Send Orders for Reprints to reprints@benthamscience.ae

RESEARCH ARTICLE

A Network Biology Approach for Assessing the Role of Pathologic Adipose Tissues in Insulin Resistance Using Meta-analysis of Microarray Datasets

Aditya Saxena[1,2,*] and Kumar Sachin[3]

[1]*Department of Biotechnology, Institute of Applied Sciences & Humanities, GLA University, Mathura (U.P.), India;* [2]*Uttarakhand Technical University, Dehradun (U.K.), India;* [3]*Department of Biochemistry and Biotechnology, S.B.S. (PG) Institute of Biomedical Sciences & Research, Dehradun (U.K.), India*

Abstract: ***Background*:** The role of adipose tissue in Insulin resistance (IR) and Type 2 Diabetes (T2D) has well been received in the biomedical community; being a precursor of T2D, identification of the molecular basis of IR is therefore, vital to elucidate T2D- pathogenesis and meta-analysis of previously conducted microarray studies provides an inexpensive approach to achieve this end.

***Methods*:** In this study, we have carried out a statistical meta-analysis of 157 microarray datasets from five independent studies and identified a meta-signature of 1,511 genes; their functional meaning was elucidated by integrated pathways-analysis. Further, a protein-protein interaction network was constructed and key genes along with their high confidence transcriptional- and epigenetic-mediators were identified using a network biology approach.

***Results*:** Various inflammation- and immune system-related pathways such as TGF-β signaling, IL7 signaling, Neutrophil degranulation, and Chemokine signaling *etc*. were enriched in sick adipose tissues; identified transcription factors, and microRNAs were also found to regulate processes relevant to IR/T2D pathophysiology.

***Conclusion*:** This study endorses the development of effective bioinformatics workflow and further grants an indication for the acceptance of adiposopathy as the root mechanistic pathology that poses risk for development of type 2 diabetes; concept of adipospathy in place of metabolic syndrome will open the possibility to design drugs, those will ameliorate adipose functions and hence proved to be more effective against Type 2 Diabetes.

ARTICLE HISTORY

Received: November 10, 2017
Revised: May 10, 2018
Accepted: July 16, 2018

DOI:
10.2174/1389202919666180726125645

Keywords: Cytoscape, Insulin resistance, Microarray, Network biology, Type 2 diabetes, Pathologic adipose tissues.

1. INTRODUCTION

Insulin resistance (IR) has been considered as a precursor of Type 2 Diabetes (T2D). Clinically, it is attributed to defective insulin signaling in its responsive tissues such as adipose, liver, and muscle.

Identification of the molecular basis of IR is, therefore, vital to elucidate the mechanism of T2D pathophysiology. Obesity has long been implicated towards the development of insulin resistance with subsequent clinical manifestations of T2D. However, metabolically healthy obese (MHO) people are clinically characterized in population, delineating the fact that it is indeed *incorrect fat distribution* rather mere *excess fat storage* that sets the stage for IR and T2D etiology; recent findings unraveling the role of adipose tissue in energy homeostasis in addition to its primary function as energy reserve further corroborate this fact [1, 2].

*Address correspondence to this author at the Department of Biotechnology, Institute of Applied Sciences & Humanities, GLA University, P.O. Box: 281 406, Mathura, India; Tel: +91-8171630011;
E-mails: aditya.saxena@gla.ac.in; aditya.235@gmail.com

Clinical association of IR with obesity is attributed to inflammation in adipose tissues due to macrophage infiltration, adipocyte hypertrophy, visceral deposition, and the defective process of adipogenesis. These molecular changes deregulate insulin signaling [3]. In addition to it, pathologic adipose tissue also impairs insulin secretion by releasing various non-glucose secretagogues *i.e.* non-esterified fatty acids, glycerol, pro-inflammatory cytokines, leptin, and adiponectin hormones. The distribution of body fat also affects the prevalence of IR. People with peripheral obesity (subcutaneous fat) are less prone to develop IR than people with central obesity (visceral fat) as secretary proteins responsible for energy production seem to predominate in visceral fat. Moreover, adipocytes present in subcutaneous and visceral regions also differ with respect to the amount of protein secretion [2].

DNA microarray is an unbiased method of investigating the genome-wide transcriptome profile in a group of cells or tissue. A number of studies have reported its use in assessing adipose biology in T2D/IR conditions like differences in gene expression across various adipose depots, between lean and obese individuals, the effect of insulin/NEFA/adipokines

1389-2029/18 $58.00+.00

on cellular physiology, different stages of adipogenesis and brown *vs*. white adipose tissue gene expression. In addition, it has also been used to unravel the effect of various environmental factors on these pathologies *viz*. drugs, toxins, diet *etc*.

