

IN-SILICO ANALYSIS ON DIFFERENTIAL RNA EXPRESSION IN OBESITY INDUCED MELANOMA TUMOR IN *MUS MUSCULUS*

*Submitted in partial fulfillment of the requirements for the award
of Bachelor of Science Degree in*

Bioinformatics and Data science

By

SUMESH S (40738021)



DEPARTMENT OF BIOINFORMATICS AND DATA SCIENCE

SCHOOL OF BIO AND CHEMICAL ENGINEERING

**SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

**Accredited with Grade “A” by NAAC | 12B Status
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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **SUMESH S (40738021)** who carried out the project entitled ' **IN-SILICO ANALYSIS ON DIFFERENTIAL RNA EXPRESSION IN OBESITY INDUCED MELANOMA TUMOR IN *MUS MUSCULUS***' under my supervision from January 2023 to May 2023.

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DECLARATION

I, **SUMESH S** hereby declare that the project report entitled '**IN-SILICO ANALYSIS ON DIFFERENTIAL RNA EXPRESSION IN OBESITY INDUCED MELANOMA TUMOR IN *MUS MUSCULUS***' done by me under the guidance of **Dr. SWETHA SUNKAR, M.Sc., M. Tech., Ph.D.**, at **SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY** is submitted in partial fulfillment of the requirements for the award of Bachelor of Science Degree in **BIOINFORMATICS AND DATA SCIENCE**.

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Abstract

Obesity has been identified as a risk factor for several types of cancer, including melanoma. In this study, we performed an in-silico analysis to identify differentially expressed genes in obesity-induced melanoma tumors in *Mus musculus*. The microarray dataset GSE211392 was selected from gene expression omnibus (GEO) database and GEO2R tool was used and identified a total of 236 differentially expressed genes (DEGs), of which 74 and 162 genes were up-regulated and down-regulated respectively. Database for annotation, visualisation and integrated discovery (DAVID) tool was used for functional annotations. Cytoscape was used for the screening of clusters and identification of hub genes. Among them, SLC22A7 and CYP1A2 were identified to have highest degree nodes from up-regulated and down-regulated gene networks respectively and hence can be used as possible molecular target. Our results indicate that alterations in gene expression and metabolic pathways induced by obesity may play a role in the development and advancement of melanoma tumors. However, additional research is required to confirm these findings and explore possible therapeutic strategies for the management of obesity-induced melanoma. The study's outcomes provide important insights into the molecular mechanisms that underlie the link between obesity and melanoma and underscore the potential of computational analysis in identifying new targets for cancer treatment.

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CHAPTER - 1

1. INTRODUCTION

Melanoma is a form of skin cancer that arises from melanocytes, which produce melanin responsible for skin, hair, and eye coloration. This cancer can manifest anywhere in the body, including non-sun-exposed regions, though it often appears on sun-exposed body areas such as arms, legs, and face. If not detected and treated early, melanoma can spread to other organs like the lymph nodes, liver, lungs, and brain, and could be fatal. Risk factors that increase the chance of developing melanoma include having many unusual or regular moles, a history of sunburn, fair skin, weakened immune system, and family history of melanoma. Treatment options for melanoma include surgical excision of the tumor along with chemotherapy, radiation therapy, or immunotherapy, depending on the stage and severity of the cancer. It is a deadly form of skin cancer that affects a significant proportion of the population worldwide. According to the International Agency for Research on Cancer, an estimated 287,723 deaths occurred worldwide due to melanoma in 2018. The incidence of melanoma has been steadily increasing over the past few decades, and it is now one of the most common forms of cancer among young adults (Ferlay et al., 2019).

Obesity refers to a medical condition in which an individual accumulates excess body fat, which can have adverse effects on their health. Typically, a BMI of 30 or above is used to diagnose obesity, which considers an individual's height and weight. Obesity is associated with numerous health problems, including type 2 diabetes, heart disease, high blood pressure, stroke, certain cancers, and sleep apnea. It can also negatively impact one's mental health and quality of life. The causes of obesity are intricate and multifactorial, with a combination of genetic, environmental, and behavioral factors contributing to its development. These factors can involve genetics,

diet, levels of physical activity, stress, sleeping patterns, and socio-economic status. Treating obesity often involves adopting healthy lifestyle habits, including increasing physical activity levels and improving dietary habits. In some cases, medication or surgery may be necessary. Preventing obesity is also important, and can be achieved by promoting healthy eating and physical activity habits, as well as creating environments that facilitate healthy behaviors.

Obesity has been identified as a risk factor for several types of cancer, including melanoma. Several studies have reported an increased risk of melanoma in obese individuals, and this association has been observed in both men and women. One study reported that obese individuals had a 30% increased risk of developing melanoma compared to individuals with a normal body mass index (BMI). The exact mechanism by which obesity contributes to the development and progression of melanoma remains poorly understood. However, several hypotheses have been proposed, including alterations in hormonal levels, immune dysfunction, and changes in the tumor microenvironment. (Flegal KM et al., 2016). There are evidences that indicate there is a link between obesity and melanoma that can impact its development and progression. Some studies have revealed that obesity can raise the risk of developing melanoma, while others have shown a higher incidence of melanoma recurrence and mortality in obese individuals.

The precise mechanisms underlying the relationship between obesity and melanoma are not entirely understood. However, it is believed that the chronic inflammation and hormonal imbalances associated with obesity may play a role in the development and advancement of melanoma. Moreover, excess adipose tissue, which is characteristic of obesity, can generate specific hormones and cytokines that can influence the growth and survival of melanoma cells.

In recent years, there has been growing interest in understanding the molecular mechanisms underlying the association between obesity and cancer. Advances in high-throughput technologies, such as gene expression profiling, have made it

possible to identify changes in gene expression that are associated with obesity and cancer. Gene expression profiling can provide insights into the molecular mechanisms underlying the association between obesity and cancer, which could help identify potential therapeutic targets.

Researchers utilize genetic modification or UV radiation exposure to induce melanoma in mice. Mouse models of melanoma are frequently utilized to investigate the disease's biology and to test various treatments. Extensive research on melanoma in *Mus musculus* has yielded crucial findings in the field of cancer research. These include identifying genetic mutations that drive melanoma progression and creating immunotherapy treatments for advanced melanoma. Overall, *Mus musculus* is an invaluable model organism for examining melanoma and other cancer types. Additionally, it is useful for developing and testing new cancer treatments.

The identification of differentially expressed genes in obesity-induced melanoma tumors could provide insights into the molecular mechanisms underlying the association between obesity and melanoma. Several studies have reported changes in gene expression associated with obesity and cancer. For example, a study reported alterations in inflammation, epigenetics, and mammary tumor growth in obese mice (Danko et al., 2016). Another study identified changes in gene expression in melanoma cell lines treated with the histone deacetylase inhibitor vorinostat (Chen et al., 2008).

There are multiple hypotheses that attempt to explain the link between obesity and melanoma. One of these hypotheses suggests that changes in hormonal levels, including insulin and insulin-like growth factor-1 (IGF-1), in obese individuals may contribute to the growth and advancement of melanoma (Danko et al., 2016). Another hypothesis proposes that obesity-related immune dysfunction could impair the immune response to melanoma cells, thereby promoting tumor growth (Wu et al., 2012). Furthermore, alterations in the tumor microenvironment, such as changes in

adipokine and cytokine signaling, may also play a role in the development and progression of melanoma in obese individuals (Sun et al., 2020).

Although the connection between obesity and melanoma has been increasingly established, the specific molecular mechanisms behind this relationship are not yet well comprehended. In order to gain a better understanding of these mechanisms, researchers can employ in-silico analysis techniques on RNA expression data from melanoma tumors induced by obesity in mice. This methodology employs computational methods to evaluate vast amounts of data, such as RNA sequencing information, to identify gene and pathway expression changes. This approach could aid in the identification of potential biomarkers and therapeutic targets for obesity-induced melanoma.

Our study provides evidence of differential gene expression in obesity-induced melanoma tumors in *Mus musculus*. The identification of novel genes and biological pathways associated with obesity-induced melanoma provides a basis for further studies aimed at understanding the molecular mechanisms underlying this association. Our findings could have important implications for the development of targeted therapies for obesity-induced melanoma.

CHAPTER - 2

2. LITERATURE SURVEY

Melanoma is a skin cancer that results from uncontrolled proliferation of melanocytes in the skin (Tsao et al., 2016). It is a highly aggressive form of skin cancer with a high mortality rate, and its incidence has been on the rise over the past few decades, particularly among young adults (Ferlay et al., 2019). Risk factors for melanoma include UV radiation exposure, family history of melanoma, fair skin, and immunosuppression (Gershenwald and Scolyer, 2018). Obesity has been identified as a risk factor for several types of cancer, including melanoma (Rastrelli et al., 2017). Numerous studies have linked obesity to an increased risk of melanoma, with this association observed in both men and women (Gamba et al., 2015; Karimi et al., 2016). For instance, one study found that obese individuals had a 30% higher risk of developing melanoma compared to those with a normal BMI (Karimi et al., 2016).

Although the exact mechanism by which obesity contributes to the development and progression of melanoma is not yet fully understood, various hypotheses have been suggested, such as hormonal changes, immune dysfunction, and alterations in the tumor microenvironment (Smith, 2021). To better understand the molecular mechanisms underlying the link between obesity and cancer, recent research has focused on identifying changes in gene expression associated with these conditions using high-throughput technologies like gene expression profiling (Kang et al., 2019). Studies investigating gene expression patterns in melanoma have shed light on the molecular mechanisms involved in its development and progression. For instance, (Van Belle et al., 2020) identified a set of genes that were expressed differently in primary melanomas than in normal skin, while (Chen et al., 2019) identified a set of genes with differential expression between melanoma cells and normal skin cells. These findings have provided valuable insights into the underlying molecular mechanisms involved in melanoma.

To comprehend the molecular mechanisms involved in the connection between obesity and melanoma, various studies have used transcriptomic approaches to identify differentially expressed genes (DEGs) linked to obesity-induced melanoma. Transcriptomics involves studying the entire set of RNA transcripts produced by a tissue or cell, providing a snapshot of gene expression levels in a given biological sample. DEGs related to cell proliferation, inflammation, and metabolism have been discovered through transcriptomic studies (Luo et al., 2021; Yang et al., 2019).

