuno-suppressant. Thus, transplantation therapy demonstrates viable but still with multiple hurdles to be a successful therapeutic regimen (Burrack, Martinov et al. 2017). This lead to the examination of the *in vitro* regeneration of β cells from various stem cell sources that can provide a sustainable solution for combating the need as a source for islet (Sneddon, Tang et al. 2018).

Stem cell therapy has gained wider recognition for its remarkable ability to support cell survival and homing capacity to the damaged tissue (Pittenger, Discher et al. 2019). Islet differentiation can be modulated by various factors especially the milieu of secreted factors by other cells, which can vary in normal as well as pathological conditions. One such remarkable element is the regenerative capacity of secreted factors from human adipose tissue-derived stem cells (hADSCs). hADSC secretome is known to orchestrate various functions like wound healing, angiogenesis, anti-inflammation, immunomodulatory, anti apoptosis etc (Vizoso, Eiro et al. 2017).

Conditioned media (CM) from stem cells have garnered much attention for regenerative potential without the involvement of the cell itself. It has also proved to have a beneficial effect on islet functionality. Various reports suggest direct or indirect co-culture of hADSC with islet having anti apoptotic effect and increased insulin secretion (Yamada, Shimada et al. 2014) (Dietrich, Crescenzi et al. 2015). Nevertheless, the application of hADSC secretome on islet differentiation is yet to be properly defined. Also, if this secretome of hADSCs can be used as an *in vivo* differentiation agent for the resident PREPs, then it can be a better alternative to transplantation.

It will exclude the risk of cell rejection and it is easy for packaging, transportation, and freeze drying (Gunawardena, Rahman et al. 2019).

On the other side, the secretome of hADSC has been shown to alter (Dzhoyashvili, Efimenko et al. 2014) under metabolically compromised conditions since the stem cell themselves are affected by a harsh micro environment (Saki, Jalalifar et al. 2013). Our lab has reported that obesity affects the metabolic profile of hADSC in the Indian population so we have also analyzed the modulatory effect of obese hADSC secretome on islet differentiation (Rawal, Patel et al. 2020). We demonstrated that expression of genes regulating the insulin – sensitivity and differentiation of pre-adipocytes to adipocytes such as CEBPA, CEBPB, CEBPD, PPAARG, SIRT1, PREF1 and SREBF1 were also reduced in obese ADSC's compared to the lean one. Thus, explaining differential status of key metabolic genes in wake of metabolic compromised state (Komal Rawal Ph.D. Thesis).

Thus, this research focuses on two major studies (i) Exploring the use of hADSC secretome in islet differentiation as a tool for regenerative medicine and (ii) Understanding the regulatory effect of altered hADSC secretome under a metabolically compromised condition on islet differentiation and functionality.

5.2 Material and Methods

5.2.1 Cell culture

Human Adipose derived stem cells were previously isolated and cryopreserved from lipoaspirates from control (BMI≤23) and obese (BMI≥25) individuals as described earlier. Human ethical approval was obtained from Banker's Heart Institute (A Human Ethical and Central Drugs Standard Control Organization (CDSCO)) approved institute (ECR/214/Inst/Guj/2013/RR-16) and Institutional ethical committee (IECHR 2016-6), Vadodara, India (Rawal, Patel et al. 2020) (Rawal, Purohit et al. 2021). hADSC were propagated at 37 °C in 5% CO2 in KnockOut^{TM} DMEM (Gibco#10829-018) supplemented with 1.0% of penicillin-streptomycin (Gibco#15140-122) and 10% FBS (Gibco#10270-106).

Mouse pancreatic resident endocrine progenitors were previously isolated from a fresh islet and cryopreserved by digestion of mouse pancreas as described earlier (Dadheech, Srivastava et al.

2015) (Srivastava, Dadheech et al. 2018) (Srivastava, Dadheech et al. 2018) and were propagated at 37 °C in 5% CO2 in DMEM high glucose (Gibco#12100-046) supplemented with 1.0% of penicillin-streptomycin (Gibco#15140-122) and 10% FBS (Gibco#10270-106).

5.2.2 Conditioned media (CM) preparation from hADSC

hADSC were seeded until 80% confluency with KnockOut™ DMEM (Gibco#10829-018) with 10% FBS. After that culture media was replaced with serum free KnockOut™ DMEM and propagated at 37 °C in 5% CO2.After 24 h of incubation, media was collected centrifuged at 4000 rpm for 30min. Supernatant was stored at -80 after filtering through 0.2mm filter until further experiment use (Fig 5.1).

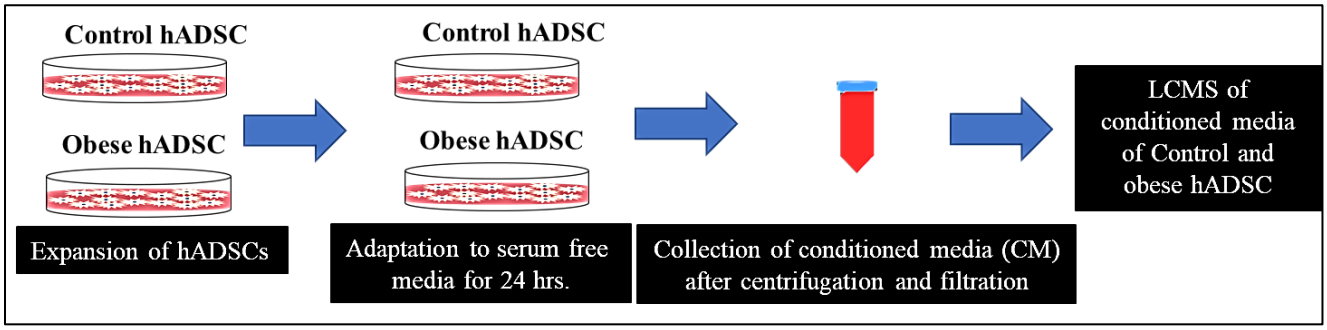


Figure 5.1 *In vitro* Plan of work for hADSC secretome collection

5.2.3 Differentiation of ILCCs from PREPs under the effect of hADSC secretome

The PREPs were differentiated into ILCCs in presence of differentiation media which consists of serum-free KnockOut™ DMEM (Gibco#10829-018) media supplemented with 1% BSA, 1X glutamax,1X Zinc acetate, 1.0% of penicillin-streptomycin, insulin (5 µg/ml), transferrin (5 µg/ml), and selenite (5 ng/ml) cocktail as previously reported. PREPs were differentiated using swertisin (15 µg/ml) as differentiating agent (Dadheech, Soni et al. 2013) (Dadheech, Srivastava et al. 2015) (Srivastava, Dadheech et al. 2018). PREPs were subjected to fifty percent of control and obese hADSC CM diluted with differentiation media till the end of islet differentiation in a 4 day protocol (Fig 5.2).

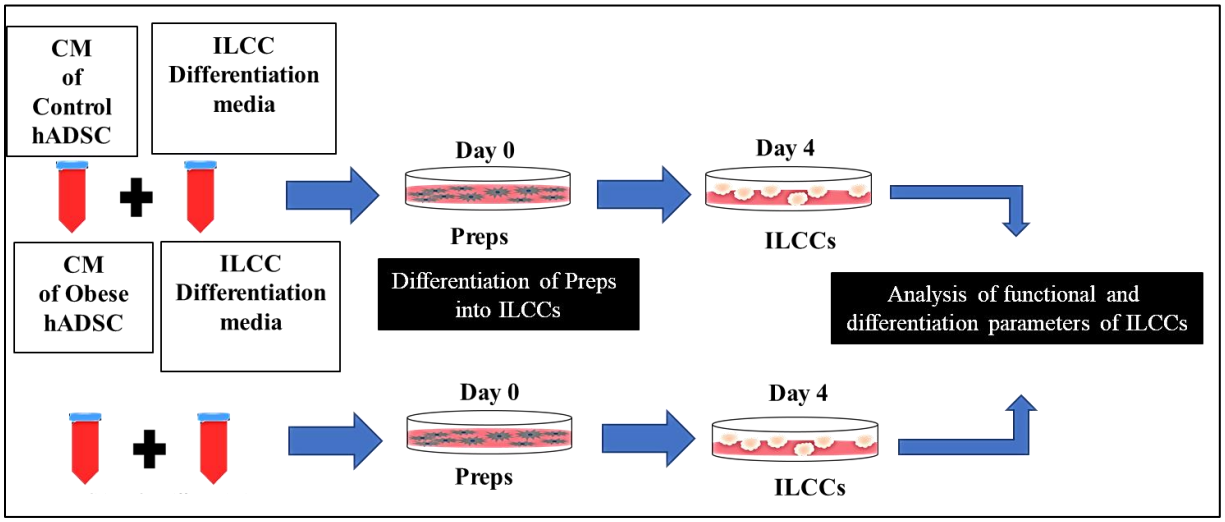


Figure 5.2 *In vitro* Plan of work of differentiation of PREP into ILCCs

5.2.4 High-performance liquid chromatography of Conditioned media (CM) of hADSC

(HPLC; LC20AD, Shimadzu) with a C4 column (Genetix) was used to analyze stromal cells derived secretome. Solvent A was composed of 0.1% Trifluoroacetic Acid (TFA) in ultrapure water, and solvent B was composed of 0.1% TFA in Acetonitrile (ACN). A linear gradient with a flow rate of 0.3 mL/min was employed, using the following gradient: 1% B (at 7 min), 60% B (at 30 min), 100% B (at 42 min) for 10 min, followed by equilibration with 1% B. Fractions were concentrated with vacuum centrifuge (Speedvac, Savant, USA) and reconstituted with distilled water (D.W.) and injected in LC system (Waters). The determination of the presence of peptides in the secretome was performed at 215 nm wavelength and data was captured by the HPLC system.