Meta-analysis of several microarray datasets is an inexpensive means that accounts for demographic variability more elegantly, increases the statistical power, and hence helps in obtaining more exact gene expression differentials. Previously one meta-analysis on microarray datasets has been conducted to assess the systemic cellular pathology in IR/T2D conditions [4].

To interpret the functional meaning of gene-list obtained from microarray studies, it is necessary to analyze it in the context of known biology and network analysis approach provides a framework to achieve this end. It offers the representation of intracellular biological systems as the network of interacting molecular components by treating them as '*nodes*' and interactions between them as '*edges*'.

In the present study, a statistical meta-analysis of 157 microarray datasets from five independent studies belonging to normal, and IR individuals and specific to adipose tissue was conducted and a meta-signature which comprised of 1,511 genes was identified.

As type 2 diabetes is ascribed to interaction between environmentally triggered weak insulin signaling with genetically programmed pancreatic β-cell dysfunction; our meta-analysis included adipose samples from individuals, those have been subjected to disparate environmental conditions such as treatment with insulin-sensitizing drug, fasting, and hyperinsulinemia- euglycemic clamp in an attempt to obtain a robust meta-signature of insulin-resistant state.

Our analysis has reported 22 cellular pathways implicated by the genes comprising meta-signature. A selected number of most significant proteins (genes) were further identified on the basis of two network metrics; sub-network derived from these proteins was extended to include high confidence transcription factors and microRNAs.

The role of these pathways and regulatory mediators in the overall IR pathophysiology was inferred by the literature search. Our findings underscore that adiposopathy *i.e. sick fat* is, in fact, a primary cause of the development of IR and T2D.

2. MATERIALS AND METHODS

We have selected five independent microarray studies from NCBI Genomic Expression Omnibus (GEO) database which have been conducted primarily to assess the transcriptional alteration in adipose tissues between normal and insulin resistant individuals (Table **1**).

Hardy O.T. *et al.* [5] generated two microarray series (GSE15773, and GSE20950) - both comprising paired samples from subcutaneous and visceral adipose tissues from BMI-matched (BMI ≥40) normal glucose tolerant, and impaired glucose tolerant individuals. As all participants were morbidly obese, their study design allowed identification of differentially expressed genes solely related to defective insulin signaling and not simply a feature of the obese state.

Soronen J. *et al.* [6] conducted another microarray study (GSE26637) for measuring gene expression differentials in subcutaneous adipose tissues between lean normal glucose tolerant, and obese impaired glucose tolerant individuals. Samples were extracted both after an overnight fast as well as after hyperinsulinemic- euglycemic clamp.

Sears D.D. *et al.* [7] conducted a large microarray series (GSE13070) comprising 364 samples from 72 individuals to ascertain the influence of an insulin-sensitizing drug, thiazolidinedione (TZD) on the transcriptional profile of skeletal muscle, and abdominal subcutaneous tissues. Samples were collected before and after hyperinsulinemic-euglycemic clamp, at baseline and after three-month TZD treatment to unravel transcriptional alteration exclusively attributed to impaired insulin signaling and therefore discounting influence of other factors.

Keller P. *et al.* [8] generated a microarray series (GSE27949) comprising type 2 diabetic, normal glucose tolerant and impaired glucose tolerant individuals to assess the role of selected miRNAs, those have been reported to be involved in the process of adipogenesis.

As all studies utilized Affymetrix-hgu33Plus2 platform, therefore our meta-analysis is expected to minimize chip to chip variation across different studies.

All microarray datasets were independently preprocessed using Bioconductor package *gcrma* [9]. A non-specific filtering step was further carried out to select high sensitivity probes using package *genefilter* [10] and Affymetrix probe ids were collapsed to Entrez ids and official gene symbols.

Meta-analysis of normalized datasets was conducted using a web-based tool NetworkAnalyst [11] which offers various meta-analysis procedures on summary level data (*P-values, gene ranks,* and *effect sizes etc*.). Fisher's method was used on gene-level log-transformed P-values to obtain a meta-signature of genes after adjustment of batch size ($P < 0.05$).

For pathway and regulatory analysis of obtained meta-signature, a network-based approach was adopted using Cytoscape [12] - a commonly used framework that is extensively used to visualize, and analyze biological data with its various apps.

Meta-genes were first imported to ClueGO app [13] and a functionally organized GO/pathway term network was constructed, considering all the three pathway databases: WikiPathways, KEGG, and Reactome ($P < 0.05$).

