SUMMARY AND CONCLUSION

SUMMARY

Obesity is associated with severe clinical manifestations like diabetes, CVD and hepatosteatosis. It is the uncontrolled expansion of adipose tissue due to excess fat accumulation and increased number of adipocytes. This expansion of adipose tissue alters adipokine levels and mitigate insulin signaling in adipose tissue. FFA serves as ligand to TLR4 which attract macrophages and infiltrate in adipose tissue to secrete more pro-inflammatory adipokines like leptin, resistin, TNFα, CRP etc. TNFα and resistin are known to induce inflammation and insulin resistance in adipose tissue.

Adipose tissue is a complex of cellular network that regulates metabolism and energy homeostasis. Obesity alters the function of adipocytes, endothelial cells, macrophages etc. There is a macrophage class-switch which enhance number of M1 macrophages from 5% to upto 80% in the adipose tissue during chronic obesity. Adipose derived mesenchymal stem cells are also affected by obesity as observed by scientist in the present decade. hADSC of obese Caucasians are reported to have compromised stemness and diminished metabolic profile. Since, Indians are metabolically obese and more susceptible to metabolic disorders and as hADSC are being applied enormously in regenerative medicine in India, an attempt was made in the present study to scrutinize the profile of hADSC along with adipose tissue of obese Indians.

We were first to attempt and scrutinize the metabolic profile of hADSC of obese Indian subjects in chapter 4. Obese Indians with BMI$\geq$25 were selected as per new consensus for obesity. hADSC of obese subjects had diminished proliferation, their pluripotency was hampered and all the key ATFs were upregulated in undifferentiated state with downregulated OTF. We for the first-time report that hADSC of obese subjects are insulin resistant as no population study depicted, that obesity induces insulin resistance in hADSC. Also, hADSC of obese Indians were pre-committed to adipogenesis as they preferred to differentiate into hypertrophic adipocytes with reduced osteocytes, which proved that hADSC had compromised metabolic profile and stemness at a very low BMI compared to those of Caucasians.

Comprehensive metabolic profile of adipose tissue of Indian obese, had demonstrated extravagant stored levels of leptin and resistin, while adiponectin levels were diminished. Lipogenic genes and insulin signaling were elevated in adipose tissue of obese subjects which denoted propensity to increase obesity and hyperinsulinemia. At

cellular level, hADSC, the most self-resilient and immune-modulatory cells were altered in obese Indian subjects. This study highlights the application of hADSC in regenerative medicine with proper scrutiny.

Obese people have high glucose, insulin, TG, CRP, LDL, leptin, resistin with reduced adiponectin, IL-4, IL-10, HDL etc. in circulation which cause metabolic disturbances. TNFα is one of the prime cytokine which is produced ubiquitously and is also produced as adipokine during positive energy state. Resistin is a cysteine rich peptide produced by mouse pre-adipocyte which facilitates maturation and lipid synthesis for *de novo* adipocytes. But under high fat diet and excessive calories, production of resistin is increased which enhance leptin levels that cause leptin resistance. Here we, explored stemness and metabolic profile of hADSC under the influence of TNFα and resistin. To ameliorate their effects, SM an iridoid, established as insulin sensitizers was used along with Met, a known insulin sensitizer as a positive control.

Effects of TNFα and resistin were observed independently on proliferation and differentiation potentials of hADSC in chapter 5. We for the first time generated TNFα and resistin induced insulin resistance hADSC model after 48H of treatment independently. Cell cycle was hampered where resistin embarked potent effects on it after 96H of treatment. Met was more efficient to rectify dysregulated cell cycle. Increase in downstream inflammatory mediators IKKβ was observed upon TNFα and resistin treatment which was ameliorated by SM and Met. As a consequence of inflammation induced by TNFα and resistin, downstream effects were observed on pluripotent genes, insulin signaling proteins and glucose uptake and adipogenic genes which were all found to be altered. However, both SM and Met efficiently ameliorated TNFα and resistin mediated alterations in stemness and metabolic profile of hADSC. Phenotypic observation revealed that TNFα inhibited both adipogenesis and osteogenesis while resistin upregulated both adipogenesis and osteogenesis. SM and Met both at 25ug/ml and 64.5ug/ml concentration respectively, inhibited adipogenesis and osteogenesis. Thus, the overall chapter signifies the deleterious effect of both TNFα and resistin on stemness and metabolic profile of hADSC which was explicitly ameliorated by SM by reducing insulin resistance and restoring stemness in hADSC.

As resistin was found to be elevated in metabolically compromised Indians and till date there are no studies being carried out on resistin's detrimental effects on fate of

hADSC, an attempt was made to understand the temporal expressions of signaling pathway proteins and targeted genes which govern adipogenic and osteogenic differentiation of hADSC. Resistin as observed perturbed fate of hADSC, thus, chapter 6. was imperative to explore the temporal expressions of transcription factors during adipogenic and osteogenic differentiation along with pSMAD1/5/8 and β catenin. Resistin treatment diminished the expressions of both pSMAD1/5/8 and β catenin in the initial time point which were upregulated again in mature adipocytes on 22^{nd}D. The ATFs were expressed in temporal fashion where CEBPβ and CEBPδ were prominent on 4^{th}D but resistin diminished their expressions, while PPARγ and CEBPα appeared on 12^{th}D, where resistin increased their expression predominantly at pre-adipocyte stage, but CEBPα was reduced on 22^{nd}D. Adiponectin gene expression was visualized on 22^{nd}D only and resistin diminished its expression, which concluded that resistin treatment targeted all the three phases of adipogenesis and stammered stemness of hADSC.