RNA sequencing (RNA-seq) is another high-throughput sequencing technology that allows for a more thorough and unbiased analysis of gene expression levels between various biological samples, providing an understanding of the underlying molecular mechanisms of the disease. Several studies have used RNA-seq to identify DEGs linked to obesity-induced melanoma in human and animal models. For example, one study used RNA-seq to examine the gene expression of subcutaneous adipose tissue and melanoma tumors in obese and lean mice and identified several DEGs involved in metabolic pathways and inflammation (Li et al., 2020).

Other high-throughput technologies, such as microarray and proteomics, have also been used to identify DEGs associated with obesity-induced melanoma. A mouse model study on obesity-induced melanoma conducted by (Kim et al., 2015) using microarray technology discovered several DEGs associated with cell adhesion, angiogenesis, and immune response. Similarly, a proteomics study conducted by (Ortega-Martinez et al., 2017) on obese mice with melanoma identified several differentially expressed proteins involved in cell proliferation and angiogenesis.

In order to gain a better understanding of the molecular mechanisms underlying the association between obesity and melanoma, several studies have used transcriptomic profiling to identify changes in gene expression associated with obesity and melanoma. Transcriptomic profiling is a powerful tool that can provide insights into the molecular mechanisms underlying disease pathogenesis and identify

potential therapeutic targets. A study used transcriptomic profiling to identify differentially expressed genes associated with obesity and melanoma in human melanoma samples (Liu et al., 2020). The study found that obesity was associated with changes in the expression of genes involved in metabolism, inflammation, and cell adhesion, which could contribute to the development and progression of melanoma.

In addition to transcriptomic profiling, several studies have used other omics technologies, such as proteomics and metabolomics, to investigate the molecular mechanisms underlying the association between obesity and cancer. For example, one study used proteomic profiling to identify differentially expressed proteins associated with obesity and melanoma in human melanoma samples (Keller et al., 2019). The study found that obesity was associated with changes in the expression of proteins involved in cell proliferation, metabolism, and the immune response, which could contribute to the development and progression of melanoma. Overall, transcriptomic profiling and other omics technologies have provided valuable insights into the molecular mechanisms underlying the association between obesity and melanoma. However, further studies are needed to validate these findings and identify potential therapeutic targets for the treatment of melanoma in obese individuals.

In conclusion, the association between obesity and melanoma is well-established, and there is growing interest in understanding the molecular mechanisms underlying this association. Gene expression profiling and in-silico methods are powerful tools for identifying differentially expressed genes and potential therapeutic targets in melanoma tumors in obese individuals. These approaches have the potential to improve our understanding of the underlying mechanisms of obesity-induced melanoma and facilitate the development of novel therapies for this disease.

CHAPTER - 3

3. AIM AND SCOPE

3.1 AIM

The objective of this research project is to perform an in-silico analysis of differential RNA expression in obesity induced melanoma tumor in *Mus musculus* using various bioinformatics tools and databases. The project aims to identify differentially expressed genes, map them to proteins, construct a protein-protein interaction network, identify hub genes, and perform functional enrichment analysis to gain insights into the biological pathways involved in the pathogenesis of melanoma in obesity.

3.2 SCOPE

Melanoma is one of the most aggressive types of skin cancer that can arise from pigment producing cells known as melanocytes. Obesity has been identified as a risk factor for the development of melanoma, however, the molecular mechanisms underlying the link between obesity and melanoma are not yet fully understood. In recent years, the advancement of high-throughput transcriptomic technologies such as RNA sequencing (RNA-Seq) and microarray has revolutionized the field of cancer research by providing insights into the molecular changes associated with different cancer types. In-silico analysis of the transcriptomic data has become an essential tool for identifying the differentially expressed genes, their functional significance, and their potential as therapeutic targets.

The project will involve the analysis of publicly available gene expression data from the Gene Expression Omnibus (GEO) database. We will use bioinformatics tools such as the String database, the DAVID tool, and Cytoscape to analyze the

differentially expressed genes and identify the key pathways and biological processes involved in the development of obesity-induced melanoma.

The outcome of this study will provide a comprehensive understanding of the molecular changes associated with obesity induced melanoma tumor in *Mus musculus*. The identification of the key hub genes and their signaling pathways will contribute to the development of new therapeutic strategies for the treatment of obesity induced melanoma. The possible clinical significance of the identified hub genes as potential biomarkers for the early detection, diagnosis and treatment of obesity induced melanoma in humans will also be explored. Overall, this project will contribute to the advancement of knowledge in the field of cancer research and will have potential clinical implications for the prevention and treatment of melanoma.

CHAPTER - 4

4. MATERIALS AND METHODS

4.1 ONLINE DATABASES AND TOOLS REQUIREMENT

1. GEO (Gene Expression Omnibus)
2. String Database (Search Tool for the Retrieval of Interacting Genes/Proteins)
3. DAVID Tool (Database for Annotation, Visualization, and Integrated Discovery)
4. Cytoscape (Software)
 - i. MCODE plugin
 - ii. cytoHubba plugin

4.2 PURPOSE OF THESE DATABASES AND TOOLS

1. **Gene Expression Omnibus (GEO):** GEO is a public repository of microarray and next-generation sequencing data. It contains a large collection of gene expression datasets and provides access to these datasets to the scientific community. In this project, GEO was used to obtain the microarray dataset of *Mus musculus* samples.

2. **String Database:** The String database is a comprehensive resource for protein-protein interactions. It contains information on physical and functional protein interactions, as well as information on gene co-expression and pathway analysis. In this project, the String database was used to map differentially expressed genes to proteins and to construct protein-protein interaction networks.

3. **DAVID Tool:** The DAVID tool (Database for Annotation, Visualization, and Integrated Discovery) is a bioinformatics resource that provides functional annotation and enrichment analysis of genes and proteins. In this project, DAVID was used to perform functional enrichment analysis of the differentially expressed genes and to identify enriched Gene Ontology (GO) terms, biological pathways, and protein domains.

4. **Cytoscape:** Cytoscape is an open-source software platform for visualizing and analyzing molecular interaction networks. In this project, Cytoscape was used to visualize protein-protein interaction networks, to perform network analysis, and to identify hub genes using the cytoHubba plugin.

5. **cytoHubba:** cytoHubba is a plugin for Cytoscape that identifies hub genes in molecular interaction networks. It provides various algorithms, such as Degree, MNC, MCC, and DMNC, to identify hub genes. In this project, cytoHubba was used to identify hub genes in the protein-protein interaction networks.

6. **MCODE:** MCODE is a plugin for Cytoscape that identifies highly interconnected regions in molecular interaction networks. It provides a clustering algorithm to identify densely connected regions in the network. In this project, MCODE was used to identify clusters of highly interconnected genes in the protein-protein interaction networks.

4.3 METHODOLOGY

4.3.1 Selection of GEO datasets

The dataset analysed in this study was freely available and retrieved from the Gene Expression Omnibus (GEO) database (<https://www.ncbi.nlm.nih.gov/geo/>). The gene expression profile GSE211392 was selected.

4.3.2 Processing of retrieved data for differentially expressed genes

The prediction of the Differentially Expressed Genes present in the selected dataset was analysed by GEO2R tool (<https://www.ncbi.nlm.nih.gov/geo/geo2r/>). The Significant genes were filtered based on the cutoff criteria of adjusted P-Value less than 0.05. From the significant genes, the up-regulated and down-regulated genes were predicted based on Log FC values such as LogFC >0.1 and LogFC < -0.1, respectively.

4.3.3 Enrichment and functional analysis of DEGs

The DEGs were further analysed for their functional and enrichment analysis using database for annotation, visualisation and integrated discovery (DAVID) tool. The DAVID tool shows gene ontology (GO) that represents enriched genes and pathway related information of genes from different databases such as KEGG, OMIM etc.,. The molecular function (MF), biological process (BP), cellular component (CC) of DEGs were categorised in GO terms (Neeharika et al., 2021). The pathway information analysis shows the involvement of DEGs in different functions dealing with obesity induced melanoma tumor related pathways, diseases, drugs and chemical substances. The cut-off criteria were used for P value as $P < 0.05$ and gene count > 2.

4.3.4 Construction of PPI network

The Search Tool for the Retrieval of Interacting Genes (STRING) database (<http://stringdb.org/>) is an online tool that facilitates the generation of protein-protein network information that includes the indirect (functional) and direct (physical) associations (Szkłarczyk et al., 2015). The networks were mapped for the identified DEGs (up-regulated and downregulated genes separately) based on the threshold of combined score > 0.4.

4.3.5 Screening of PPI networks for modules and analysis of hub genes

Cytoscape (version 3.9.1) (<http://www.cytoscape.org/>), is an open-source software platform for visualizing and analyzing complex networks, such as biological pathways, gene regulatory networks, and protein interaction networks. The mapped networks were exported to cytoscape and visualised for the further analysis. The molecular complex detection (MCODE) plug-in was used to determine the highly interconnected regions as modules from PPI network (degree cut-off). cytoHubba, a plug-in Cytoscape was used to predict the hub genes in PPI network based on the parameter degree.

CHAPTER - 5

5. RESULTS AND DISCUSSION

5.1 DEG analysis

The genes expressing differentially in the dataset related to obesity induced melanoma tumor (GSE211392) was analysed using GEO2R tool. The criteria to select the significant genes were the probability value P which should be less than 0.05 and Log fold change values. The cut off criteria for up-regulated and down-regulated genes was Log FC > 0.1 and Log FC < -0.1, respectively. The GEO2R analysis for GSE211392 showed 250 genes of which all 250 genes were found to be significant based on the above mentioned criteria. In this, 236 genes were differentially expressed of which 74 were up-regulated and 162 genes were down-regulated. Top 30 genes which are up-regulated and downregulated along with logFC value and P-Value are provided in Table 5.1 and 5.2 respectively.

