Identifying biological markers for insulin resistance using high-throughput sequencing data (RNA-seq) from dermal endothelial cells to establish a safe diagnostic test

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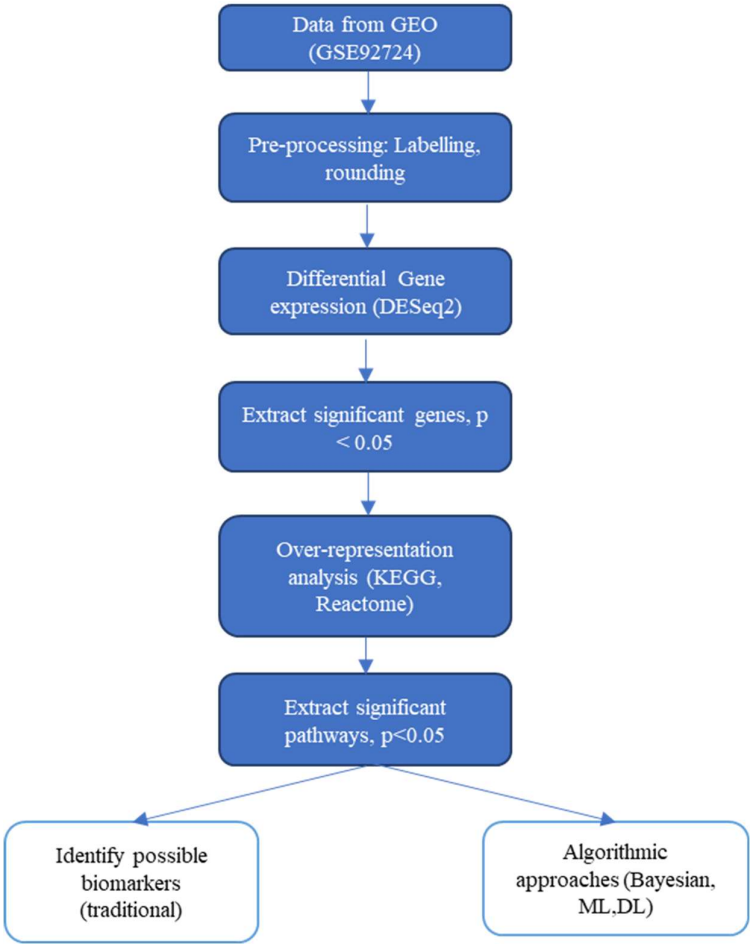
1. Introduction

With the emergence of RNA sequencing (RNA-seq) technologies, RNA-based biomolecules hold expanded promise for their diagnostic, prognostic and therapeutic applicability in various diseases, including cancers and infectious diseases. Detection of gene fusions and differential expression of known disease-causing transcripts by RNA-seq represent some of the most immediate opportunities. Here, I propose a novel diagnostic technique for insulin resistance (Type II diabetes) based on differential gene expression of RNA-seq data from dermal endothelial cells. Endothelial cells are easily extractable from human skin and modern procedures have made it a relatively painless process.

2. Experimental Setup



3. Results

3.1 Differential Gene Expression

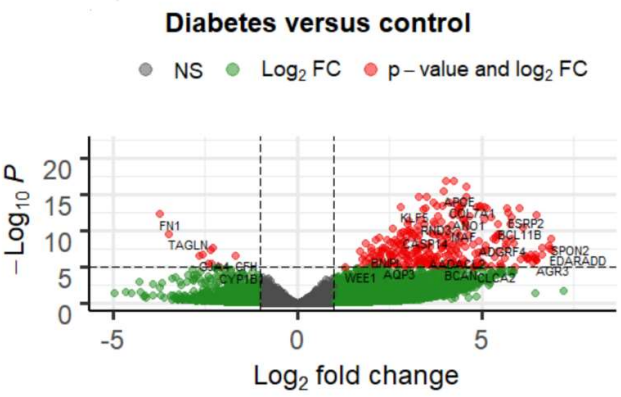


Fig 1. Volcano plot (Red genes are significanty expressed)

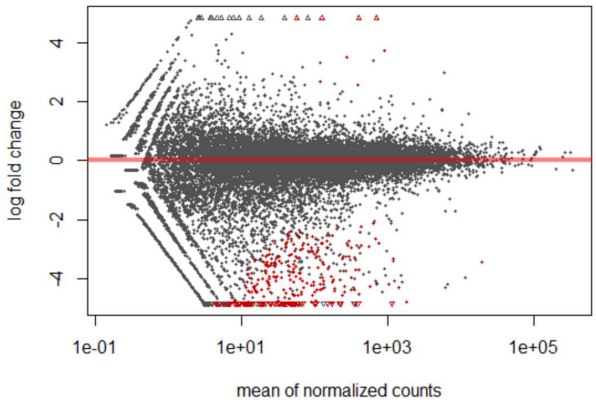


Fig 2. MA plot (Red genes are significantly expressed)