During osteogenesis, it was observed that resistin significantly downregulated pSMAD1/5/8 complex with alterations of β catenin on just 6^{th}D of osteogenesis. RUNX2 was observed on 6^{th}D which was diminished on resistin treatment while, OSTERIX was highly upregulated on both 6^{th}D and 14^{th}D of osteogenesis. Moreover, SIRT1 which drives RUNX2 expression was upregulated subsequently, but resistin inhibited its expression as observed on 14^{th}D of osteogenesis. These result concluded that resistin altered BMP but not much of WNT signaling proteins, which produced osteocytes that mimicked subchondral obese phenotype defects. Further, major focus was on effects of SM and Met on signaling pathways and target adipogenic and osteogenic genes in mature adipocytes and osteocytes obtained from resistin mediated inflamed hADSC. Significant proteins of BMP, WNT and insulin signaling were observed during both the differentiation. Resistin altered expressions of pSMAD1/5/8 complex which was rectified by SM in mature adipocytes and osteocytes after 22D and 14D of differentiation respectively. β catenin levels were altered by resistin both in adipocytes and osteocytes and SM restored them. Insulin signaling proteins IR and pAKT were upregulated in adipocytes from resistin treated hADSC but downregulated in osteocytes which denotes their participation in resistin mediated differentiation. SM reversed the effects of resistin on the expressions of these proteins. Resistin upregulated CEBPβ, CEBPδ and PPARγ but diminished CEBPα in matured

adipocytes. Functional adipokines adiponectin and leptin were measured where, resistin treatment downregulated adiponectin and upregulated leptin. These, results revealed that resistin elevated adipogenesis but hampered maturation of adipocytes and induced insulin resistance in adipocytes. SM effectively ameliorated functions of adipocytes and insulin resistance in adipocytes. Also, resistin upregulated osteogenesis but stammered its maturation by inhibiting SIRT1 expressions which is required for functionality and insulin sensitivity of osteocytes. SM effectively upregulated SIRT1, IR and pAKT in osteocytes.

Therefore, this chapter explored for the first-time repercussions of resistin on key signaling proteins and their respective adipogenic and OTFs, mitigating differentiation potentials of hADSC.

CONCLUSIONS

1. The study conducted in the present Ph.D. thesis addresses repercussion of obesity on stemness and its metabolic profile both at clinical and experimental levels. It is the first study which demonstrates that hADSC of obese Indians are highly vulnerable at a very low BMI compared to those of Caucasian population. Induction of insulin resistance in undifferentiated hADSC is the first report in any population. High inflammation with concomitant reduced pluripotent markers and differentiation potentials of hADSC alarms the precarious application of these cells isolated from obese donors.
2. It is the first report as per our knowledge which demonstrated that both TNFα and resistin independently induced insulin resistin thus, we successfully generated TNFα and resistin induced insulin resistance hADSC model.
3. As per our knowledge for the first-time repercussions of TNFα and resistin were observed on pluripotent genes OCT4, SOX2 and NANOG which were differentially altered by both the adipokines.
4. TNFα and resistin instilled inflammatory mediators, affected proliferation, stemness and metabolic profile of hADSC. Induction of insulin resistance might have contributed in loss of pluripotency and self-renewal of the stem cells.
5. TNFα and resistin both affected adipogenesis and osteogenesis in opposite manner where TNFα diminished while resistin increased the differentiation in hADSC.

These could be potentially attributed to the observations that they altered pluripotent genes in different manners which regulate differentiation of hADSC.

6. It could be proposed that TNFα mimicked metabolically obese non-obese phenotype while resistin supports metabolically obese obese phenotype and thus, the two inflammatory adipokines can be used as indicators to detect differentiate the type of obesity which could be explored as good biomarker candidates.
7. Resistin temporally altered key signaling proteins and downstream ATF and OTF respectively which rendered dysfunctional adipocytes and osteocytes that could lead to obesity and degenerative diseases.
8. Further, as a therapeutic approach, SM was found to be a potent insulin sensitizer as it abrogated resistin mediated inflammation, insulin resistance in undifferentiated and differentiated hADSC, thus rendered protection to the cells and restored lost pluripotency of hADSC.
9. This is the first study where; SM has been explored for its insulin sensitizer effect and anti-adipogenic activity in hADSC. Thus, SM is a potential antiadipogenic insulin sensitizer which could be a potential antidiabetic and anti-obesity phyto-compound used for treatment of diabetes and obesity.
10. Since, SM rendered protection to hADSC and restored pluripotency, it can be used for improving efficacy of hADSC in regenerative therapy.