Table 5.1 Top 30 up-regulated genes

Gene symbol	adj.P.Val	P.Value	t	B	logFC
SERPINA4-PS1	1.14E-04	3.50E-07	5.38	6.35	1.9275
SLC22A7	1.23E-07	7.45E-11	7.13	14.26	1.0717
GM15401	1.39E-06	1.51E-09	6.53	11.44	1.0385
SERPINA12	5.95E-05	1.50E-07	5.57	7.14	1.0339
ELOVL3	5.20E-04	2.58E-06	4.93	4.49	0.9978
CSAD	4.07E-07	3.45E-10	6.83	12.82	0.8676
GNA14	2.87E-05	6.35E-08	5.76	7.94	0.8516

Up-regulated

GM2788	9.53E-04	5.37E-06	4.76	3.81	0.7219
SLC16A6	2.57E-07	1.92E-10	6.94	13.37	0.6685
PROK1	2.63E-06	3.21E-09	6.38	10.73	0.661
PEG3	9.09E-05	2.61E-07	5.45	6.62	0.6418
CES3A	1.22E-06	1.24E-09	6.57	11.62	0.628
TSC22D1	3.63E-05	8.34E-08	5.7	7.69	0.6277
INHBA	4.44E-07	3.87E-10	6.8	12.71	0.5873
PKLR	5.76E-09	1.81E-12	7.83	17.74	0.5737
SLC13A3	2.27E-04	8.63E-07	5.18	5.51	0.5737
BMYC	9.91E-04	5.78E-06	4.74	3.75	0.5007
TRIM7	9.85E-04	5.69E-06	4.74	3.76	0.4896
CYP2D40	8.94E-05	2.49E-07	5.46	6.67	0.4735
PAQR9	1.03E-04	3.09E-07	5.41	6.47	0.4723
A930018M24RIK	8.37E-04	4.56E-06	4.8	3.97	0.4574
INHBC	8.93E-06	1.45E-08	6.07	9.32	0.4534
TRP53INP2	5.82E-04	2.94E-06	4.9	4.37	0.4523
IGF2BP2	2.87E-05	6.38E-08	5.76	7.94	0.4411
SLC17A4	5.20E-06	7.55E-09	6.2	9.93	0.4257
ABCA2	9.37E-04	5.23E-06	4.76	3.84	0.4219
NRP1	4.71E-08	2.28E-11	7.35	15.37	0.4199
GPD1	3.01E-08	1.17E-11	7.48	15.99	0.4198
AOX3	8.33E-05	2.26E-07	5.48	6.76	0.408
MBL1	1.32E-04	4.15E-07	5.35	6.19	0.4027

Table 5.2 Top 30 down-regulated genes

Down-regulated

Gene symbol	adj.P.Val	P.Value	t	B	logFC
GM3776	2.28E-10	4.96E-14	-8.5	21.11	-1.2057
ACMSD	1.53E-14	7.42E-19	-10.5	31.48	-1.1762
RANBP3L	3.41E-04	1.48E-06	-5.06	5.01	-0.9659
VLDLR	4.74E-04	2.20E-06	-4.97	4.64	-0.8967
NNMT	5.01E-04	2.43E-06	-4.94	4.55	-0.8729
GSTT3	7.89E-10	1.91E-13	-8.25	19.85	-0.7608
1810053B23RIK	2.01E-04	6.95E-07	-5.23	5.71	-0.7569
RCAN2	5.01E-04	2.45E-06	-4.94	4.54	-0.7563
GSTA1	2.24E-04	8.45E-07	-5.19	5.53	-0.7034
CYP2A5	5.41E-04	2.69E-06	-4.92	4.45	-0.7016
GSTT2	3.01E-08	1.24E-11	-7.47	15.94	-0.6957
GLS2	3.63E-19	8.78E-24	-12.53	41.97	-0.637
RDH16	1.15E-03	6.87E-06	-4.7	3.59	-0.631
S100G	7.20E-04	3.78E-06	-4.84	4.14	-0.6209
ADORA1	1.36E-06	1.42E-09	-6.54	11.5	-0.5663
SLC16A5	4.59E-05	1.11E-07	-5.64	7.42	-0.566
SLC7A2	1.54E-07	1.04E-10	-7.06	13.94	-0.5561
GLT1D1	5.80E-08	3.09E-11	-7.3	15.08	-0.5472
GM10639	4.97E-04	2.38E-06	-4.95	4.57	-0.542
UPP2	1.73E-04	5.77E-07	-5.27	5.88	-0.5389
ABCC4	7.36E-04	3.94E-06	-4.83	4.1	-0.5376
AGXT2L1	7.28E-04	3.84E-06	-4.84	4.12	-0.5308
TRIB3	6.76E-04	3.52E-06	-4.86	4.21	-0.5153
OAT	1.54E-07	1.03E-10	-7.06	13.96	-0.5122

GM9992	9.09E-05	2.60E-07	-5.45	6.63	-0.5085
DQX1	8.99E-07	8.48E-10	-6.65	11.98	-0.4998
COX7A1	5.21E-06	7.69E-09	-6.2	9.91	-0.4986
ROPN1L	5.20E-04	2.58E-06	-4.93	4.49	-0.4829
UGDH	3.65E-04	1.64E-06	-5.03	4.91	-0.4648
ALDH1B1	3.23E-07	2.58E-10	-6.88	13.09	-0.4502

5.2 Functional enrichment of DEGs

Gene set enrichment analysis is one of the key aspects of gene expression data analysis. Identifying the association of DEGs with a specific biological process or molecular function is the primary objective of gene enrichment analysis and is mainly based on functional annotation of DEGs. The gene ontology (GO) terms that comprises the standardised annotation of genes is most commonly used for enrichment analysis. The GO terms were generally divided under three categories namely biological process (BP), cellular component (CC), and molecular function (MF). The functional enrichment is obtained by using the software DAVID to evaluate the GO categories and KEGG pathways for both up-regulated and down-regulated genes. Analysis was done for up-regulated and down-regulated genes separately.

The functional enrichment is obtained by using the software DAVID to evaluate the GO categories, KEGG and Reactome pathways for both up-regulated and down-regulated genes. Analysis was done for up-regulated and down-regulated genes separately. The up-regulated genes were found to be majorly enriched in Biological Process (BP) especially the transmembrane transport, gluconeogenesis, positive regulation of peptidyl-tyrosine phosphorylation, negative regulation of peptidase activity, sodium ion transport, anion transport. The genes enriched in Cellular Components (CC) include extracellular region, integral component of plasma membrane, apical plasma membrane, basolateral plasma membrane, lysosome. This was followed by the presence of these up-regulated genes carrying Molecular

Functions (MF) such as identical protein binding, transmembrane transporter activity, protein homodimerization activity, symporter activity, growth factor activity. The Reactome pathways highlighted that the DEGs are engaged in Transport of small molecules, Adaptive Immune System, SLC-mediated transmembrane transport, Transport of bile salts and organic acids, metal ions and amine compounds Extracellular matrix organization.

Likewise, analysis for down-regulated genes is done based on functionality in three Gene Ontology (GO) terms and are found to be enriched in BP, CC and MF. And these genes were mainly involved in Biological Process (BP) like lipid metabolic process, metabolic process, fatty acid metabolic process, tricarboxylic acid cycle, urea cycle. These genes are enriched in Cellular Components (CC) such as cytoplasm, cytosol, mitochondrion, intracellular membrane-bounded organelle, endoplasmic reticulum. These genes were also engaged in Molecular Functions (MF) such as identical protein binding, oxidoreductase activity, transferase activity, catalytic activity, macromolecular complex binding. The KEGG pathways disclosed that the DEGs are engaged in metabolic pathways like Metabolic pathways, Biosynthesis of amino acids, Valine, leucine and isoleucine degradation, Carbon metabolism, Thermogenesis.