LCMS of Conditioned media and **Yield**, **Morphometry** analysis and **DTZ** staining of ILCCs were performed as per the methodology described in **Chapter 4.**

Immunocytochemistry of C peptide and glucagon, **DCFDA** staining for ROS generation, **FDA/PI** staining for cell death has been performed as per methodology described in **Chapter 4.** We performed **Protein extraction** and **western blotting** as per methodology described in **Chapter 4.**

5.3 RESULTS

To study the crosstalk between hADSC and islet via trophic effects, we selected the shortest route of islet differentiation from pancreatic resident endocrine progenitors for studying the effects on islet differentiation and functionality. We investigated the effect of hADSC secretome of control and obese under normal and pathological conditions to analyze the effect on islet pathobiology.

5.3.1 Characterization of proteins of hADSC secretome from control and obese

To enhance our understanding of the characterization of different secretory products from the conditioned media of hADSC from control and obese individuals, conditioned media was subjected to HPLC analysis. The results showed the elusion of chromatographic peaks at different time intervals.

In Fig 5.3, the peak patterns of secretome of control group were analyzed which were similar in case of control secretome groups. Fig 5.4 shows a similar peak pattern in obese secretome group. Whereas in Fig 5.5 when comparing peaks between control and obese, strikingly peculiar peptide peaks were observed around 35,37,41,48 min. Thus, the data suggests a similar peak pattern for individual control and obese groups while different and highly specific secretory profile of peak pattern between control and obese hADSC secretome.

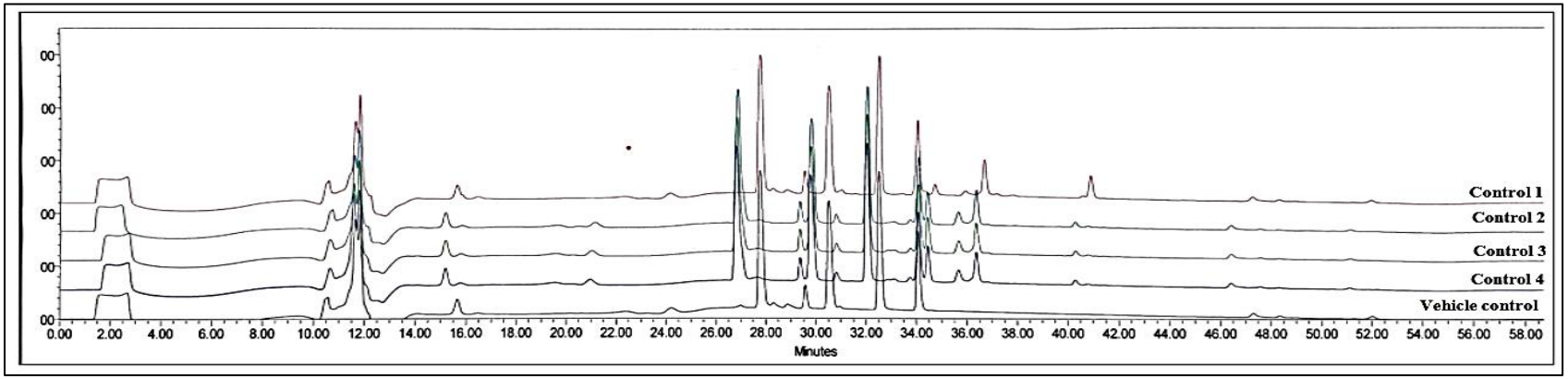


Figure 5.3 HPLC Chromatogram of secretome from Control hADSC

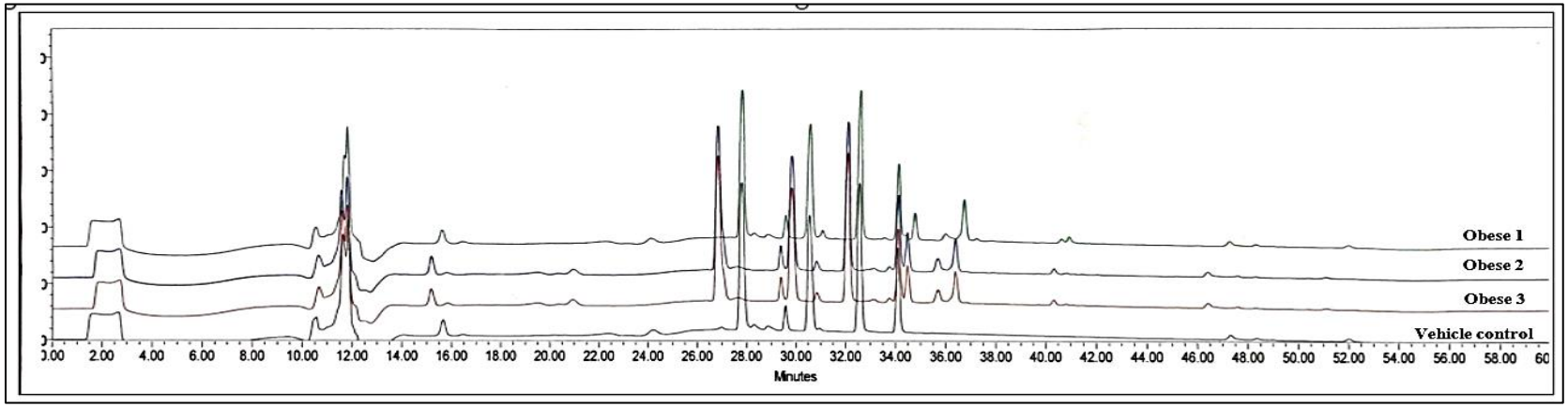


Figure 5.4 HPLC Chromatogram of secretome from Obese hADSC

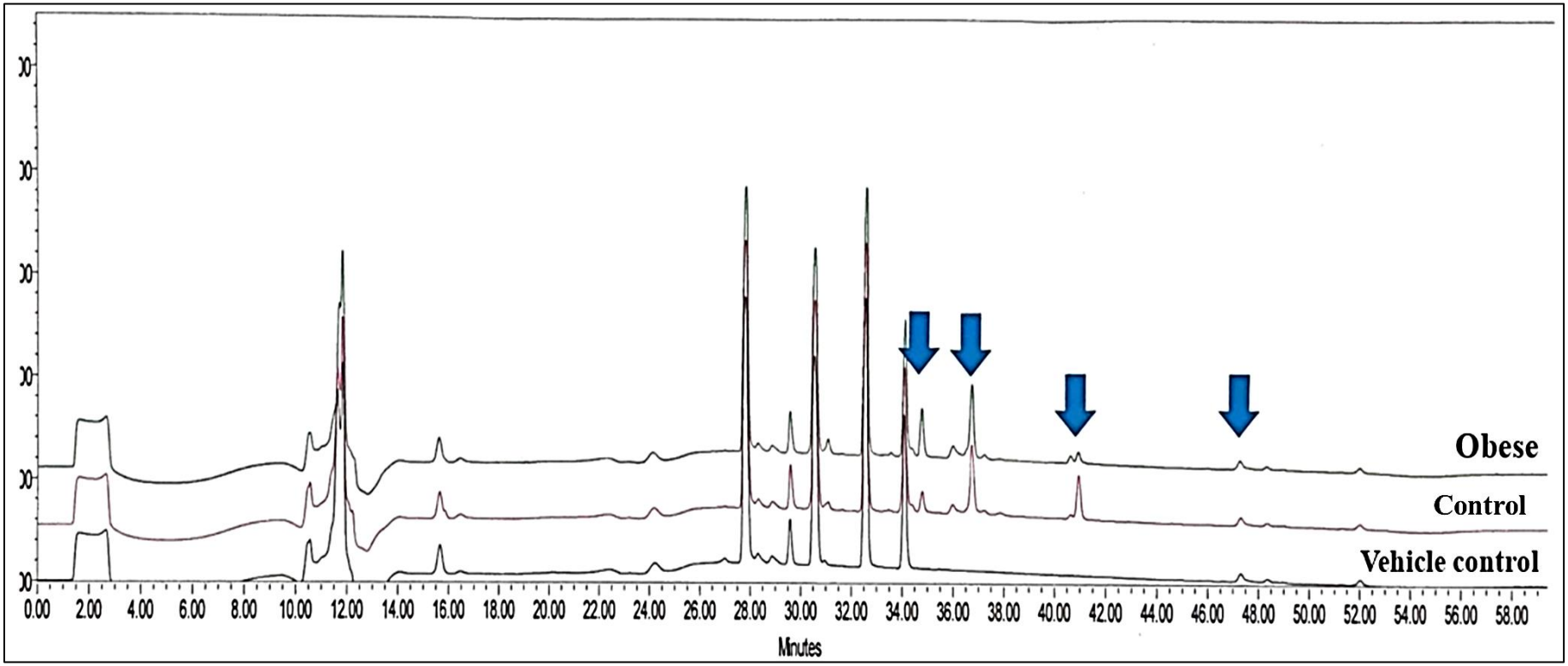


Figure 5.5 HPLC Chromatogram of secretome from Control and Obese hADSC. The graph represents the specific peak difference between the secretome. Distinct peaks in comparison to the vehicle control and secretomes were highlighted with blue arrow

To identify and characterize different secretory products from the conditioned media of hADSC from control and obese individuals, conditioned media was further subjected to LCMS analysis. A noteworthy difference in chromatogram was observed for both the secretome samples which substantiates the finding of altered secretome. A varying pattern of peaks was observed at different retention times. The highest chromatographic peak was observed at 48.10 min and 48.28 min in conditioned media of hADSC from control and obese respectively (Fig 5.6).