To identify most important regulatory mediators of these meta-genes, a functional interaction network for protein products of these genes was created using stringApp that provides access to STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) database [14]. Two sub-networks those comprised of top 20 genes based on their *degree-centrality,* and *bottleneck-centrality* respectively were created using cytohubba app [15] and subsequently merged to obtain a sub-network of proteins (genes). CyTargetLinker app [16] was then used to extend this sub-network with regulatory information (miRNA-targets, and TF-targets).

Highly significant top 10 miRNAs and all transcription factors were selected considering the degree-centrality of each node using NetworkAnalyzer [17].

Table 1. Details of microarray sample used in meta-analysis.

S. No.	GSE	Adipose Site	Normal: IGT
1	GSE15773 (G1)	subcutaneous	4:5 (obese)
2	GSE15773 (G2)	visceral	5:5 (obese)
3	GSE20950 (G1)	subcutaneous	10:9 (obese)
4	GSE20950 (G2)	visceral	10:10 (obese)
5	GSE26637(G1)	Subcutaneous	5:5 (lean: obese) (fasting state)
6	GSE26637(G2)	Subcutaneous	5:5 (lean: obese) (hyperinsulinemic-euglycemic state)
7	GSE13070	Subcutaneous	28:29 (thiazolidinedione-treated with hyperinsulinemic-euglycemic state)
8	GSE27949	subcutaneous	11:11
Total			157

3. RESULTS

NetworkAnalyst identified 17 and 546 differentially expressed (DE) genes (*P<0.05*) in GSE26637 (G1) and GSE26637 (G2) respectively, indicating there exist large transcriptional differences between insulin sensitive-lean and insulin resistant-obese phenotypes, whereas other studies did not report any DE genes.

The subsequent analysis reported a meta-signature of 1,511genes on the basis of combined P-value and t-statistics of differential expression across all datasets (Supplementary Table **S1**).

Protein-protein interaction network of these meta-genes using stringApp connected 1,335 of these genes with 11 additional linker proteins.

We further identified twenty-seven hub proteins in this network by selecting two topological properties - *degree-centrality,* and *bottleneck-centrality*; both are highly correlated. However, proteins with high degree-centrality tend to be '*party hubs*' and connect similar biological processes whereas proteins with high bottleneck-centrality tend to be '*date hubs*' and connect diverse processes and therefore have a strong tendency to be products of essential genes [18].

The 27 proteins identified were : LRRK2, MMP9 FGF2, PTPRC, MYC, TLR4, CTNNB1, RAC2, JUN, PIK3CG, ACACB, IL8, VEGFA, ITGAM, EHMT1, SYK, IL1B, LYN, MAPK1, FOS, ESR1, PIK3C3, PLEK, ACTA2, OASL, XPO1 and KI.

A Regulatory Interaction threshold of 2 was selected in CyTargetLinker app while extending this sub-network to include high confidence miRNAs and transcription factors that resulted in the identification of 43miRNAs and 3 transcription factors (Table **2**; Fig. **1**).

An elaborate literature searching for assessing the implication of pathways and regulatory agents have proved fruitful in elucidating some aspects of pathologic adipose tissue induced insulin resistance.

3.1. Pathways Analysis

ClueGO pathway analysis enriched 299 pathways (Supplementary Table **S2**) represented by highly suggestive 22 term-leading pathways (Table **3**).

Enrichment of various immune system related pathways such as 'B Cell Receptor Signaling Pathway', 'Chemokine signaling pathway', 'IL1 and megakaryocytes in obesity', 'Interleukin-7 signaling', 'Neutrophil degranulation', 'HTLV-I infection', 'Leishmaniasis', 'Pertussis', and '*Staphylococcus aureus* infection' indicates that adipose tissue is indeed inflamed and that it may likely contributes toward the development and progression of IR-phenotype.

The most significant pathway term representing 146 pathways and also consistent with IR-phenotype was '*IRS-mediated signaling*'. Another pathway term '*MAPK Signaling Pathway*' has also well studied downstream of insulin receptor that mediates most of the anabolic effects of insulin signaling such as cell proliferation, differentiation, and gene expression. These phenotype suggestive pathways indicated fruitfulness of our bioinformatics approach.

Table 2. MicroRNA-targets and TF-targets reported in CyTargetLinker analysis.

S. No.	Name	Biological Type	Degree	Betweenness Centrality
1	hsa-miR-181c	Micro- RNAs	22	0.001665
2	hsa-miR-181a		21	0.002106
3	hsa-miR-181d		21	0.002078
4	hsa-miR-338-5p		20	0.002038
5	hsa-miR-181b		20	0.001665
6	hsa-miR-302a		19	0.002023
7	hsa-miR-302b		19	0.002023
8	hsa-miR-302c		19	0.002023
9	hsa-miR-520a-3p		19	0.002023
10	hsa-miR-520b		19	0.002023
1	Spi-1/PU.1	Transcription Factor	11	3.2421E-4
2	ALDOA		6	1.4985E-4
3	SRF		5	0

'*Neutrophil degranulation'* is considered the hallmark of adipose inflammation as these are one of the first cells recruited to the site of inflammation. Neutrophils release elastase which is a protease that degrades Insulin Receptor Substrate (IRS), and adaptor proteins, leading to attenuation of insulin signaling [19].