Figure 5.1 Enrichment analysis of up-regulated genes: Biological process

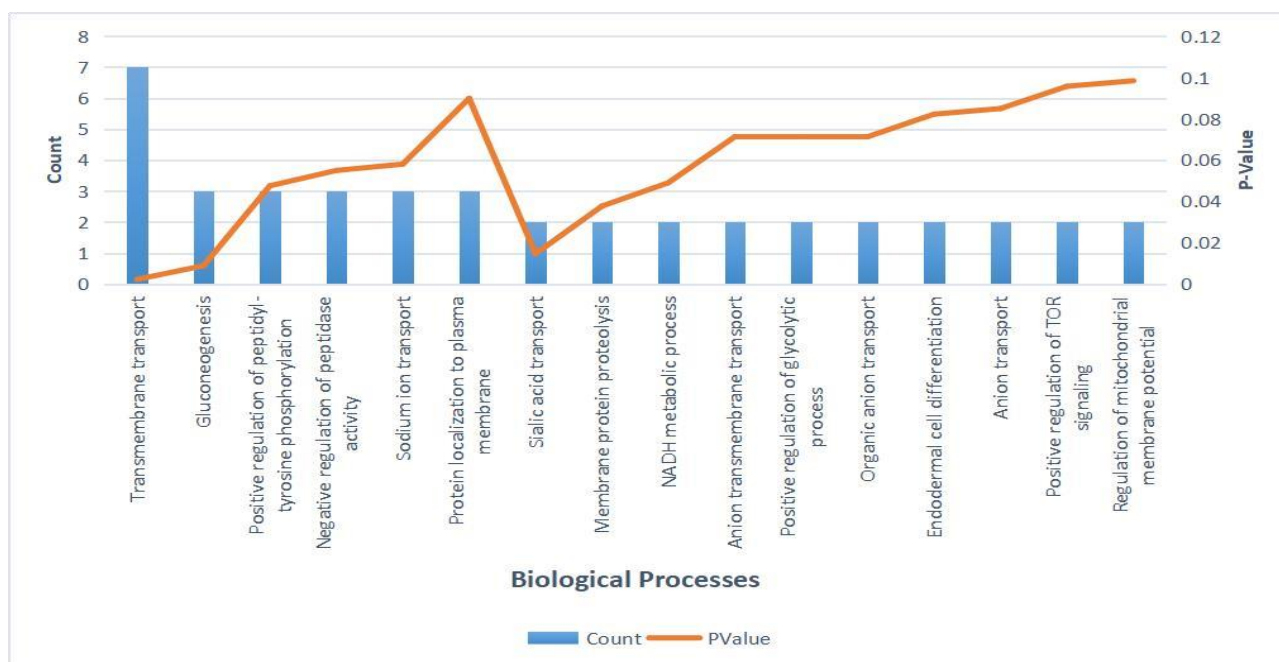


Figure 5.2 Enrichment analysis of up-regulated genes: Cellular component

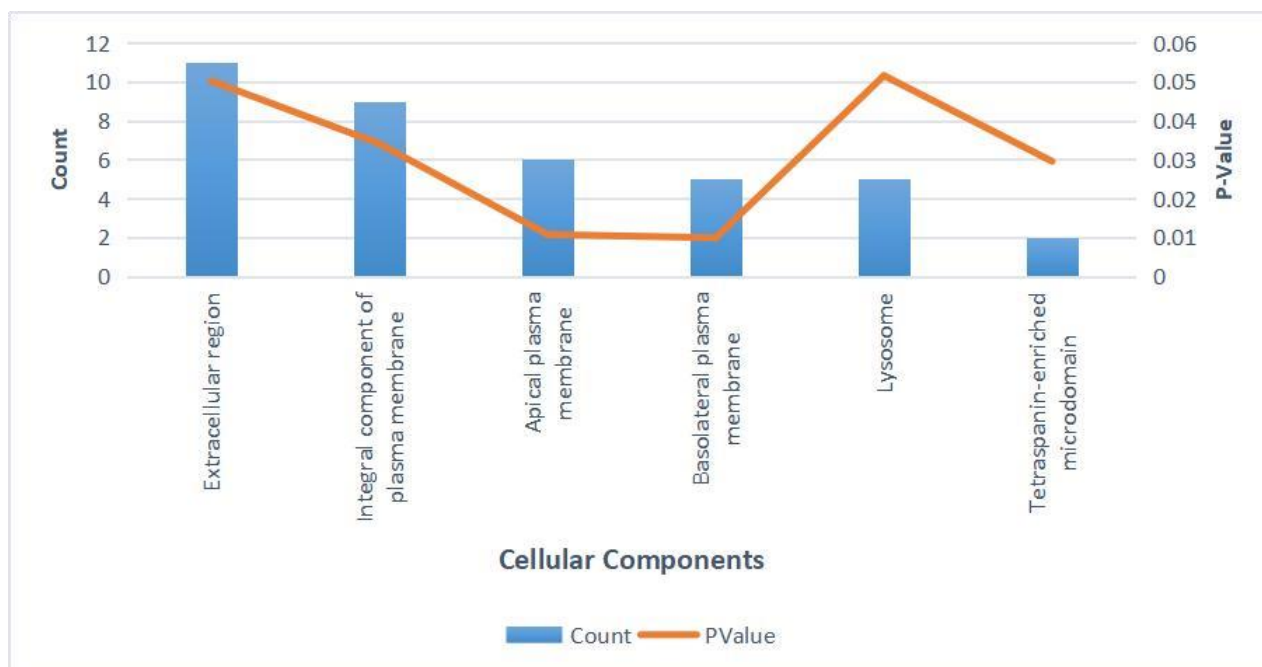


Figure 5.3 Enrichment analysis of up-regulated genes: Molecular function

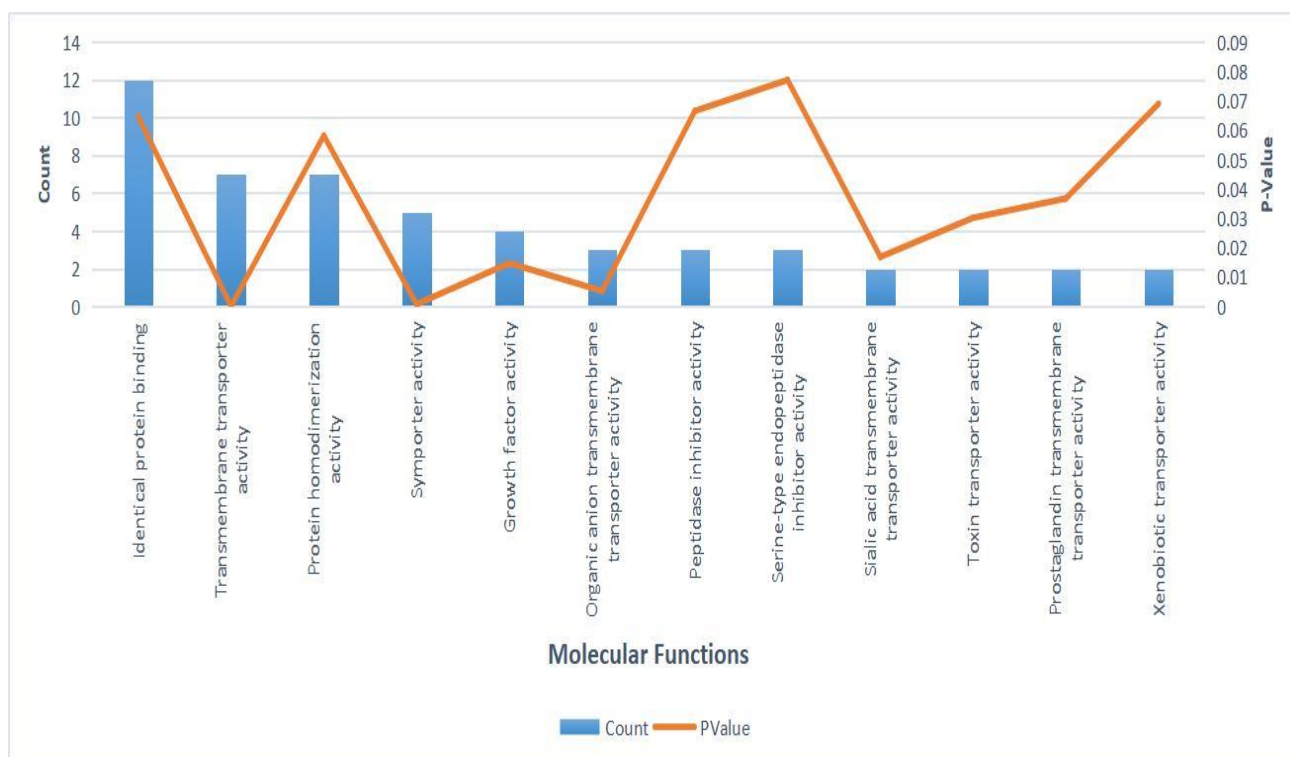


Figure 5.4 Enrichment analysis of up-regulated genes: Reactome pathway