Intriguing correlation is with the abundance of proteins which demonstrated differential expression of proteins in conditioned media of hADSC with a majority of the proteins overexpressing in obese. We categorized these proteins using gene ontology analysis (Panther database (Thomas, Campbell et al. 2003)) for their functional classification into molecular function, biological process, cellular component, protein class, and pathway. When analyzed for molecular function, most of the proteins considerably fall under catalytic activity (GO:0003824) followed by binding (GO:0005488) and molecular function regulator (GO:0098772). Biological processes categorized proteins majorly into the cellular process (GO:0009987) followed by metabolic process (GO:0008152), biological regulation (GO:0065007) and localization (GO:0051179). Cellular component classified proteins evidently for Cellular anatomical entity (GO:0110165) and intracellular (GO:0005622). Proteins were categorized under protein classes as transfer/carrier protein (PC00219), defense/immunity protein (PC00090), chromatin/chromatin-binding, or -regulatory protein (PC00077), translational protein (PC00263) protein-binding activity modulator (PC00095) with maximum proteins in metabolite interconversion enzyme (PC00262) category. For pathway analysis proteins were classified into Wnt signaling pathway (P00057), Nicotinic acetylcholine receptor signaling pathway (P00044), FAS signaling pathway (P00020), Inflammation mediated by chemokine and cytokine signaling pathway (P00031), Cytoskeletal regulation by Rho GTPase (P00016), Blood coagulation (P00011) (Fig 5.7). These findings reinforce that there is an appreciable difference in the secretome which is an effect seen of subjugation of the hADSC to metabolically compromised environment.

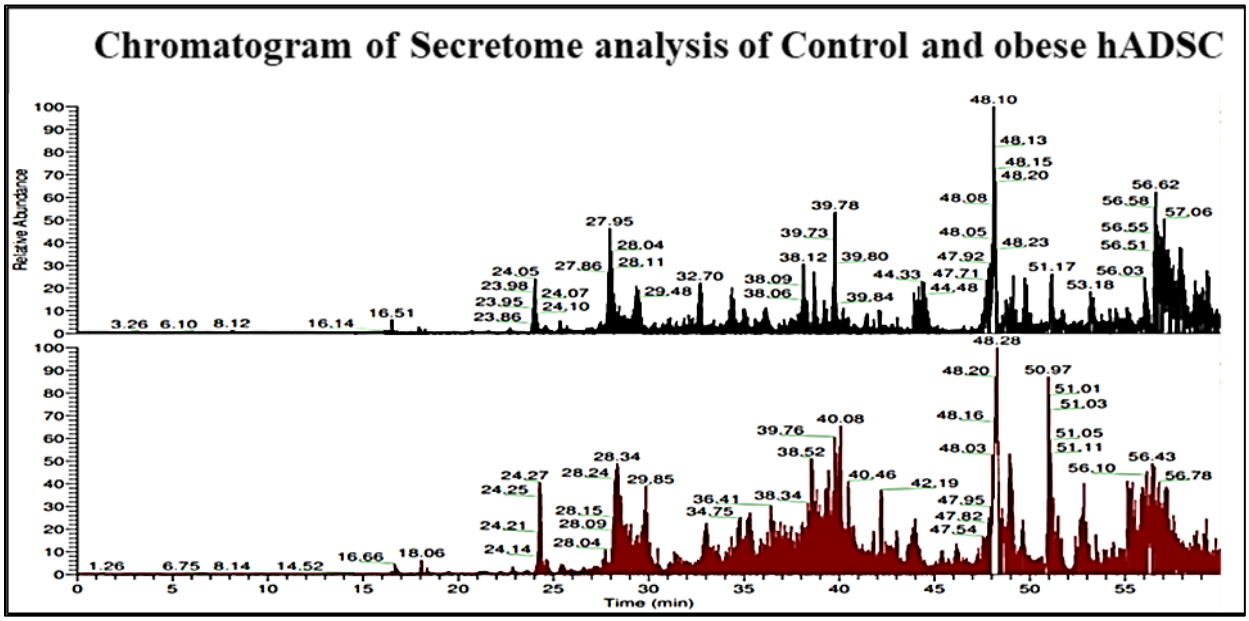


Figure 5.6 Differential characterization of protein from secretomes Chromatogram of secretomes of hADSC from control and obese

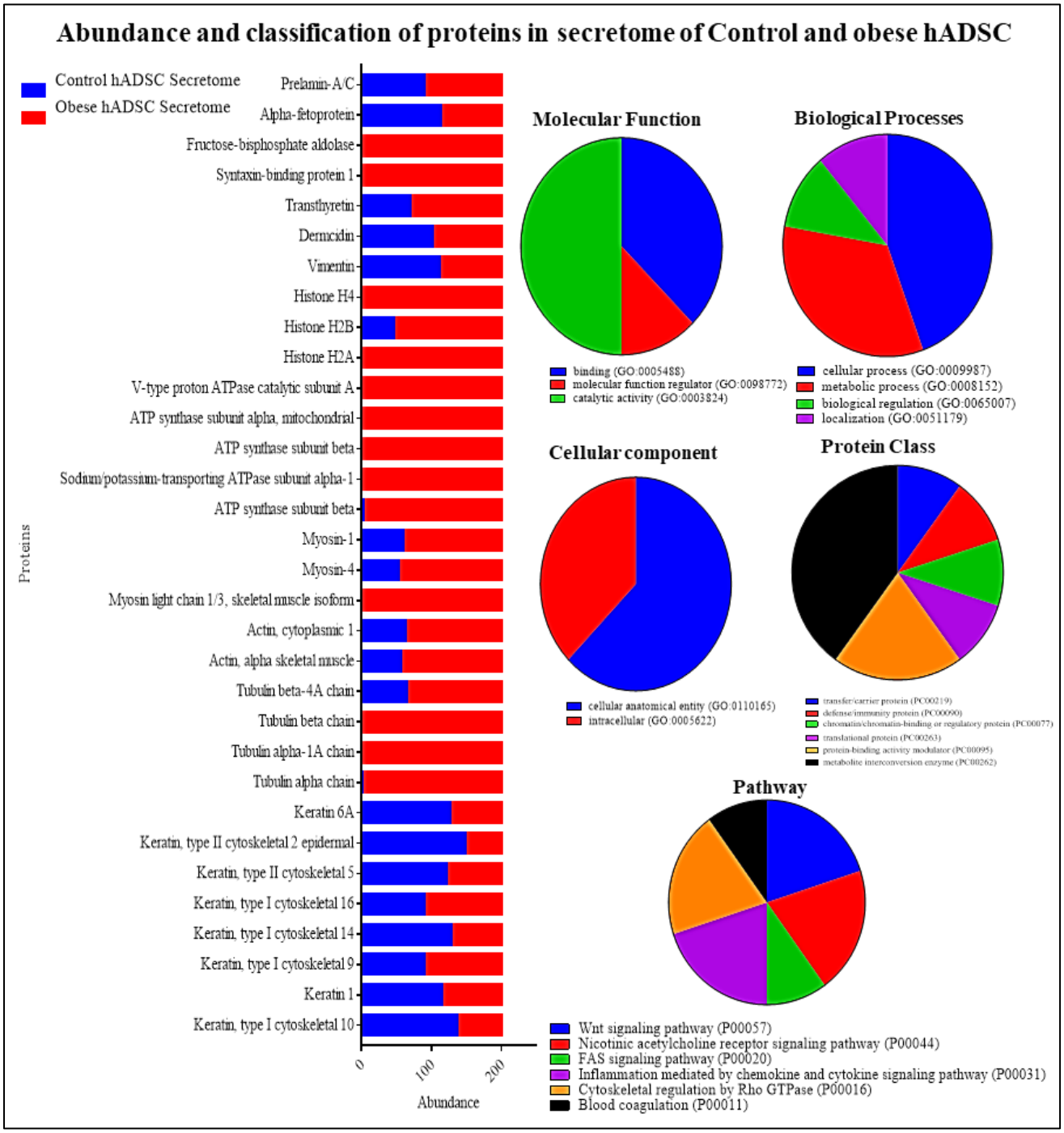


Figure 5.7 Differential characterization of protein from secretomes) Graph showing abundance and classification of proteins present in secretomes of hADSC from control and obese.

5.3.2 ILCCs from PREPs under control hADSC secretome demonstrates optimal functional and phenotypic characterization

Owing to the fundamental interest in analyzing islet differentiation under the effect of secretory products of hADSC, PREPs were allowed to differentiate with control and obese hADSC secretome in a 4 day protocol supplemented with islet differentiation media combined with secretomes (Fig 5.8). Cluster formation was monitored microscopically for 4 days and more participation of PREPs, maximum cell aggregation, and zone of activation was observed in ILCCs under the effect of control hADSC secretome as a contrast to obese pointing effectively towards endocrine reprogramming.