Another enriched pathway, '*Chemokine signaling*' has also been reported to be associated with insulin resistance and obesity. Overnutrition-induced hypertrophic *preadipocytes* and *adipocytes* secrete a large amount of a chemokine Monocyte Chemoattractant Proteins (MCP)-1/CCL2 [20]. CCL2 causes the infiltration of monocytes *via* binding to their C-C motif Chemokine Receptor 2 (CCR2) into obese adipose tissue wherein they differentiate into macrophages and induce the process of inflammation by expressing pro-inflammatory cytokines and thereby lead to insulin resistance state.

'*IL1 and megakaryocytes in obesity',* and *'Interleukin-7 signaling'* further point towards the link between IR and pro-inflammatory signaling. One study has demonstrated enhanced glucose homeostasis and insulin sensitivity in IL-7 receptor (IL-7R) KO mice [21].

Pro-inflammatory signaling is mediated by IKK/NF-κB kinases. It is interesting to note that insulin signaling also activates these kinases through PI3K/Akt pathway. Therefore, it has been argued that inflammatory signaling actually constitutes a negative feedback loop in insulin signaling that may lead to IR phenotype particularly in conditions such as tissue inflammation [22].

Another pathway '*TGF-β signaling pathway'* is also central in regulating glucose and energy homeostasis. It inhibits proliferation of pancreatic β-Cell by controlling the expression of p27, a master regulator of cell cycle [23]. It also suppressesIRS-1 phosphorylation, [24] and insulin/insulin-like growth factor-1 mediated adipogenesis process. Experimental inhibition of TGF-β1 signaling has been reported to protect mice from obesity, Insulin resistance, and diabetes, conferring it an attractive target for therapeutic interventions [24].

3.2. microRNA Analysis

MicroRNAs are the molecular regulators that fine-tune the expression of protein-encoding genes as per the need of the organism. Many metabolic diseases including type 1 and type 2 diabetes, [25] cancer, and autoimmune disorders have been reported to be associated with changes in microRNA expression.

All the four members of miR-181 a, b, c, and d were present in top 10 miRNAs. miR-181c is reported to regulate Hippo signaling pathway which controls cell proliferation and apoptosis and have been implicated in a wide variety of cancer including pancreatic cancer [26]. Insulin, in addition to glucose and lipid metabolism shows mitogenic effects through MAPK/ERK pathway and increased insulin production to counteract diminished insulin signaling is a characteristic feature of IR phenotype and may be linked to miR-181c.

miR-181a has been reported to negatively correlate with high levels of IL6, macrophage infiltration, or metabolic conditions such as type 2 diabetes [27] and therefore, might be considered an important therapeutic target. It has been reported that insulin itself down-regulates expression of some miRNAs including miR-181d as its target genes were supposed to involve in insulin signaling [28]. Another miRNA, miR-181b has also been reported to ameliorate insulin-mediated glucose homeostasis by regulating endothelial function in adipose tissue [29].