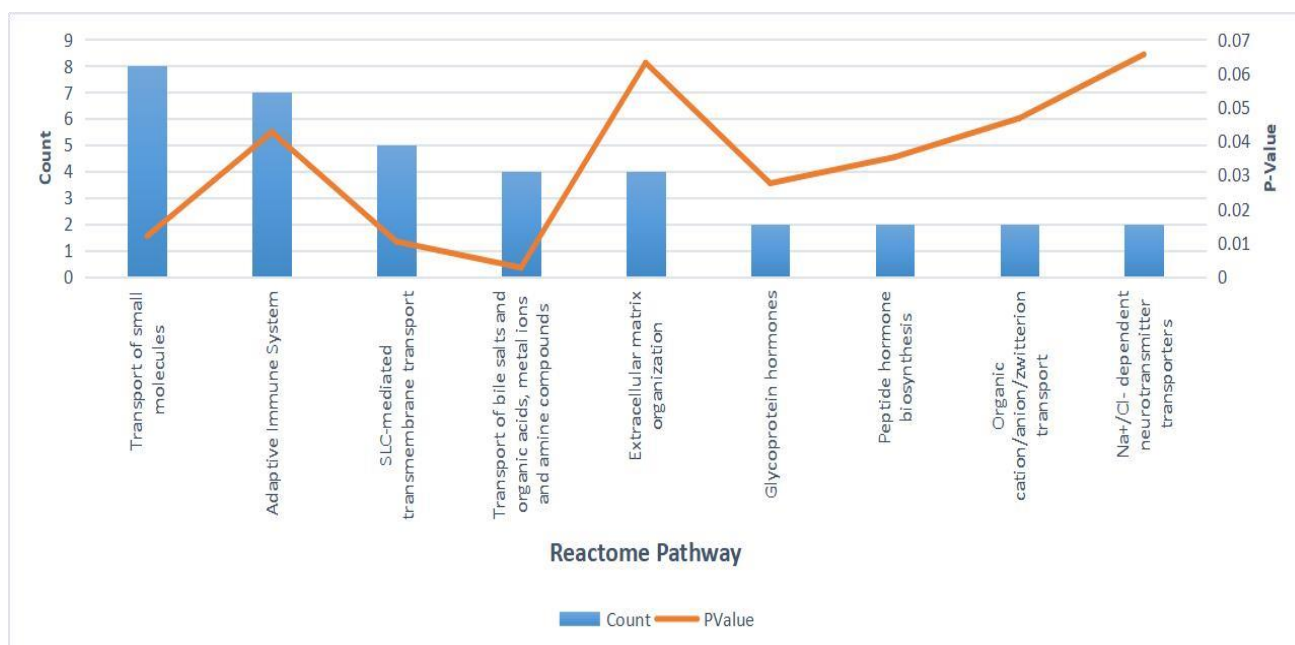


Figure 5.5 Enrichment analysis of down-regulated genes: Biological process

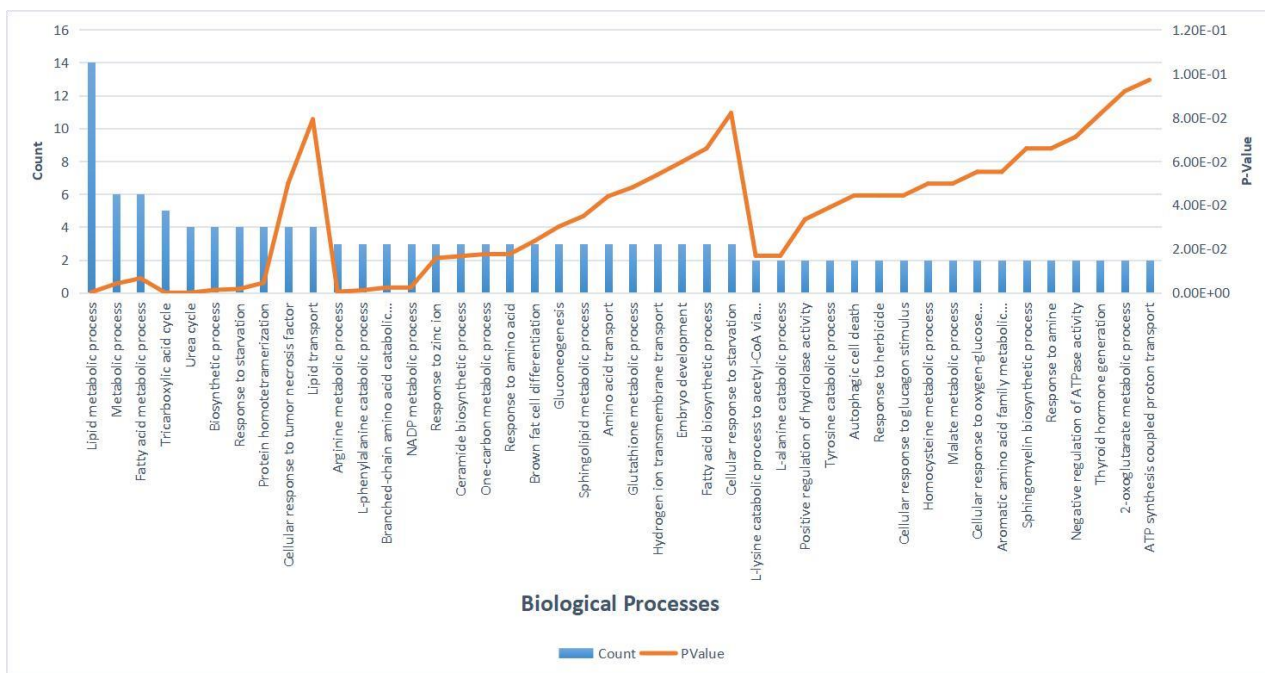


Figure 5.6 Enrichment analysis of down-regulated genes: Cellular component

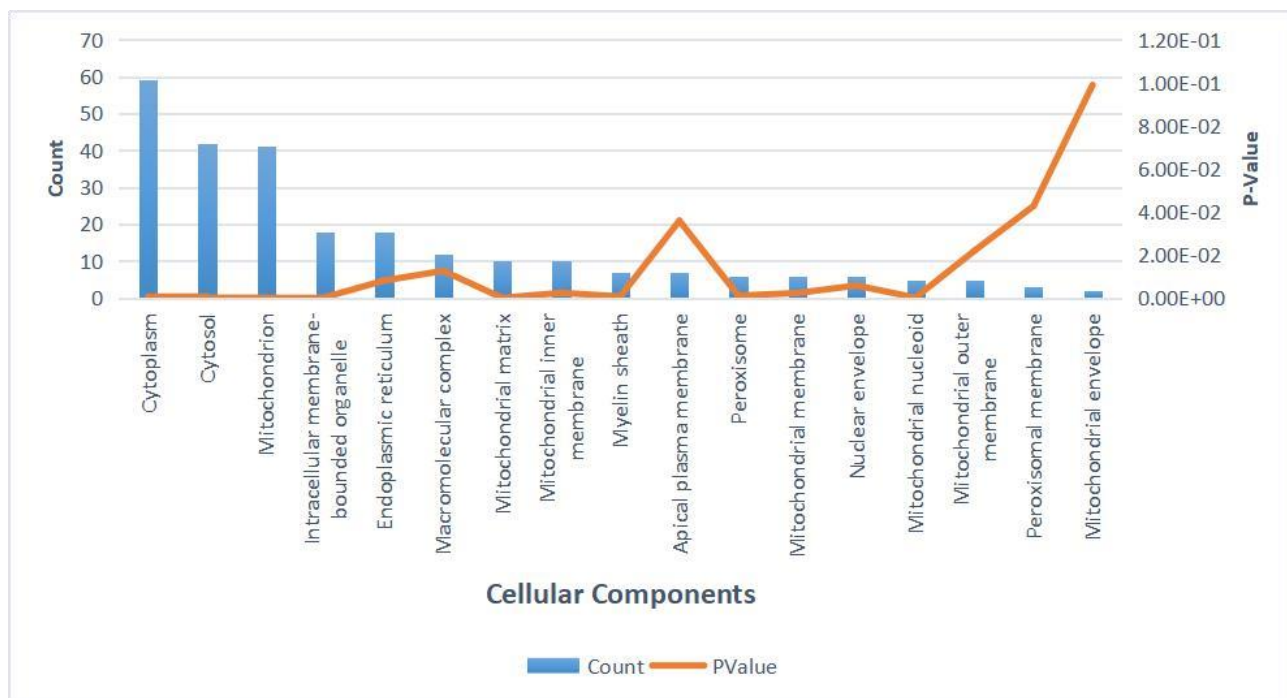


Figure 5.7 Enrichment analysis of down-regulated genes: Molecular function

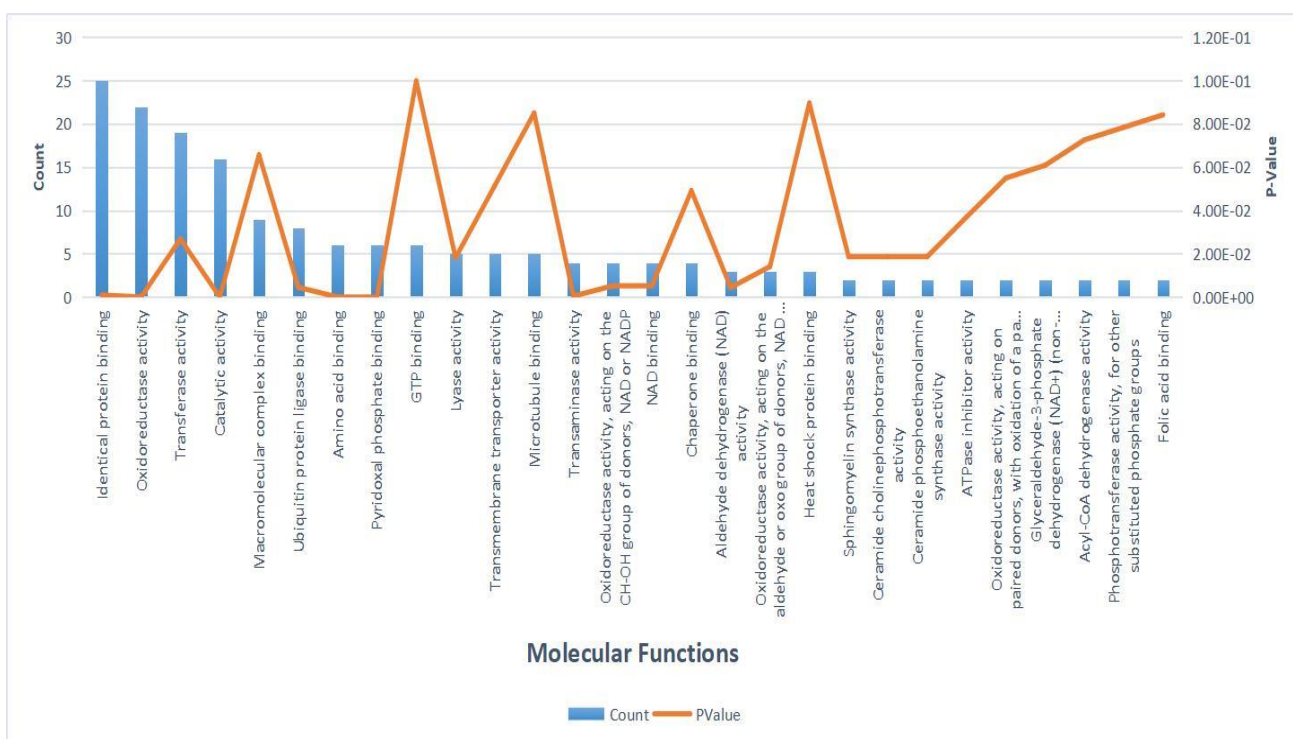
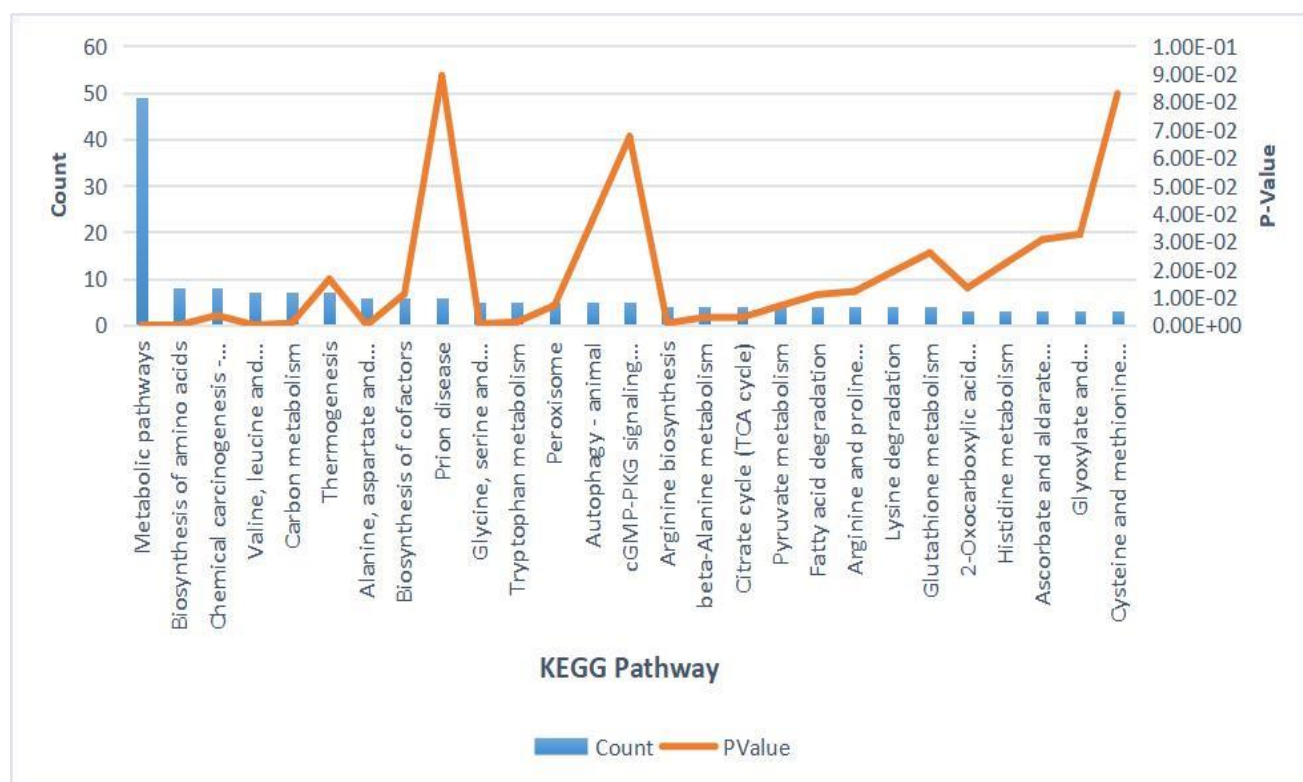