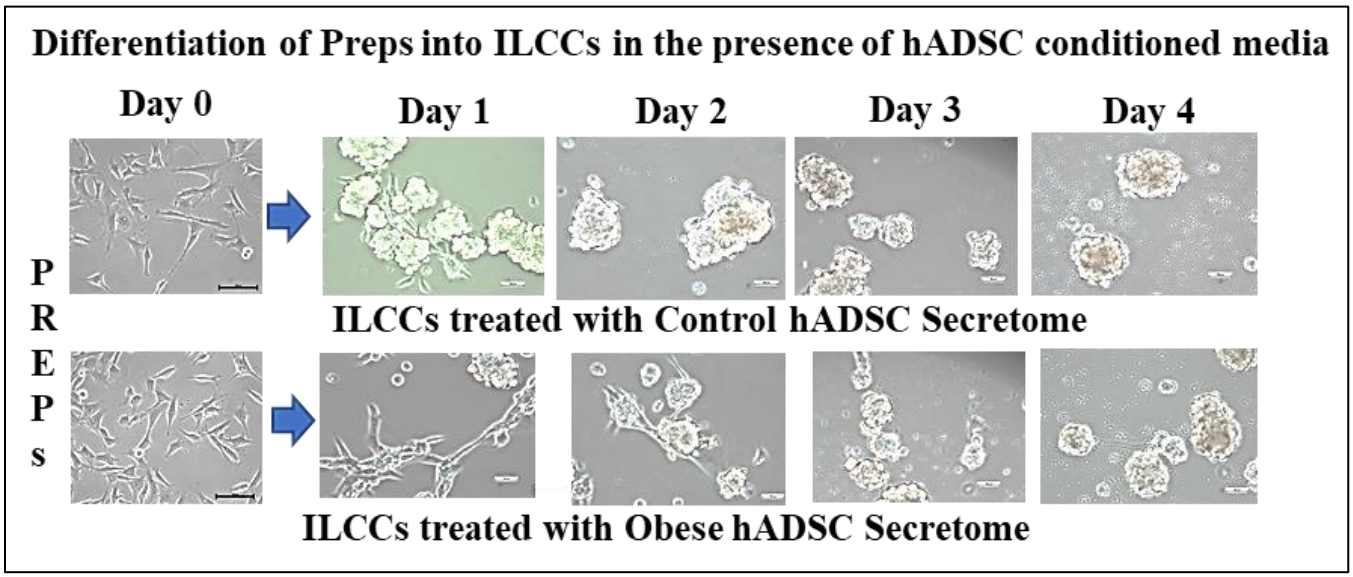


Figure 5.8 Functional analysis of ILCCs in the presence of hADSC conditioned media ILCCs were differentiated from PREPs in presence of control and obese secretomes of hADSC. Representative images of temporal differentiation of ILCC from day 0 to day 4 with control and obese hADSC secretomes.

We further moved for confirmation of the maturation of ILCCs. DTZ stains insulin positive cells as it binds to zinc present in insulin granules and presents a crimson colour. A brighter red colour was observed, as zinc sequestration was present within insulin positive granules for ILCCs under the effect of control hADSC secretome as contrast to obese. For further considerable insight into the maturation of ILCCs we immunostained the clusters with C peptide and glucagon (Fig 5.10). It was noteworthy to observe intense red and green staining in ILCCs under the effect of control hADSC secretome as contrast to obese confirming more insulin positive content. The critical correlation of immunocytochemistry of ILCCs pointed towards incomplete and immature functional differentiation under obese hADSC secretome. Further, we counted the ILCCs in both groups and observed more yield in the obese hADSC secretome group. Most importantly though the yield was higher, the population of ILCCs was heterogeneous with most ILCCs falling in 100-250 um range and were scattered with loose edges in obese hADSC secretome whereas more compact and spherical were found in control. These results offered compelling evidence of the inadequacy of functional maturation of ILCCs when reinforced with secretory products of obese hADSC secretome (Fig 5.9).

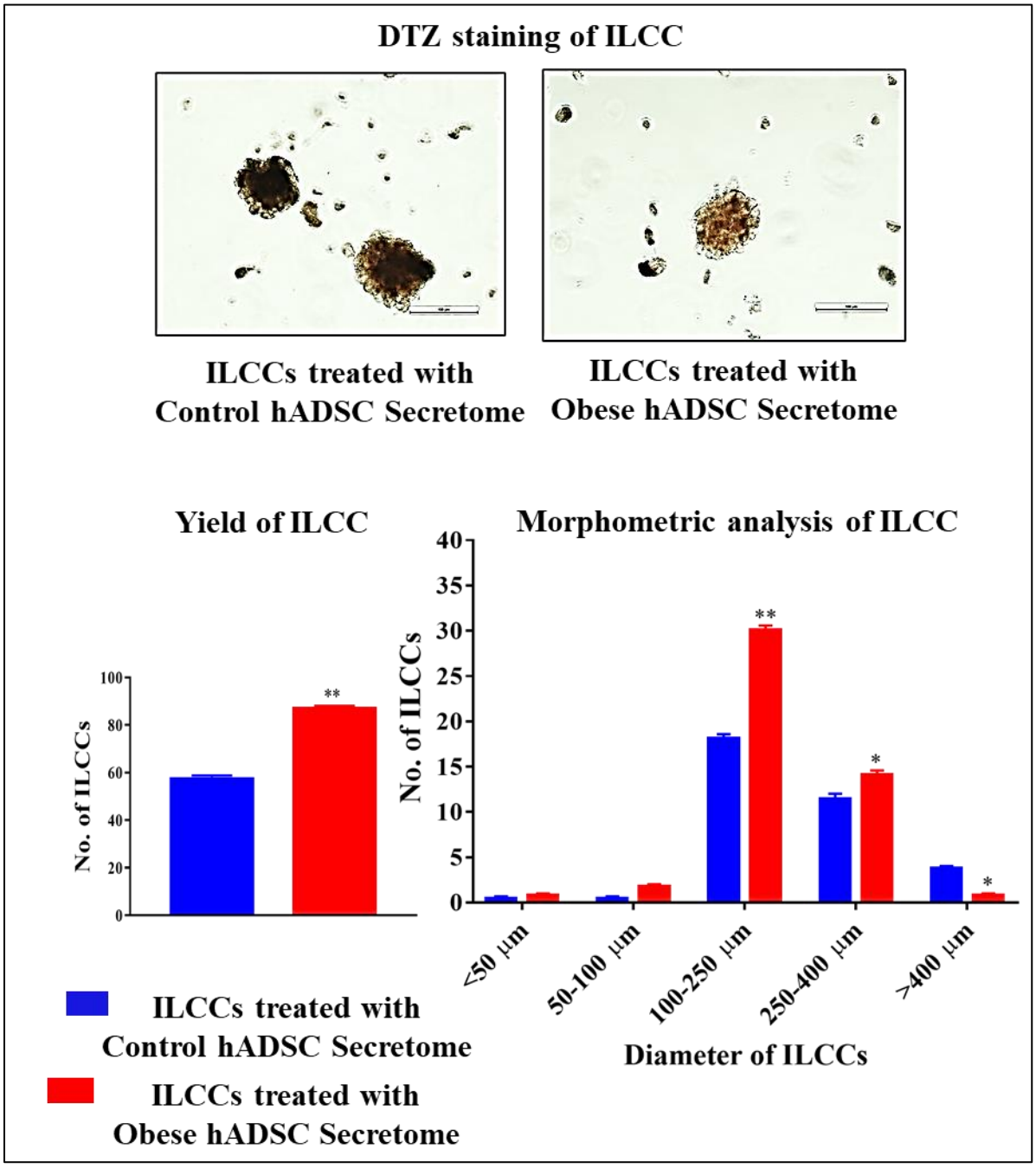


Figure 5.9 Functional analysis of ILCCs in the presence of hADSC conditioned media ILCCs were differentiated from PREPs in presence of control and obese secretomes of hADSC. Functional parameters like DTZ staining, Yield and Morphometric analysis was done (magnification 20X)