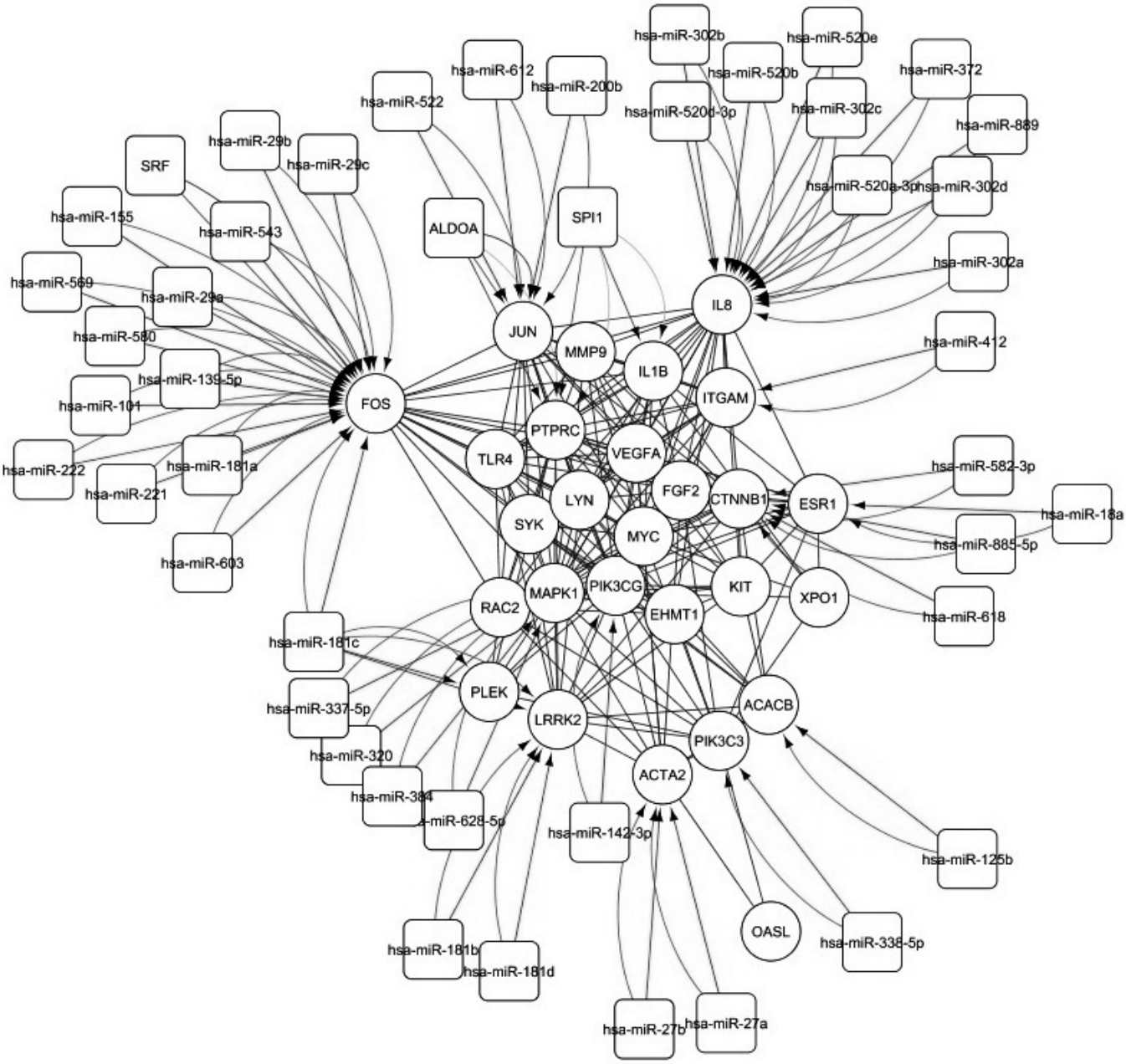

Fig. (1). Sub-network of hub proteins (*from meta-signature*) along with their regulatory microRNAs, and Transcription Factors.

MicroRNA, miR- 338-5p is also involved in adipose biology by regulating the expression of three proteins - Peroxisome proliferator-activated receptor- γ, Peroxisome proliferator-activated receptor- γ coactivator (PGC)-1α, and Myogenic factor5α, those are involved in the browning of white adipose tissue [30].

miR-302a has been reported to be a negative regulator of peroxisome proliferator-activated receptor γ -mediated adipogenesis; [31] miR-302b inhibits insulin-like growth factor signaling [32] and therefore, both have potential to diminish insulin signaling mediated glucose homeostasis.

3.3. Transcription Factor Analyses

Transcription Factors (TF) are the single largest protein family that regulates gene expression. The TF-analyses reported Spi-1/PU.1 with highest degree-centrality in our network. Expression of this TF was reported in mature adipocytes, it induces expression of NADPH oxidase, and pro-inflammatory cytokines (TNFα, IL-1β, and IL-6). NADPH oxidase activates Reactive Oxygen Species (ROS); ROS cause oxidative stress leading to Insulin resistance and Type 2 diabetes [33].

Another TF, SRF has been reported to associate with insulin resistance, and might contribute to T2D pathogenesis; a high level of SRF gene expression in human and mouse skeletal muscle is a signature of insulin resistance. Its pharmacological inhibition has been reported improved glucose uptake and tolerance in insulin-resistant mice *in vivo*. Thus, it might also be an attractive therapeutic target [34].

Table 3. Term-leading pathways from CluGO analysis.