Figure 5.8 Enrichment analysis of down-regulated genes: KEGG pathway

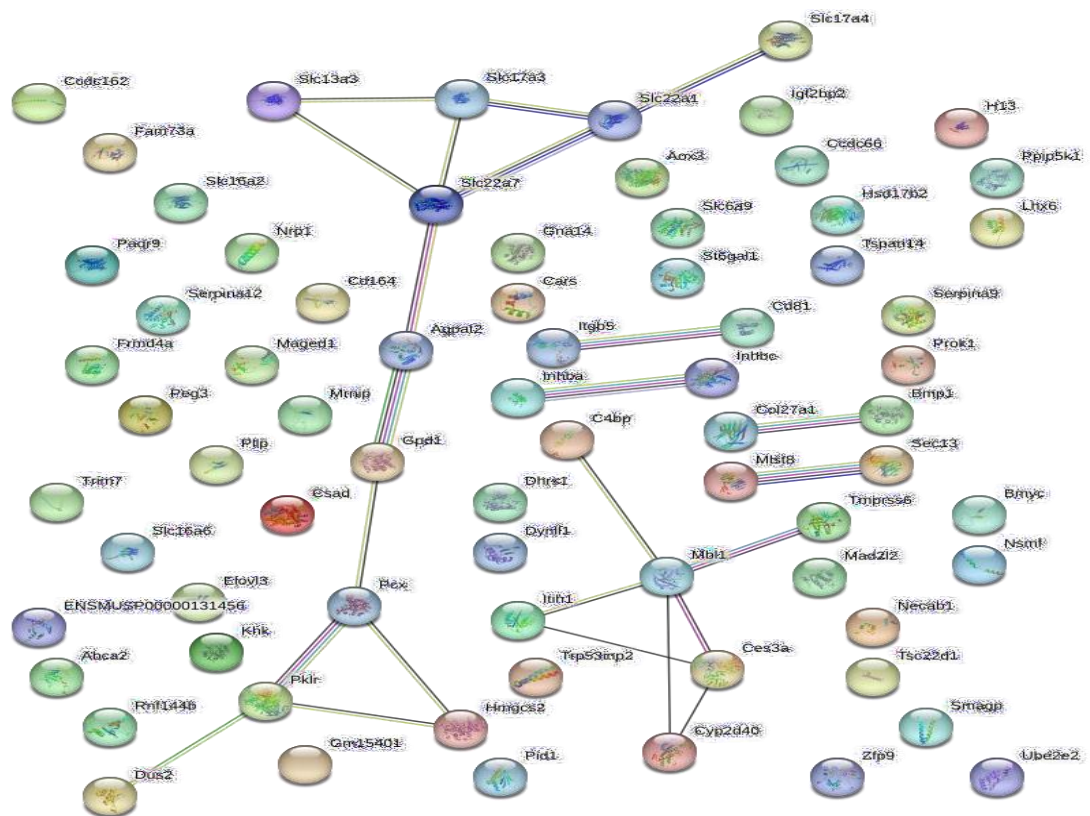


5.3 Construction of PPI network

The up-regulated and down-regulated genes were uploaded separately in STRING database v11.5. The up-regulated genes network was mapped with 72 nodes and 24 edges on an average node degree of 0.667. The average local clustering coefficient and PPI enrichment p-value were 0.262 and 0.000409, respectively.

The down-regulated genes network was constructed with the 155 nodes and 302 edges on an average node degree of 3.9. The average local clustering coefficient and PPI enrichment p-value were 0.423 and $< 1.0e-16$, respectively. Further, the mapped networks were exported to the cytoscape for further analysis.

Figure 5.9 Enrichment Network of differentially expressed genes:
up-regulated gene network



5.4 Screening of modules and analysis of hub genes in PPI network

The networks were visualised in the cytoscape for screening of modules and hub genes individually. MCODE plug-in was used for predicting the highly interconnected regions in networks. The results were analysed based on their MCODE score (>3) and nodes (≥ 4).

In the up-regulated genes network, totally three clusters were shown, out of which only 1 cluster satisfied the criteria. The cluster 1 was shown with a score of 3.333 that includes 4 nodes and 5 edges, out of which SLC13A3 node was called as Seed (highest scoring node).

Similarly, in the down-regulated genes network, totally three clusters were shown, out of which only 1 cluster satisfied the criteria. The cluster 1 was shown with a score of 8.471 that includes 18 nodes and 72 edges, out of which GNMT node was called as Seed (highest scoring node).

Figure 5.11 MCODE cluster for up-regulated genes

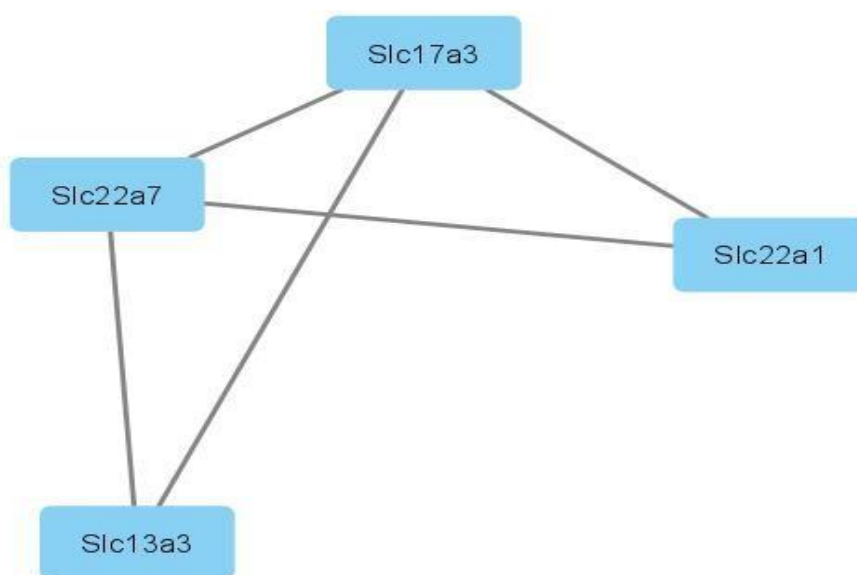
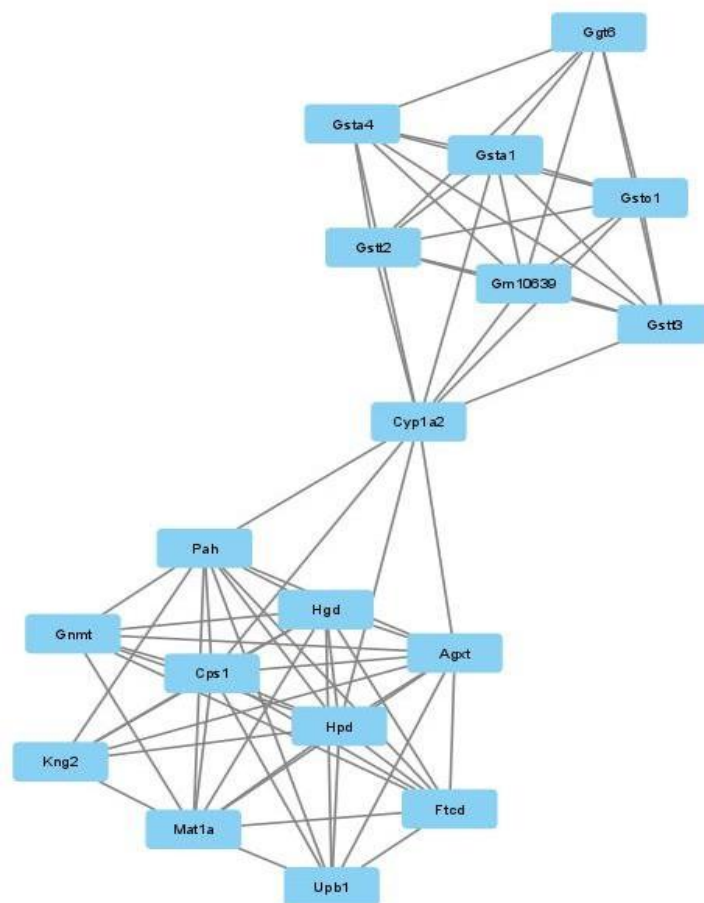


Figure 5.12 MCODE cluster for down-regulated genes



CytoHubba is a Cytoscape plugin that provides a set of algorithms for identifying network hubs and important nodes in protein-protein interaction networks (PPIs). The plugin allows users to perform several types of analyses, including identifying topological centralities.

Hub genes identification was performed by cytoHubba based on 12 algorithms for up-regulated and down-regulated gene network separately, which are Maximal Clique Centrality (MCC), Degree, Maximum Neighborhood Component (MNC), Edge Percolated Component (EPC), Density of Maximum Neighborhood Component

(DMNC), Bottleneck, EcCentricity, Closeness, Radiality, Stress, Betweenness, Clustering Coefficient (CC). The up-regulated gene network was analysed for the hub genes with node degree as parameter. From the network the hub genes identified were as SLC17A3, SLC22A7, SLC22A1, SLC13A3. The Gene Ontology (GO) annotations and pathways of these genes were analysed. These hub genes are mainly involved in transmembrane transport in Biological process (BP), plasma membrane in Cellular component (CC), transmembrane transporter activity in Molecular function (MF), Transport of small molecules in Reactome pathway (Pathway).