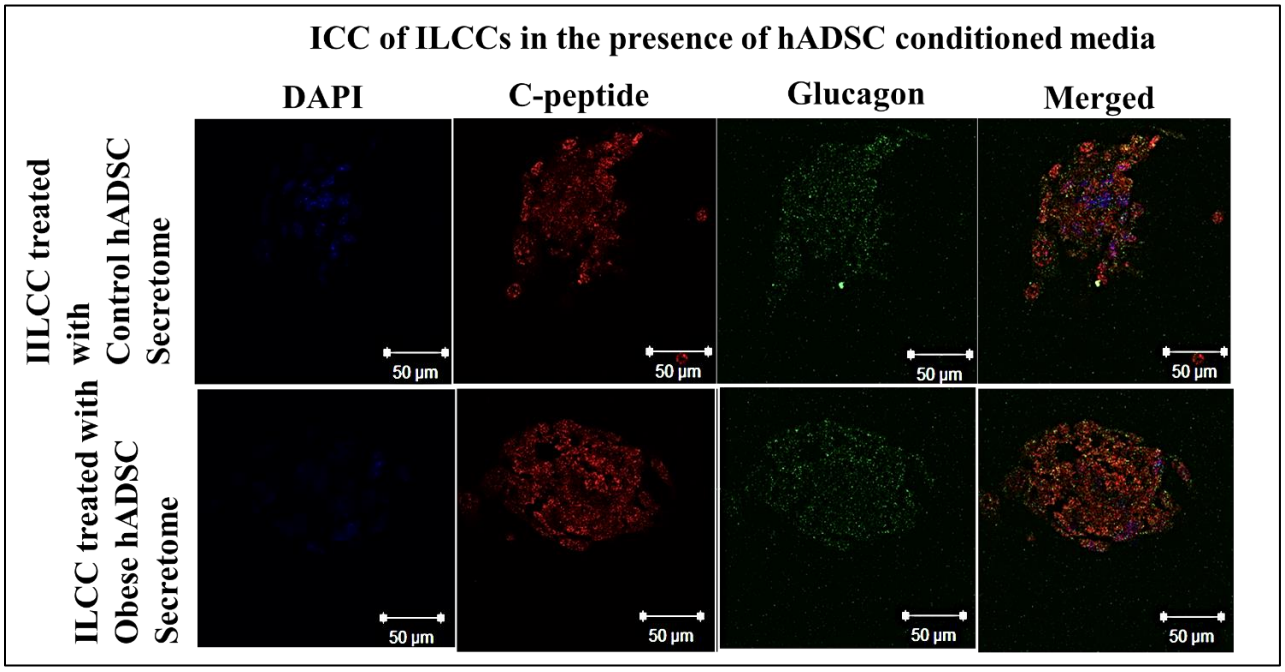


Figure 5.10 Immunocytochemistry of C-peptide and Glucagon were done in ILCC (magnification 63X).

5.3.3 ILCCs from PREPs under control hADSC secretome demonstrates high islet cell survival and integrity

The discrepancies in islet functionality led our investigation to estimate the levels of intracellular ROS generation of ILCCs in both groups. So we performed DCFDA staining which shows the intensity of intracellular oxidative stress (Fig 5.11). ILCCs with low green intensity were observed with control hADSC secretome as contrast to obese with higher green fluorescent intensity, suggesting the evident potential of control secretome for cytoprotection against ROS generation as compared to increased levels of intracellular oxidative stress in obese secretome.

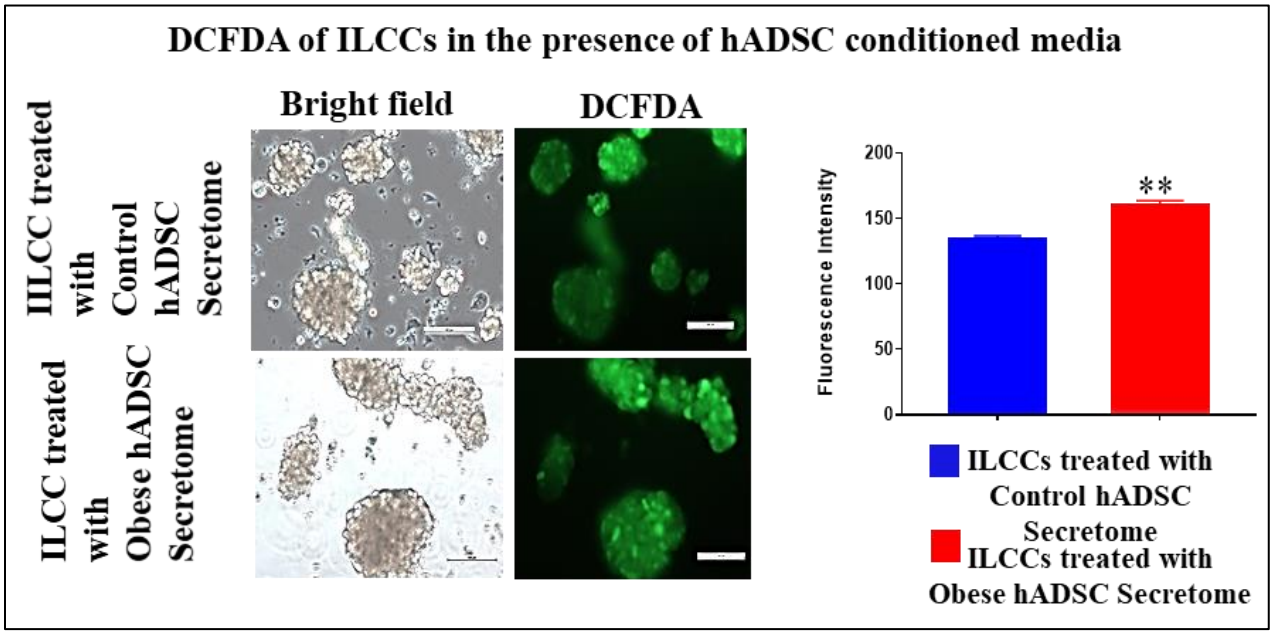


Figure 5.11 Islet survival and integrity parameter of ILCCs in the presence of hADSC conditioned media ILCC were differentiated from day 0 to day 4 under the influence of secretomes of hADSC. Parameter like ROS measurement by DCFDA analysis

To substantiate further findings, we evaluated the viability of ILCCs by FDA/PI staining (Fig 5.12). ILCCs with control hADSC secretome were observed to have more green fluorescent intensity compared to obese which suggests more viable cells since FDA stains live cells shown by green fluorescence. While dead cells are stained red by PI dye which was more evident in ILCCs with obese hADSC secretome. This finding pointed towards the conviction of discordance of islet pathobiology under normal and pathological conditions.

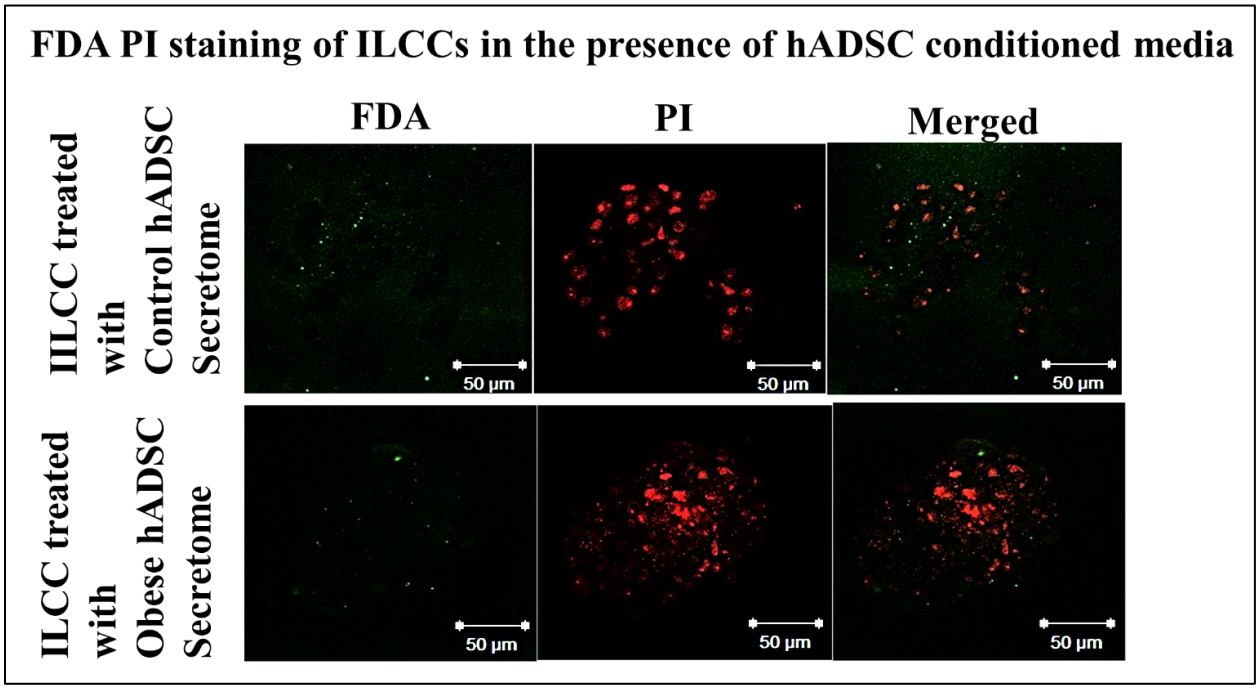


Figure 5.12 Islet survival and integrity parameter of ILCCs in the presence of hADSC conditioned media ILCC were differentiated from day 0 to day 4 under the influence of secretomes of hADSC. Islet survival parameter by FDA PI staining was done

Further cell death analysis was carried which correlated favorably with our findings. Caspase-3 and Parp1 are reliable markers for cell death (Brentnall, Rodriguez-Menocal et al. 2013) (Ko and Ren 2012). Inflammatory stressors, intracellular ROS, and oxidative stress cause DNA damage which activates caspase-3 (apoptotic marker) and Parp1 (DNA damage marker) and are hence associated with cell death. Within 4 day protocol of islet differentiation, we analyzed their expression on day 0, day 2, and day 4. We observed higher expression of both the markers on day 4 indicating ascend of ILCCs into cell death in both groups. Parp1 expression increased on Day 2 in both control and obese groups compared to Day 0 and non-significant expression change was observed in Day 4 control and obese. Caspase 3 expression increased on Day 4 compared to Day 2 in control but non-significant expression change was observed in Day 2 and Day 4 obese suggesting the fate of differentiation and molecular rearrangement of participating and non-participating PREPs into cluster formation to yield viable and functional islet (Fig 5.13). This result substantiates the finding of altered islet biology under different secretome conditions.