S. No.	Pathways
1	B Cell Receptor Signaling Pathway
2	Chemokine signaling pathway
3	Degradation of the extracellular matrix
4	Estrogen Receptor Pathway
5	Estrogen signaling pathway
6	GPVI-mediated activation cascade
7	Generic Transcription Pathway
8	HTLV-I infection
9	Hemostasis
10	IL1 and megakaryotyces in obesity
11	IRS-mediated signalling
12	Interleukin-7 signaling
13	Leishmaniasis
14	Ligand-dependent caspase activation
15	MAPK Signaling Pathway
16	Neutrophil degranulation
17	Pertussis
18	Platelet activation
19	Proteoglycans in cancer
20	Spinal Cord Injury
21	Staphylococcus aureus infection
22	TGF-beta Signaling Pathway

The concept of adiposopathy is gaining incremental acceptance in scientific community due to the appreciation of endocrine and immune potential of adipose tissue. It is an insulin-responsive organ, at the same time it also secrets fat-derived metabolites, hormones, enzymes, cytokines and other factors that have an effect on insulin activity in other organs and may even affect insulin secretion by the pancreatic β-cells.

Our analysis reported enrichment of microRNAs - regulating lipid metabolism and insulin secretion processes, transcription factors - promoting inflammatory processes, and molecular pathways - attenuating insulin signaling, insulin secretion, adipocyte differentiation, and promoting macrophage/monocyte infiltration in adipose tissue leading to tissue inflammation, thus, endorsing the concept of adiposopathy as root mechanistic cause of IR.

Our analysis also reflects the effectiveness of the proposed bioinformatics approach in elucidating the pathophysiology of IR, in particular, and of any medical condition, in general. We have identified a meta-signature of genes across five microarray studies; these proteins enriched highly suggestive pathways. Extension of these proteins with their regulatory players further clued their probable role in overall pathology.

CONCLUSION

The present study reported effective bioinformatics workflows identified molecular processes responsible for the derangement of adipose functions and also identified 27 key genes based on their topological properties in the protein-protein interaction network that was derived from meta-signature. These hub proteins may have the potential to act as IR Biomarkers or drug targets for the development of therapeutics.

Our study reinforces the notion that adiposopathy lies in the root mechanistic pathology that poses risk for development of type 2 diabetes, cardiovascular complications and dyslipidemia; [35] appreciation of this fact will direct scientific efforts towards designing of therapeutics those not only reduce fat mass (adiposity) but will also correct fat dysfunctions. In addition, this adipocentric (fat tissue as a central cause) paradigm of metabolic disease will also underscore the significance of lifestyle modification to reduce excess body fat for curing metabolic disorders.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

No funding received. AS is indebted to Vice Chancellor, GLA University, Mathura.

SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's website along with the published article.

REFERENCES

[1] Guilherme, A.; Virbasius, J.V.; Puri, V.; Czech, M.P. Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. *Nat. Rev. Mol. Cell. Biol.,* **2008**, *9*(5), 367-377.

[2] Kahn, S.E.; Hull, R.L.; Utzschneider, K.M. Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature,* **2006**, *444*(7121), 840-846. Available from: https://www.nature.com/articles/nature05482

[3] Mathur, S.K.; Jain, P.; Mathur, P. Microarray evidences the role of pathologic adipose tissue in insulin resistance and their clinical implications. *J. Obes.,* **2011**, *2011*, 587495. Available from: https://www.hindawi.com/journals/jobe/2011/587495/

[4] Saxena, A.; Sachin, K.; Bhatia, A.K. System level meta-analysis of

microarray datasets for elucidation of diabetes mellitus pathobiology. C*urr. Genomics,* **2017**, *18*(3), 298-304.

[5] Hardy, O.T.; Perugini, R.A.; Nicoloro, S.M.; Gallagher-Dorval, K.; Puri, V.; Straubhaar, J.; Czech, M.P. Body mass index-independent inflammation in omental adipose tissue associated with insulin resistance in morbid obesity. *Surg. Obes. Relat. Dis.,* **2011**, *7*(1), 60-67.

[6] Soronen, J.; Laurila, P.P.; Naukkarinen, J.; Surakka, I.; Ripatti, S.; Jauhiainen, M.; Olkkonen, V.M.; Yki-Järvinen, H. Adipose tissue gene expression analysis reveals changes in inflammatory, mitochondrial respiratory and lipid metabolic pathways in obese insulin-resistant subjects. *BMC Med. Genomics,* **2012**, *5*, 9. Available from: https://bmcmedgenomics.biomedcentral.com/articles/10. 1186/1755-8794-5-9

[7] Sears, D.D.; Hsiao, G.; Hsiao, A.; Yu, J.G.; Courtney, C.H.; Ofrecio, J.M.; Chapman, J.; Subramaniam, S. Mechanisms of human insulin resistance and thiazolidinedione-mediated insulin sensitization. *Proc. Natl. Acad. Sci.,* **2009**, *106*(44), 18745-18750.