Figure 5.13 Up-regulated network hub genes identification performed by cytoHubba based on 12 algorithms

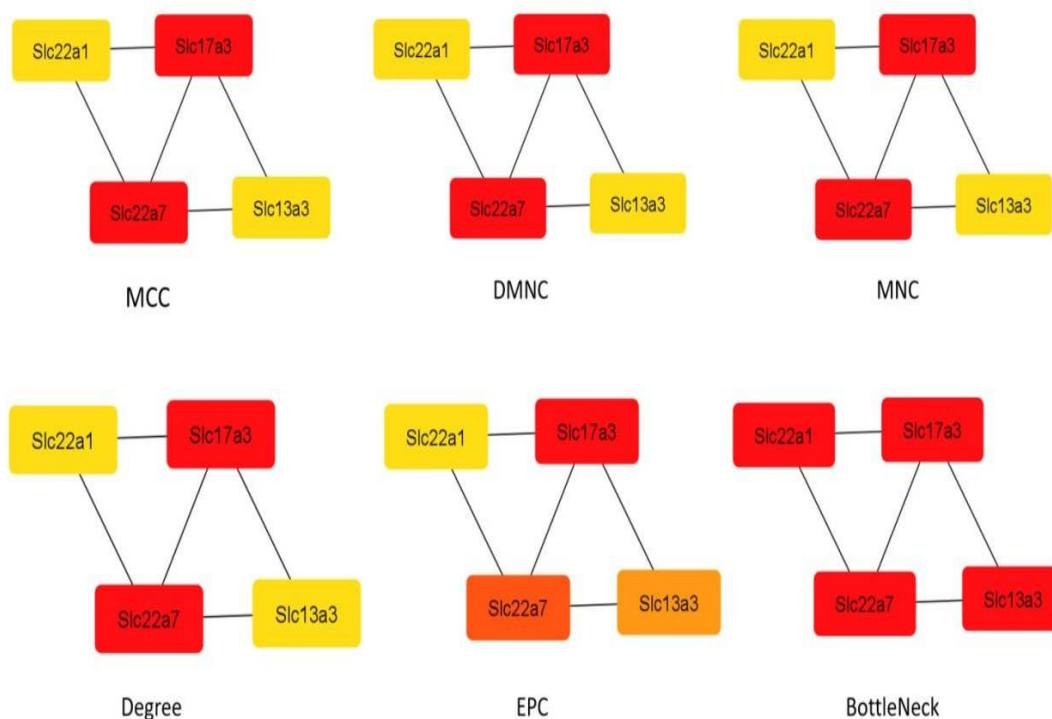
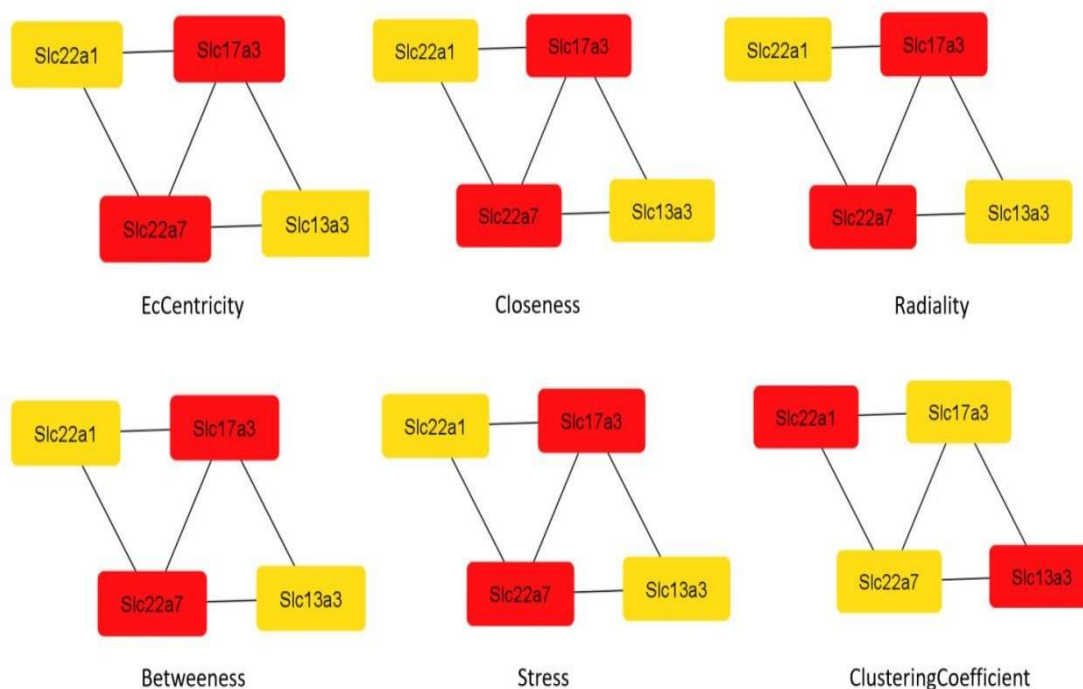


Figure 5.13 Up-regulated network hub genes identification performed by cytoHubba based on 12 algorithms (continued)



Similarly, the down-regulated gene network was analysed for the hub genes with node degree as parameter. From the network the hub genes identified were as CYP1A2, CPS1, PAH, HPD, AGXT, MAT1A, HGD, FTCD, GNMT, GSTA4. Further, the Gene Ontology (GO) annotations and pathways of these genes were analysed. These hub genes are mainly involved in metabolic process and L-phenylalanine catabolic process in Biological process (BP), cytoplasm in Cellular component (CC), identical protein binding and transferase activity in Molecular function (MF), Metabolic pathways in KEGG pathway (Pathway).

Figure 5.14 Down-regulated network hub genes identification performed by cytoHubba based on 12 algorithms

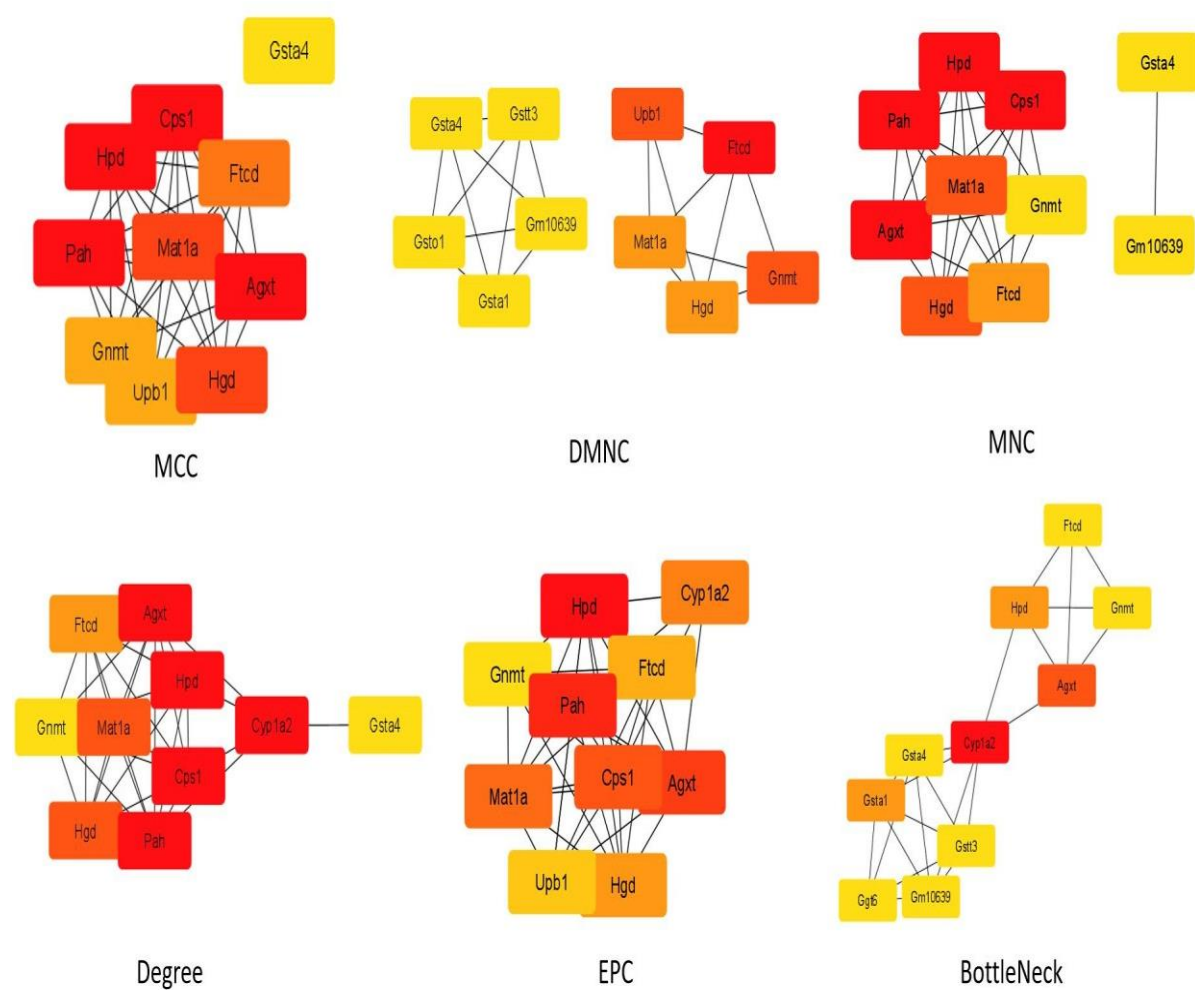
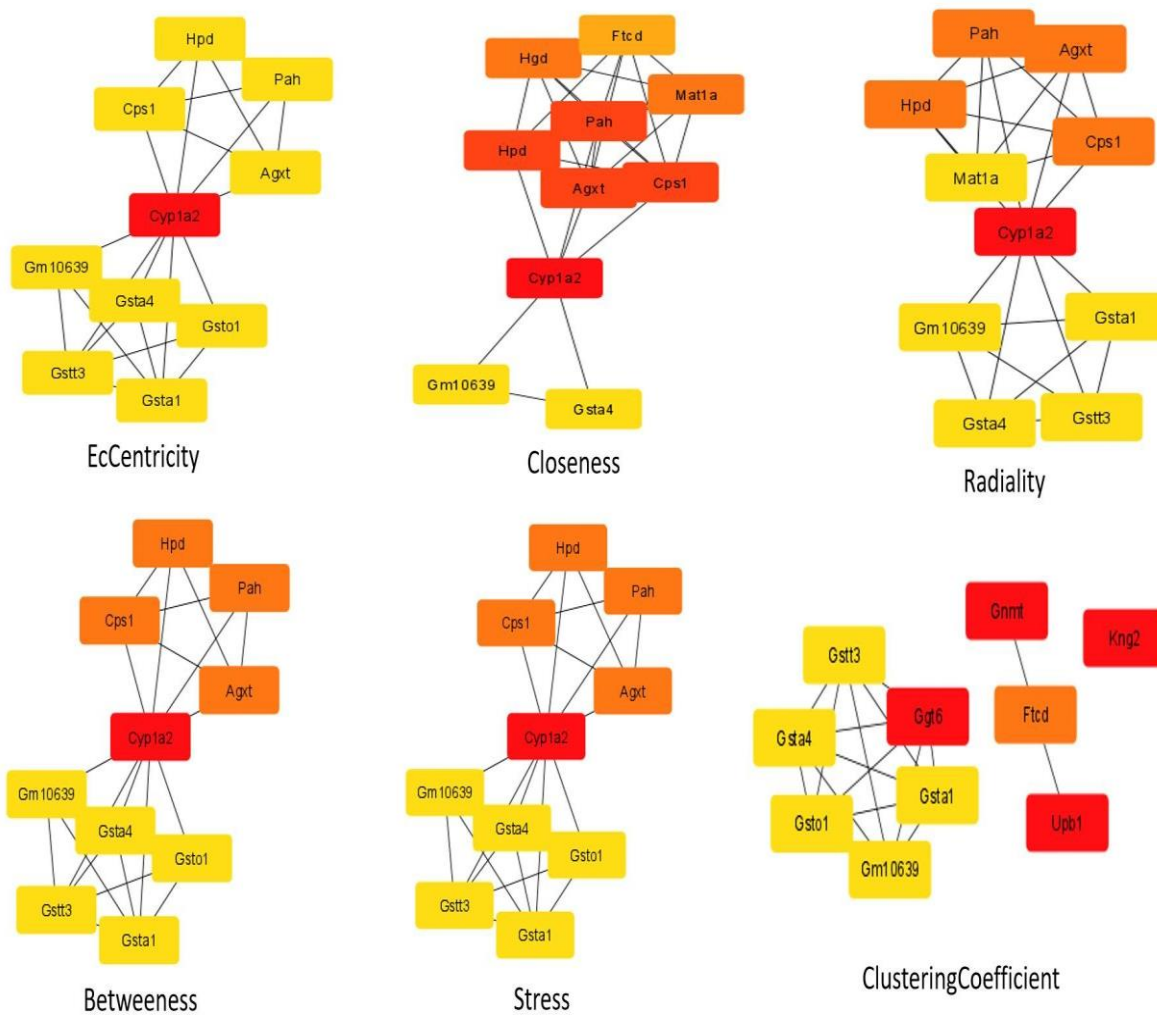