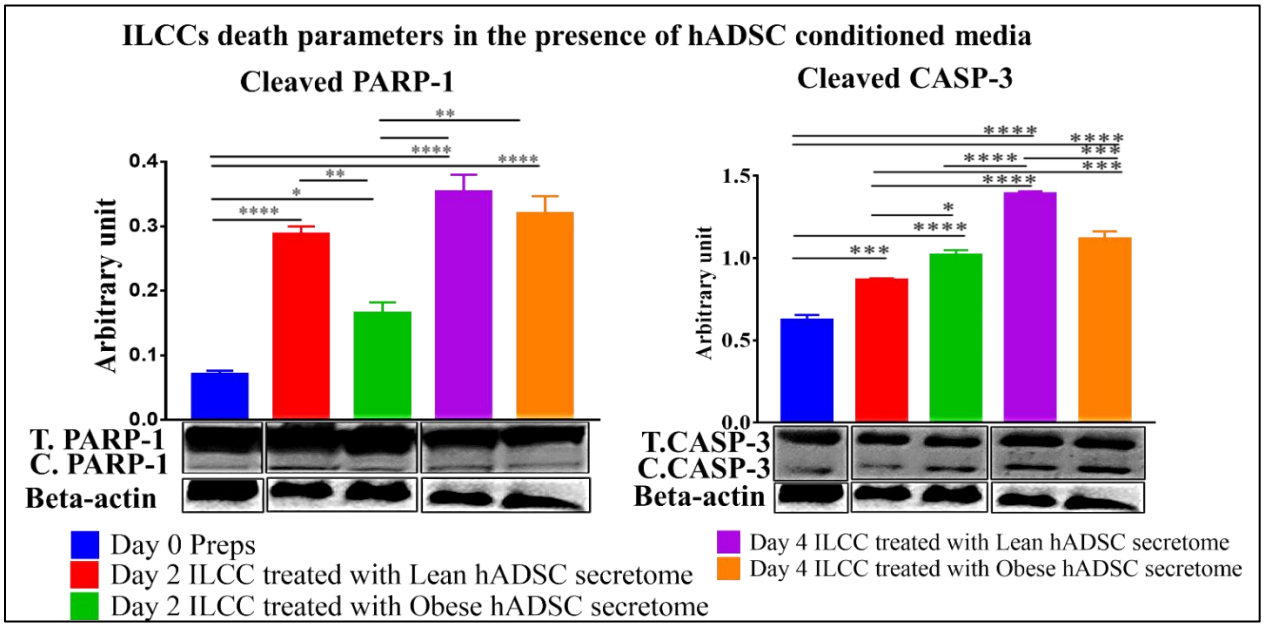


Figure 5.13 PARP-1 and CASP-3 expression by western blotting were done to check islet cell death. Densitometric analysis was done normalized to Beta-actin and expressed as arbitrary unit± S.E.M. N=3, Significance is expressed as p-value *<0.05, ** <0.01, * <0.001, **** <0.0001**

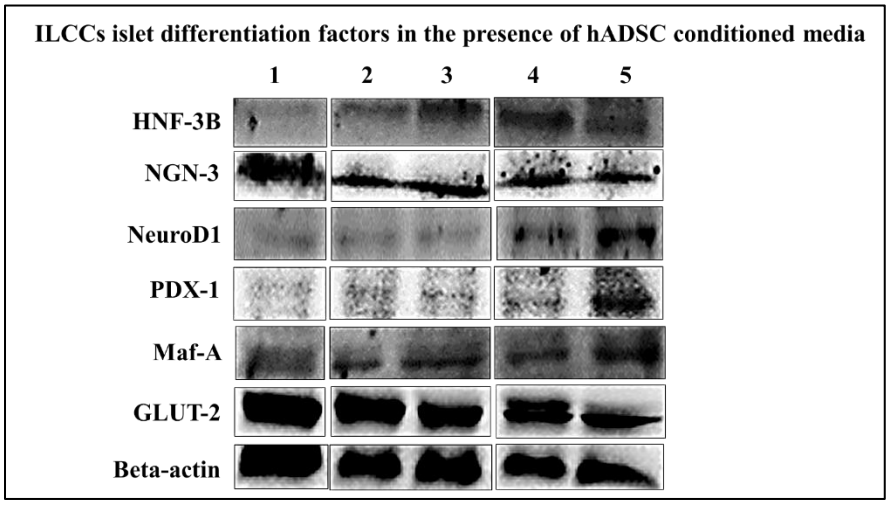
5.3.4 ILCCs from PREPs under control hADSC secretome demonstrates high fidelity towards differentiation

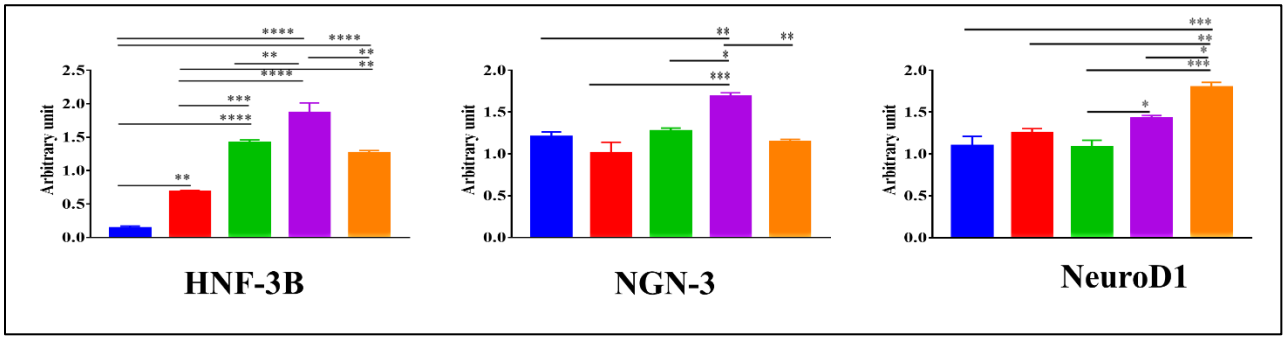
To understand the implications of the fate of islet differentiation under the effect of control and obese hADSC secretome, we performed immunoblotting of key transcription factors which regulate islet differentiation at various stages. A series of a cascade of temporal transcriptional regulation propels pancreatic progenitors towards their inclination for islet differentiation. Stepwise differentiation of islet places FOXA2 or HNF-3B as a pioneer factor. So, we analyzed the expression of FOXA2 or HNF-3B which plays a role in the development and is an important upregulator of PDX-1 which is redundant for pancreatic development (Lee, Cho et al. 2019). Expression increased on day 4 compared to day 2 in control and decreased expression was found on day 4 compared to day 2 in obese. The expression gradually increased from day 0 to day 4 through day 2 under the effect of both the secretome propelling the PREPs towards differentiation. Increased expression is observed at day 2 but eventually decreases on day 4 in obese compared to

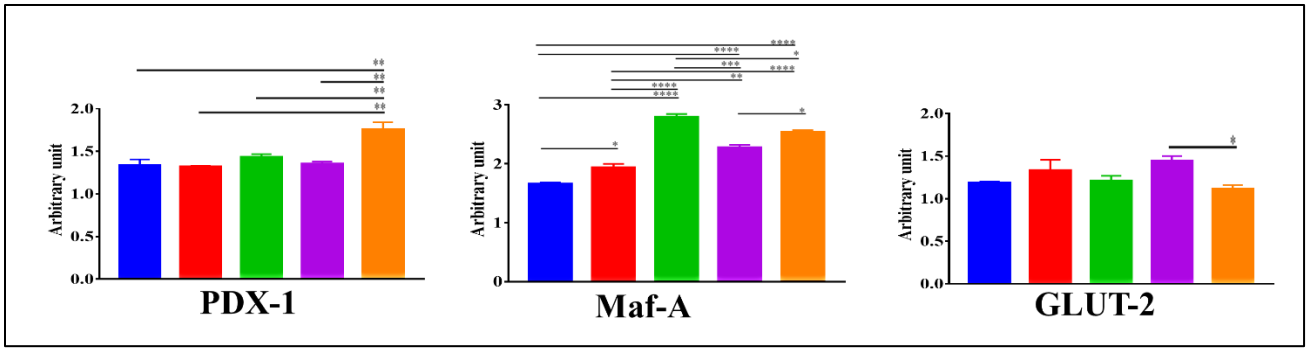
control. We then analyzed the expression of PDX-1 protein which is necessary for initiation of differentiation and it was found unchanged right from day 0 to day 4 in both groups except for upregulated expression in day 4 ILCCs compared to all the other groups. Next, we analyzed the master regulator NGN-3 (Zhu, Liu et al. 2017) which is a redundant marker of progenitor state. The expression was considerably high on day 0.