[8] Keller, P.; Gburcik, V.; Petrovic, N.; Gallagher, I.J.; Nedergaard, J.; Cannon, B.; Timmons, J.A. Gene-chip studies of adipogenesis-regulated microRNAs in mouse primary adipocytes and human obesity. *BMC Endocr. Disord.,* **2011**, *11*, 7. Available from: https://bmcendocrdisord.biomedcentral.com/articles/10.1186/1472-6823-11-7

[9] Wu, J.; Gentry, R. gcrma: Background adjustment using sequence information. R package version 2.44.0. 2016. Available from: https://bioconductor.org/packages/release/bioc/html/gcrma.html

[10] Gentleman, R.; Carey, V.; Huber, W.; Hahne, F. Genefilter: Methods for filtering genes from high-throughput experiments. R package version 1.54.2. 2016. Available from: https://bioconductor.statistik.tu-dortmund.de/packages/3.3/bioc/html/genefilter.html

[11] Xia, J.; Gill, E.E.; Hancock, R.E.W. NetworkAnalyst for statistical, visual and network-based meta-analysis of gene expression data. *Nat. Protoc.,* **2015**, *10*(6), 823-844.

[12] Shannon, P. Cytoscape: A software environment for integrated models of biomolecular interaction networks. *Genome Res.,* **2003**, *13*(11), 2498-2504.

[13] Bindea, G.; Mlecnik, B.; Hackl, H.; Charoentong, P.; Tosolini, M.; Kirilovsky, A.; Fridman, W.H.; Pagès, F.; Trajanoski, Z.; Galon, J. ClueGO: A Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. *Bioinformatics,* **2009**, *25*(8), 1091-1093.

[14] Szklarczyk, D.; Franceschini, A.; Wyder, S.; Forslund, K.; Heller, D.; Huerta-Cepas, J.; Simonovic, M.; Roth, A.; Santos, A.; Tsafou, K.P.; Kuhn, M.; Bork, P.; Jensen, L.J.; von Mering, C. STRING v10: Protein-protein interaction networks, integrated over the tree of life. *Nucl. Acids Res.,* **2014**, *43*(D1), D447-D452.

[15] Chin, C.-H.; Chen, S.-H.; Wu, H.-H.; Ho, C.-W.; Ko, M.-T.; Lin, C.-Y. cytoHubba: Identifying hub objects and sub-networks from complex interactome. *BMC Syst. Biol.,* **2014**, *8*(Suppl 4), S11. Available from: https://bmcsystbiol.biomedcentral.com/articles/10.1186/1752-0509-8-S4-S11

[16] Kutmon, M.; Kelder, T.; Mandaviya, P.; Evelo, C.T.A.; Coort, S.L. CyTargetLinker: A cytoscape app to integrate regulatory interactions in network analysis. *PLoS One,* **2013**, *8*(12), e82160. Available from: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0082160

[17] Doncheva, N.T.; Assenov, Y.; Domingues, F.S.; Albrecht, M. Topological analysis and interactive visualization of biological networks and protein structures. *Nat. Prot.,* **2012**, *7*(4), 670-685.

[18] Yu, H.; Kim, P.M.; Sprecher, E.; Trifonov, V.; Gerstein, M. The importance of bottlenecks in protein networks: correlation with gene essentiality and expression dynamics. *PLoS Computat. Biol.,* **2007**, *3*(4), e59. Available from: http://journals.plos.org/plos compbiol/article?id=10.1371/journal.pcbi.0030059

[19] Gough, N.R. Neutrophils suppress insulin signaling. *Sci. Signal.,* **2012**, *5*(243), ec250. Available from: http://stke.sciencemag.org/content/5/243/ec250

[20] Xu, L.; Kitade, H.; Ni, Y.; Ota, T. Roles of chemokines and chemokine receptors in obesity-associated insulin resistance and nonalcoholic fatty liver disease. *Biomolecules,* **2015**, *5*(3), 1563-1579.

[21] Lee, M.; Song, S.J.; Choi, M.S.; Yu, R.; Park, T. IL-7 receptor deletion ameliorates diet-induced obesity and insulin resistance in mice. *Diabetologia,* **2015**, *58*(10), 2361-2370.

[22] Stafeev, I.S.; Vorotnikov, A.V.; Ratner, E.I.; Menshikov, M.Y.; Parfyonova, Y.V. Latent inflammation and insulin resistance in adipose tissue. *Int. J. Endocrinol.,* **2017**, *2017*, 5076732. Available from: https://www.hindawi.com/journals/ije/2017/5076732/

[23] Suzuki, T.; Dai, P.; Hatakeyama, T.; Harada, Y.; Tanaka, H.; Yoshimura, N.; Takamatsu, T. TGF-β signaling regulates pancreatic β-cell proliferation through control of cell cycle regulator p27 expression. *Acta Histochem. Cytochem.,* **2013**, *46*(2), 51-58.