Figure 5.14 Down-regulated network hub genes identification performed by cytoHubba based on 12 algorithms (continued)



The identified hub genes were observed to play crucial roles in cancer studies, as reported in multiple sources. SLC17A3, SLC22A7, SLC22A1, and SLC13A3 are all members of the solute carrier (SLC) gene family, which encode for transporters that regulate the movement of small molecules, such as ions and nutrients, across cellular membranes. These genes have been implicated in various biological processes, including metabolism and cancer. Studies have shown that SLC22A7 can regulate the sensitivity of melanoma cells to chemotherapy agents, such as doxorubicin and melphalan (Jiang et al., 2019).

Additionally, SLC22A7 has been linked to drug resistance in cancer cells. SLC17A3 has been linked to the regulation of glutamate release, which can impact the growth and proliferation of cancer cells (Wang et al., 2020). Additionally, SLC17A3 has been found to be overexpressed in several types of cancer, including prostate cancer and non-small cell lung cancer (Chen et al., 2019; Kim et al., 2019). SLC22A1 is involved in the transport of organic cations, such as neurotransmitters and drugs, across cellular membranes. Alterations in SLC22A1 expression have been linked to several diseases, including cancer and diabetes (Sweet et al., 2019). In melanoma, SLC22A1 has been implicated in the uptake of the chemotherapeutic agent dacarbazine (DTIC) (Xu et al., 2017). SLC13A3 is a transporter that regulates the uptake and efflux of sulfate and other anions across cellular membranes. Dysregulation of SLC13A3 has been linked to several metabolic disorders, including obesity and diabetes (Li et al., 2020). SLC13A3 has also been implicated in the progression of hepatocellular carcinoma (Wu et al., 2020).

Mutations in PAH (Phenylalanine hydroxylase) have been linked to phenylketonuria, a metabolic disorder characterized by elevated levels of phenylalanine in the blood. Phenylketonuria has been associated with an increased risk of melanoma (Pichler et al., 2019). CPS1 encodes for carbamoyl phosphate synthetase 1, which is involved in the urea cycle and amino acid metabolism. CPS1 has been implicated in the development and progression of several cancers, including hepatocellular carcinoma and glioblastoma (Chen et al., 2019; Hui et al., 2020).

CYP1A2 is a gene that encodes for a cytochrome P450 enzyme that is involved in the metabolism of various xenobiotics and endogenous compounds, including carcinogens. Dysregulation of CYP1A2 has been associated with an increased risk of various cancers in humans, including lung, bladder, and breast cancer (Liu et al., 2018; Wang et al., 2019). It is possible that dysregulation of this gene may contribute to the development or progression of the disease. Obesity is a complex condition that is associated with various metabolic and hormonal changes, which can influence the expression and activity of many genes, including CYP1A2 (Alonso-Magdalena et al., 2019). GSTA4 is one of the downregulated hub genes identified in the present study, according to the study by Zhang et al. (2018). GSTA4 has been shown to be involved in the detoxification of reactive oxygen species (ROS), which are known to be involved in the pathogenesis of melanoma (Yin et al., 2017). Thus, the downregulation of GSTA4 in obesity-induced melanoma in mice may lead to an increase in ROS levels and oxidative stress, which can promote tumorigenesis.

MAT1A encodes for an enzyme that is involved in the synthesis of S-adenosylmethionine (SAdMe), a molecule that plays a crucial role in various cellular processes, including methylation, transsulfuration, and polyamine synthesis. Obesity has been shown to be associated with alterations in SAdMe metabolism. Studies have suggested that obesity may lead to a decrease in SAdMe levels and alterations in the expression of genes involved in SAdMe metabolism (Kharbanda, 2018). SAdMe has been shown to have anti-inflammatory and anti-cancer effects, and alterations in SAdMe metabolism have been associated with the development and progression of various types of cancer (Lu et al., 2019).

HPD encodes an enzyme involved in the tyrosine catabolic pathway and is responsible for the conversion of 4-hydroxyphenylpyruvate to homogentisate. The tyrosine catabolic pathway is important for the biosynthesis of melanin, which is a pigment that provides color to the skin, hair, and eyes. Altered melanin synthesis has been associated with various skin disorders, including melanoma. A study

suggested that alterations in the tyrosine catabolic pathway may contribute to melanoma progression (Fang et al., 2019)

The results from the present study demonstrates specific genes in obesity induced melanoma tumor in *Mus musculus* that were differently expressed (upregulated or downregulated) using in-silico approach. According to the results of the study, the gene SLC22A7 had the highest degree of connectivity within the up-regulated gene network, while CYP1A2 had the highest degree of connectivity within the down-regulated gene network. The identification of these genes suggests that they may be potential targets for the management of obesity-induced melanoma condition in mice.

CHAPTER - 6

6. SUMMARY AND CONCLUSION

The project aimed to conduct an in-silico analysis of differential RNA expression in obesity-induced melanoma tumors in *Mus musculus*. The study used the Gene Expression Omnibus (GEO) database, DAVID tool, String database, and Cytoscape software to identify potential biomarkers and pathways involved in the development of obesity-induced melanoma tumors. The project began with a comprehensive literature review that highlighted the role of obesity in the development and progression of melanoma tumors. Several studies have reported a positive correlation between obesity and the incidence of melanoma, and have suggested that obesity alters the tumor microenvironment and promotes the growth and metastasis of melanoma cells. This literature review provided the necessary background knowledge for the project and identified the knowledge gaps that the project aimed to address.

The next step involved the acquisition and analysis of data from the GEO database. The differential expression analysis identified several differentially expressed genes (DEGs) in the obesity-induced melanoma tumors. The DEGs were further analyzed using the DAVID tool to identify enriched gene ontology (GO) terms and pathways. The GO analysis identified several biological processes, cellular components, and molecular functions that were significantly enriched in the DEGs. To identify potential biomarkers and targets for therapy, the DEGs were further analyzed using the String database and Cytoscape software to construct gene networks.

Overall, this project provides insights into the molecular mechanisms involved in the development and progression of obesity-induced melanoma tumors. The findings of this study could have significant clinical implications, as they provide a basis for the

development of diagnostic and therapeutic strategies for obesity-induced melanoma tumors. However, it should be noted that the results of this study are based on in-silico analysis, and further experimental validation is needed to confirm these findings.

In conclusion, the in-silico analysis on differential RNA expression in obesity induced melanoma tumor in *Mus musculus* using the GEO database, String database, DAVID tool, and Cytoscape software allowed us to identify a set of potential biomarkers. The hub genes identified from the up-regulated gene network were SLC17A3, SLC22A7, SLC22A1, and SLC13A3, while the top 10 hub genes identified from the down-regulated gene network were CYP1A2, CPS1, PAH, HPD, AGXT, MAT1A, HGD, FTCD, GNMT, and GSTA4. These genes can serve as potential targets for the development of diagnostic or therapeutic strategies for obesity-induced melanoma tumors. Further experimental validation is needed to confirm the findings of this in-silico analysis.

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