Islet needs to determine and maintain its identity. Few of the transcriptional factors are important exclusively for maintaining the endocrine identity. For NeuroD (Gu, Stein et al. 2010) exclusive expression was observed on Day 4 in both control and obese groups compared to day 0 and day 2. Upregulation was observed in obese group compared to control on day 4. On day 1 Maf-A (He, Juhl et al. 2014) expression was increased as compared to other groups. On day 2 and day 4 expression was increased in obese compared to control. Day 2 and day 4 control is upregulated than day 2 control. Day 4 obese is upregulated than day 4 control and obese. Finally, GLUT-2 (Thorens 2015) which is the most important glucose transporter was downregulated in day 4 obese islet (Fig 5.14).

This result further strengthens the fact that islet differentiation was indeed altered by obese secretome in a detrimental manner lingering and delaying maturation and differentiation and thus yielding metabolically dysfunctional and sub-optimal islet like cell clusters. These results give indisputable evidence for differential regulation of islet differentiation under normal and pathological conditions.







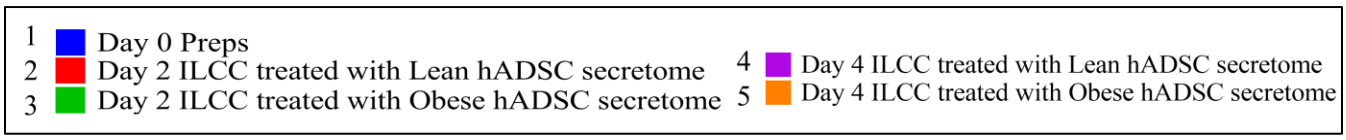


Figure 5.14 Temporal analysis of proteins involved in islet differentiation Differentiation of ILCC took place from day 0 to day 4 from PREPs with secretomes of hADSC.Western

blotting of HNF-3B,NGN-3,NeuroD1,PDX-1, Maf-A and GLUT-2.Densitometric analysis was done normalized to Beta-actin and expressed as arbitrary unit± S.E.M. N=3, Significance is expressed as p-value *<0.05, ** <0.01, * <0.001, **** <0.0001**

5.4 Discussion

The present study provides considerable insight into the effects of hADSCs-CM on islet differentiation through biological regulation. hADSC-CM provides a beneficial effect on islet functionality. hADSC cell free derivatives provide a range of therapeutic applications in regeneration and repair from wound healing to skin aging, to scar regeneration and neuroprotection. The enormous clinical applications make hADSC-CM a reliable therapeutic option (Cai, Li et al. 2020). Direct or indirect crosstalk between the pancreatic islet and hADSC has also given insight into the functional relationship between the two. Bhang et al. studied both direct and indirect effects of hADSC on rat islet viability and functionality and found that apoptotic activity of islet decreased with increased insulin secretion when subjected to hADSCs-conditioned medium (Bhang, Jung et al. 2013). But the present study not only highlights islet functionality but also the important aspect of hADSC in islet differentiation.

The metabolically harsh environment detrimentally affects stem cells. The secretome of the cell gets altered in cases of obesity along with the characteristic of hADSCs (Saki, Jalalifar et al. 2013). Considerable insight is followed in the recent study to explore the effects of hADSC CM under normal and pathological conditions which ultimately affect islet biology.

It is very critical to note that the islet pathophysiology was detrimentally affected by secretome from hADSC from obese. These discrepancies were further supported by the differential expression of proteins found in the secreted milieu of the hADSCs. Most of the proteins were classified under different protein classes which gave an overview of the generalization of broad categories of the proteins. This widens our knowledge of secretome alterations under pathological conditions. We have later considerably correlated the protein expression with subsequent effect on islet differentiation and functionality.

The Cluster formation with more participation of PREPs, maximum cell aggregation and zone of activation in ILCCs under the effect of control hADSC secretome has been observed as contrast to obese. This points towards the confirmation of the maturation of ILCCs. with higher expression of C peptide and glucagon, an unprecedented marker of islet functionality (Leighton, Sainsbury et al. 2017) Various reports have shown that *in vitro* exposure of islets to hADSC secretome enhances islet functionality and viability (Dietrich, Crescenzi et al. 2015). Population of ILCCs was heterogenous with higher yield under obese hADSC secretome with mostly ILCCs falling under 100-200 um range (Bertera, Balamurugan et al. 2012) These results reinforces the fact of islet cell dysfunctionality prevails under undesirable effect of obese hADSC secretome.

Islet with obese secretome had more fluorescence staining from DCFDA. In a glucotoxic environment accumulation of ROS is observed in islet since there is an inherent lack of scavenging mechanism (Bindokas, Kuznetsov et al. 2003). Fluorescein diacetate/propidium iodide (FDA/PI), demonstrates reduced differential staining of intact islets (Barnett, McGhee-Wilson et al. 2004). Therapeutic effects of hADSC are impaired in metabolically compromised conditions (Peng, Yang et al. 2017). Caspase 3, an apoptotic executor (Brentnall, Rodriguez-Menocal et al. 2013) and PARP-1, DNA repair enzyme (Ko and Ren 2012) which gets activated and directs cells towards apoptosis were found upregulated in islet with obese secretome. Yamada et al.demonstrated enhanced functional and survival of porcine islets with indirect coculture with hADSC (Yamada, Shimada et al. 2014). This concurs well with the decreased islet cell viability which emphasizes the fact of reaching limited functional and vital potential by the islets.

The remarkable correlation from this study is between the normal and pathological condition and the effect it has on islet differentiation. The observation to emerge from the data is the developmental effect on an islet which is unexplored in other studies.

HNF-3B plays a very important role in controlling the morphogenesis of the pancreas. Lee et al. demonstrated that lack of HNF-3B in beta cells makes the mice severely hypoglycemic and has dysregulated insulin secretion (Lee, Sund et al. 2002). In our study, it is demonstrated that obese secretome causes a decrease in expression of HNF-3B which causes dysregulated maturity of the newly formed islet.

PDX-1 is an important differentiation factor that is redundant in maintaining beta cell identity along with beta cell differentiation and islet maturation (Kaneto, Miyatsuka et al. 2007). PDX-1 was found overexpressed in day 4 islet under obese secretome. Overexpression of PDX-1 enhanced maturation but fail to respond to increased glucose. HNF-3B is also responsible for PDX-1 transcription which itself is differentially expressed under effect of secretome (Lee, Sund et al. 2002). It also governs pancreas morphogenesis and generation of NGN-3+endocrine progenitors which is differentially expressed under secretome effect. It is reported that abrogated formation of islets results with disrupted expression of NGN-3 early in mouse pancreas development since NGN-3 is important for endocrine cell development of the islets (Rukstalis and Habener 2009).

NeuroD is overexpressed in obese secretome compared to control. NeuroD maintains the functional identity of beta cells and is a requisite for immature to mature transition during the beta cell developmental stage (Gu, Stein et al. 2010).Maf-A was overexpressed in islets under obese secretome. He et al reported inhibition of differentiation during enforced expression of Maf-A in endocrine progenitors (He, Juhl et al. 2014). GLUT-2 was less expressed in islets when subjected to obese secretome which is essential for glucose sensing and insulin secretion (Thorens 2015).

The secretome comprised of different proteins which were differentially expressed and regulated in control and obese hADSC secretome. The majority of keratin proteins were expressed in control hADSC secretome and lesser expressed in obese hADSC secretome. Blessing et al reported a reduced number of insulin vesicles in pancreatic beta cells by transgenic targeting of K1 or K10 which ultimately leads to diabetes (Blessing, Rüther et al. 1993). The absence of keratins leads to dysfunctional glucose uptake as keratin dynamics affect glucose transporters. (Vijayaraj, Kröger et al. 2009).

We found an abundance of vimentin in control secretome and lesser in obese. Krivova et al speculated vimentin as an early endocrine pancreas disorder marker. Hypertrophy and hyperplasia of islet was associated with the presence of vimentin and glycogen or insulin positive cells in islets of macrosomic infants from both diabetic and nondiabetic mothers (Krivova, Proshchina et al. 2018). Expression of vimentin was associated with loss of islet cell identity since it indicated plasticity and dedifferentiation. Moreover, also associated with reduced expression of Nkx6.1 and

PDX-1 though they were not apoptotic. All these lead to beta cell dysfunction leading to metabolic disorder (Roefs, Carlotti et al. 2017). Vimentin was less expressed in control secretome and Wang et al demonstrated that some nestin positive cells are heterogeneous which may lack co-expression with vimentin (Wang, Li et al. 2005).

Myosin was abundantly present in obese secretome compared to control secretome. Intracellular movement of hormone granules in pancreatic islet is redundant for regulatory secretion of hormones which is controlled by Ca2+/CaM-dependent phosphorylation of Myosine light chain (Cheney, Riley et al. 1993). This phosphorylation mechanism also facilitates lower Ca2+ requirements than needed for the release of secretory granules (Iida, Senda et al. 1997). Most of the ATP synthase subunits were not found in control secretome but abundantly present in obese. Li et al. observed decreased expression of ATP synthase β-subunit (ATPsyn-β) in pancreas islets of rat model of PCOS with T2DM which when upregulated by transfection also improves ATP content in islet (Li, Li et al. 2017). ATP synthase subunits were exclusively expressed in obese secretome. Increased expression is observed to be a compensatory response to enhanced metabolism under hyperglycemic condition (Leguina-Ruzzi, Vodičková et al. 2020).

Dermicidin, a stress induced protein, was abundant in control secretome than obese. DCN2 is found in the serum of diabetic patients which is associated with disease pathogenesis. It increases insulin resistance and is ultimately responsible for lowering GLUT4 expression (Bhattacharya, Khan et al. 2017).