[24] Yadav, H.; Quijano, C.; Kamaraju, A.K.; Gavrilova, O.; Malek, R.; Chen, W.; Zerfas, P.; Zhigang, D.; Wright, E.C.; Stuelten, C.; Sun, P.; Lonning, S.; Skarulis, M.; Sumner, A.E.; Finkel, T.; Rane, S.G. Protection from obesity and diabetes by blockade of TGF-beta/Smad3 signaling. *Cell Metab.,* **2011**, *14*(1), 67-79.

[25] Chakraborty, C.; Doss, C.G.P.; Bandyopadhyay, S.; Agoramoorthy, G. Influence of miRNA in insulin signaling pathway and insulin resistance: micro-molecules with a major role in type-2 diabetes. *Wiley Interdiscip. Rev. RNA,* **2014**, *5*(5), 697-712.

[26] Chen, M.; Wang, M.; Xu, S.; Guo, X.; Jiang, J. Upregulation of miR-181c contributes to chemoresistance in pancreatic cancer by inactivating the Hippo signaling pathway. *Oncotarget,* **2015**, *6*(42), 44466-44479.

[27] Estep, M.; Armistead, D.; Hossain, N.; Elarainy, H.; Goodman, Z.; Baranova, A.; Chandhoke, V.; Younossi, Z.M. Differential expression of miRNAs in the visceral adipose tissue of patients with non-alcoholic fatty liver disease. *Ailment Pharmacol. Ther.,* **2010**, *32*(3), 487-497.

[28] Granjon, A.; Gustin, M.P.; Rieusset, J.; Lefai, E.; Meugnier, E.; Güller, I.; Cerutti, C.; Paultre, C.; Disse, E.; Rabasa-Lhoret, R.; Laville, M.; Vidal, H.; Rome, S. The microRNA signature in response to insulin reveals its implication in the transcriptional action of insulin in human skeletal muscle and the role of a sterol regulatory element-binding protein-1c/myocyte enhancer factor 2C pathway. *Diabetes,* **2009**, *58*(11), 2555-2564.

[29] Sun, X.; Lin, J.; Zhang, Y.; Kang, S.; Belkin, N.; Wara, A.K.; Icli, B.; Hamburg, N.M.; Li, D.; Feinberg, M.W. MicroRNA-181b improves glucose homeostasis and insulin sensitivity by regulating endothelial function in white adipose tissue novelty and significance. *Circul. Res.,* **2016**, *118*(5), 810-821.

[30] Arias, N.; Aguirre, L.; Fernández-Quintela, A.; González, M.; Lasa, A.; Miranda, J.; Macarulla, M.T.; Portillo, M.P. Erratum to: MicroRNAs involved in the browning process of adipocytes. *J. Physiol. Biochem.,* **2016**, *72*(3), 523-524.

[31] Jeong, B.-C.; Kang, I.-H.; Koh, J.-T. MicroRNA-302a inhibits adipogenesis by suppressing peroxisome proliferator-activated receptor γ expression. *FEBS Lett.,* **2014**, *588*(18), 3427-3434.

[32] Lee, C.-C.; Chen, P.-H.; Ho, K.-H.; Shih, C.-M.; Cheng, C.-H.; Lin, C.-W.; Cheng, K.T.; Liu, A.J.; Chen, K.C. The microRNA-302b-inhibited insulin-like growth factor-binding protein 2 signaling pathway induces glioma cell apoptosis by targeting nuclear factor IA. *PloS One,* **2017**, *12*(3), e0173890. Available from: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0173890

[33] Lin, L.; Pang, W.; Chen, K.; Wang, F.; Gengler, J.; Sun, Y.; Tong, Q. Adipocyte expression of PU.1 transcription factor causes insulin resistance through upregulation of inflammatory cytokine gene expression and ROS production. *Am. J. Physiol. Endocrinol. Metab.,* **2012**, *302*(12), E1550-E1559.

[34] Jin, W.; Goldfine, A.B.; Boes, T.; Henry, R.R.; Ciaraldi, T.P.; Kim, E.Y.; Emecan, M.; Fitzpatrick, C.; Sen, A.; Shah, A.; Mun, E.; Vokes, V.; Schroeder, J.; Tatro, E.; Jimenez-Chillaron, J.; Patti, M.E. Increased SRF transcriptional activity in human and mouse skeletal muscle is a signature of insulin resistance. *J. Clin. Invest.,* **2011**, *121*(3), 918-929.

[35] Bays, H.; Abate, N.; Chandalia, M. Adiposopathy: Sick fat causes high blood sugar, high blood pressure and dyslipidemia. *Fut. Cardiology,* **2005**, *1*(1), 39-59.