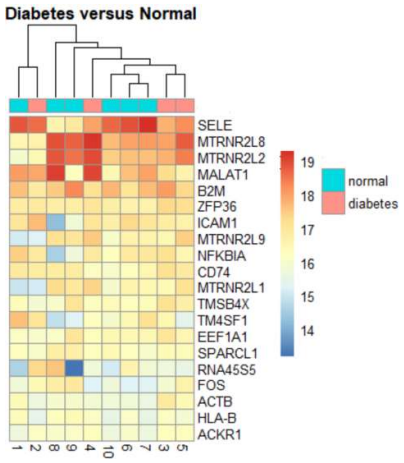


Fig 3. Heatmap of top 20 differentially expresed genes

3.2 Over-representation analysis

Table 1. Significant pathways from KEGG database (Top 5)

S.No	Database	Pathway	Genes
1	KEGG	Cytokine-cytokine receptor interaction	TNFRSF18;IL1RN;TNFRSF19;ACKR3;CCL27;CXCL14;IL17RE;IL20;IL20RA;IL20RB;IL22RA1;BMPR1A;CD4;IL18;CCL20;IL10RA;IL7;LIF;IL1B;TNF
2	KEGG	Signaling pathways regulating pluripotency of stem cells	DLX5;ISL1;OTX1;ID4;LIF;FGFR2;FGFR3;AXIN2;FZD10;WNT10B;FZD7;WNT16;WNT5B;WNT4;WNT3;BMPR1A
3	KEGG	Cell adhesion molecules (CAMs)	CDH3;CNTN1;NLGN4Y;NEO1;PVRL1;PTPRF;SDC4;CLDN4;CLDN8;SDC1;CD4;CD86;CLDN1;CDH1;VCAM1;ITGA4
4	KEGG	Transcriptional misregulation in cancer	HPGD;PAX3;BCL11B;UTY;SPINT1;ARNT2;MAF;CEBPA;JUP;SPI1;WNT16;CD86
5	KEGG	PI3K-Akt signaling pathway	MYB;IL7;LPAR3;NTF3;NTF4;PPP2R2C;LPAR5;THBS2;THBS4;ERBB4;LAMB4;COL4A6;LPAR1;ERBB3;ITGB6;FGF18;FN1;ITGA4;FGFR2;FGFR3;BCL2;PDGFC

Table 2. Significant pathways from REACTOME database (Top 5)

S.No	Database	Pathway	Genes
1	REACTOME	Formation of the cornified envelope	CSTA;DSC1;DSC3;PRSS8;SPINK5;PKP3;KLK8;KRT80;EVPL;CASP14;KRT1;KRT10;KRT15;KRT19;KRT31;ST14;PERP;KRT5;KRT14;DSG1;DSG3;PKP1;JUP
2	REACTOME	Metabolism of amino acids and derivatives	DCT;PHGDH;PSAT1;HAL;PRODH;TYR;TYRP1;BBOX1;CRYM;HAAO;CKB;CKMT1B;ASPA;CKMT1A;FOLH1;FOLH1;GATM;ASPG;DUOX1;MAT1A;SLC7A5
3	REACTOME	Signaling by Receptor Tyrosine Kinases	MST1R;NTF3;PDGFC;THBS4;SPINT1;SPINT2;PTPN3;NTF4;TNS4;ESRP2;FGFBP1;THBS2;JUP;NCF2;ESRP1;PTK6;COL27A1;APOE;VAV3;S100B;ERBB3;NRG1;NRG2;ERBB4;FGFR2;FGFR3;FGF18;FN1
4	REACTOME	Gap junction assembly	GJB6;GJB4;GJA4;GJB1;GJB3;GJB5;TUBB4A
5	REACTOME	Creatine metabolism	CKB;CKMT1B;GATM;CKMT1A

3.3 Cellular response to hypoxia versus insulin resistance

The differentially expressed genes obtained from 3.1 were analysed to see if any of the genes were involved in cellular response to hypoxia. After the analysis using the Gene Ontology (GO) database, the following genes were determined to be differentially expressed in diabetes and involved in tissue hypoxia. In total, 12 genes were obtained.

Table 3. Differentially expressed genes in insulin resistance involved in cellular response to hypoxia (First 5)

S.No	Gene	Description	Function of interest
1	ADAM8	Disintegrin and metalloproteinase domain-containing protein 8	cellular response to hypoxia
2	AQP3	Aquaporin-3	cellular response to oxygen-glucose deprivation
3	ARNT2	Aryl hydrocarbon receptor nuclear translocator 2	response to hypoxia
4	BCL2	Bcl2-associated agonist of cell death	cellular response to hypoxia
5	CRYAB	Alpha-crystallin B chain	response to hypoxia