Transthyretin was abundant in obese secretome which is an amyloid fibril protein. Transthyretin reactive cells including beta cells are found in islets from type-2 diabetic patients since diabetes alters the expression of transthyretin (Westermark and Westermark 2008).

Fructose biphosphate aldolase was exclusively expressed in obese secretome. Gerst et al suggested increased expression of ALDOB resulting from hyperglycemia, is associated with lower insulin secretion in human beta cells (Gerst, Jaghutriz et al. 2018).

In addition to the proteins, several studies have also found bioactives, cytokines, growth factors, circulating DNA, RNA and extra cellular vesicles etc. to be a part of the secreted milieu, so there

is a possibility that such small molecules could have contributed to some extent to the observed outcomes apart from the proteins discussed in the current study (Cai, Li et al. 2020) (Wright, Uchida et al. 2014) (Lee, Lee et al. 2014) (Lopatina, Bruno et al. 2014). Thus the present study gives an account of crosstalk of hADSC and islets which widens the understanding of secretome therapeutics and investigates islet functionality and differentiation under pathological effect.

5.5 Summary

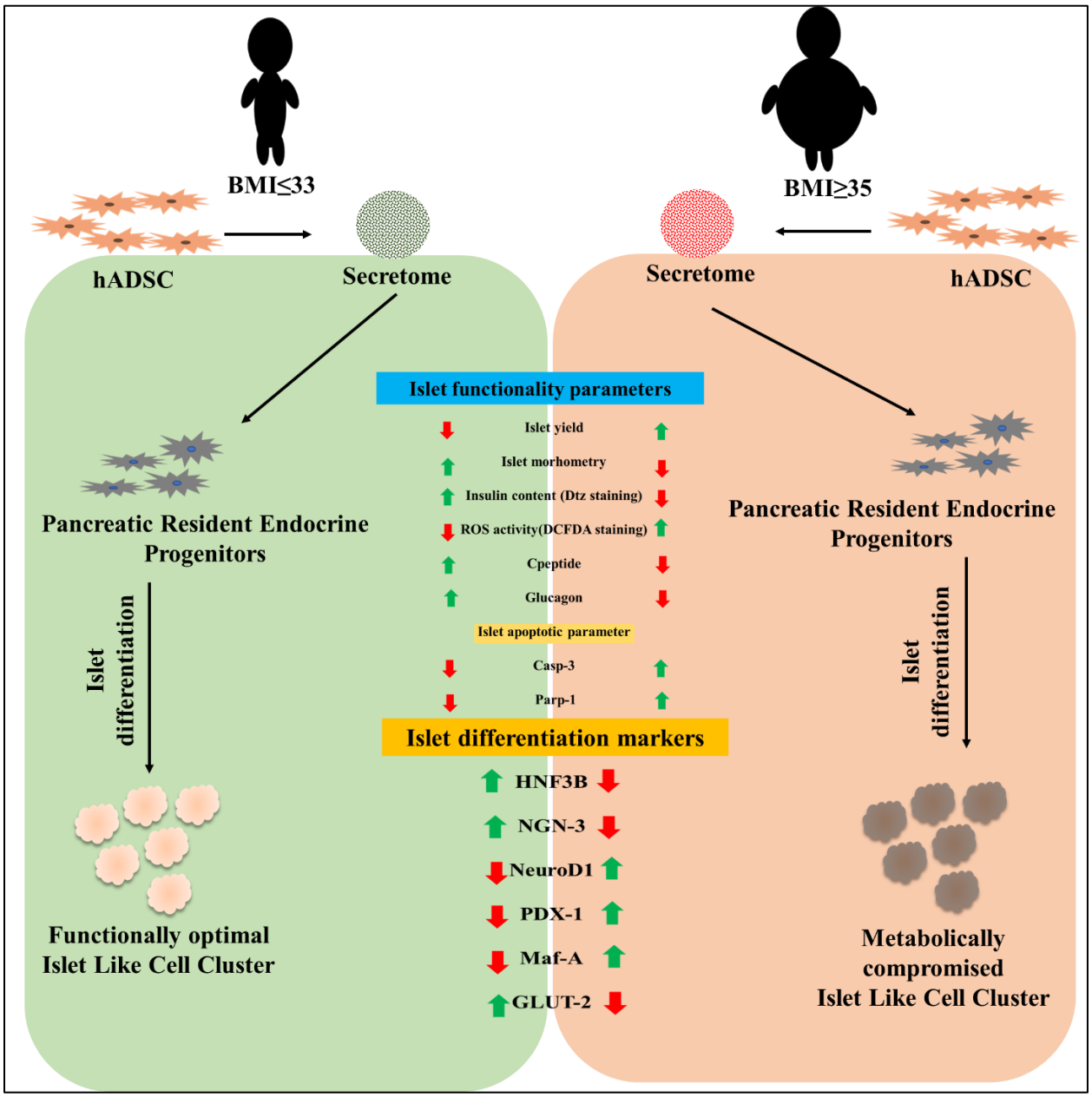


Figure 5.15 Summary of Chapter 5

Cellular therapy is the best approach for regenerative medicine. However, the cell transplantation techniques are invasive and subjected to multilevel hurdles. So an alternative secretome administration for treating pancreatic islet etiopathology in diabetes would be an effective

therapeutic approach. This study will add to the knowledge on effect of altered hADSC secretome on islet functionality and differentiation which will provide greater understanding of cross talk between hADSC from adipose tissue and pancreatic islets. Therefore, the present study would aid array of information on how hADSC secretome can be a potent therapy for diabetes. Thus, we are aiming that secretome therapy can be approached therapeutically and ease the burden of diabetes in healthcare management (Fig 5.15).

Chapter 5: Effect of secretome of skeletal muscle on islet differentiation & functionality

Chapter 5 is published in 'Experimental Cell Research' Journal.

(Full length paper can be found in the 'Publications' section of the Thesis)

Experimental Cell Research 410 (2022) 112970

Contents lists available at ScienceDirect

Experimental Cell Research

journal homepage: www.elsevier.com/locate/yexcr

Research article

Influence of metabolically compromised Adipose derived stem cell secretome on islet differentiation and functionality

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ARTICLE INFO

Keywords:
hADSC secretome
Islet functionality
Islet differentiation

ABSTRACT

Islet integrity plays a major role in maintaining glucose homeostasis and thus replenishment of damaged islets by differentiation of resident endocrine progenitors into neo islets regulates the islet functionality. Islet differentiation is affected by many factors including crosstalk with various organs by secretome. Adipose derived stem cells (ADSC) secrete a large array of factors in the extracellular milieu that exhibit regulatory effects on other tissues including pancreatic islets. The microenvironment of metabolically compromised human ADSCs (hADSCs) has a detrimental impact on islet functionality. In the present study, the role of secretome was studied on the differentiation of islets. Expression of key transcription factors like HNF-3B, NGN-3, NeuroD, PDX- 1, Maf-A, and GLUT-2 involved in development were differentially regulated in obese hADSC secretome as compared to control hADSC secretome. Islet like cell clusters (ILCCs) functionality and viability were critically hampered under obese hADSC secretome with compromised yield, morphometry, lower expression of C-peptide and Glucagon as well as higher ROS activity and cell death parameters. This study provides considerable insights on two major findings which are (i) exploring the use of hADSC secretome in islet differentiation and (ii) understanding the regulating effect of altered hADSC secretome under a metabolically compromised condition.

1. Introduction

Loss of functional pancreatic β cells is the underlying cause of type 1 diabetes mellitus which leads to multiple long-term complications. The critical contributor to this pathology is the autoimmune attack on functional beta cells. Apart from exogenous insulin treatment, current therapies include islet transplantation from cadaveric donors. But owing to exceeding demands placed on organ transplantation, there is still considerable uncertainty of the availability of donors, rejection post transplantation, and the need for immuno-suppressant. Thus, transplantation therapy demonstrates viable but still with multiple hurdles to be a successful therapeutic regimen [1]. This lead to the examination of the *in vitro* regeneration of β cells from various stem cell sources that can provide a sustainable solution for combating the need as a source for islet [2].

Stem cell therapy has gained wider recognition for its remarkable ability to support cell survival and homing capacity to the damaged tissue [3]. Islet differentiation can be modulated by various factors especially the milieu of secreted factors by other cells, which can vary in normal as well as pathological conditions. One such remarkable element is the regenerative capacity of secreted factors from human adipose tissue-derived stem cells (hADSCs). hADSC secretome is known to orchestrate various functions like wound healing, angiogenesis, anti-inflammation, immunomodulatory, anti apoptosis etc [4].

Conditioned media (CM) from stem cells have garnered much attention for regenerative potential without the involvement of the cell itself. It has also proved to have a beneficial effect on islet functionality. Various reports suggest direct or indirect co-culture of hADSC with islet having anti apoptotic effect and increased insulin secretion [5,6]. Nevertheless, the application of hADSC secretome on islet differentiation is yet to be properly defined. Also, if this secretome of hADSCs can be used as an *in vivo* differentiation agent for the resident PREPs, then it can be a better alternative to transplantation. It will exclude the risk of cell rejection and it is easy for packaging, transportation, and freeze

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https://doi.org/10.1016/j.yexcr.2021.112970
Received 2 November 2021; Received in revised form 6 December 2021; Accepted 8 December 2021
Available online 9 December 2021
0014-4827/