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Protein-protein interaction network analysis of obesity

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ABSTRACT

Obesity is a multifactorial disease that its molecular bases are very complex. Due to its complexity, there is no efficient medication for obesity. In this paper, protein analysis based on protein–protein interaction (PPI) network is illustrated and discussed in details to better understand obesity-underlying mechanism. The number of 45 proteins that their expressions are changed in obesity is interacted by Cytoscape Software and the hub and bottleneck proteins are introduced. The findings showed that UBC, AKT1, and T53 are hub-bottlenecks in the obesity network as their centrality are the highest. The enrichment analysis identified biological processes that possibly are important in obesity. It can be concluded that central elements of the network may play important role in obesity mechanism; however, further researches are required.

Keywords: Obesity, Protein-protein interaction network analysis (PPI), Differentially expressed proteins, Gene ontology (GO), Hub-bottleneck elements.

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Introduction

Obesity prevalence has been increased in the world. One of the important concerns about obesity is the susceptibly to other diseases including heart disease, diabetes (typeп), liver disease, cancer, and Alzheimer's disease (1, 2). In addition, people with obesity are more prone to poorer quality of life, lower self-confidence, social problems, and depression (3). Another thing is high health-care cost for obesity, increases each year (4, 5). Obesity risk factors are known as history of obesity, molecular origin, environment, metabolism, behavior, culture, and socio-economic status (6). Many molecular factors correlate to obesity risk. Genomic, proteomic, and metabolomic studies showed divers contribution of significant elements in obesity pathophysiology (2). Interaction between molecular and environmental agents leads to obesity different phenotypes. In this regard, proteomic researches have provided novel information about the obesity functional elements. Proteins that proved differentially expression in people with obesity may be prominent for further investigations. Identifying proteins individually is valuable for

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disease molecular profile comprehension. However, understanding how these proteins relate to each other and their systematic performance is also important to be evaluated. The mechanisms of different phenomenon such as disease condition in organisms are depended on how different proteins interact and influence their linked biological process (7). Dysregulation of protein expression may affect different pathways and leads to distinct phenotypes (8). Therefore, by examining protein-protein interaction networks, many major details of molecular mechanisms may be achieved. The proteomic study by Mohamed Abu-Farha, et al, on proteome profile of obese people compared to lean subjects showed many proteins with significant expression changes in obese people (9). In this study, these proteins are analyzed for PPI network and gene ontology annotations.

Materials and Methods

The proteomic study of obesity identified 45 proteins that may play an important role in obesity. These proteins showed differential expression pattern with at least Fold ≥1.5 when comparing lean with obese people. For network analysis, these proteins were included in the network construction by the application of Cytoscape 3.4.0 as an open source network analyzer tool (10). STRING Database (DB) was the interaction source for PPI analysis by Cytoscape. Comprehensive information from both experimental and predicted interactions from different databases with a probabilistic confidence score is applied by string source (11). The network was extracted from string DB with the confidence cut off of 0.4. A number of 100 protein were added to the network and then the essentials were assessed by the Network Analyzer. This application is well integrated in Cytoscape and computes a comprehensive list of simple and complex topology parameters using resourceful graph algorithms (12). Analysis of network revealed centrality of some elements. These elements are evaluated based on degree and betweenness centralities. In the PPI map, the nodes represent proteins and edges showed physical interactions (13). Number of linked edges to a node is the degree. The number of shortest paths passing in each node is considered as Betweenness centrality (12). Proteins with highest centralities could be from initial proteins or enriched proteins. Proteins with high degree and betweenness values can be described as hub-bottlenecks. For gene ontology analysis, 'ClueGO plug-in' was used. The biological process and cell compartment evaluation are done by this plug-in. ClueGO presents gene ontology ranging from general to very specific terms. Grouping is based on significant functionally association between terms and gene-sets. Fusing relevant terms with associated proteins reduces the redundancy. Kappa score is applied to calculate the strength of terms grouping (14, 15) and adjusted to 0.5 (medium) for GO analysis. In biological process (BP) analysis, the minimum number and percentage of genes per terms were adjusted to 3 and 4 as the default option, respectively. Min and max levels of the annotation was 3 and 8 as the default, respectively. The *p*-value was also set to ≤0.05. The correction method for *p*-value ≤0.05 was Bonferroni step down method. The enrichment/depletion test of terms was set to two-sided (enrichment/depletion) based on hypergeometric.

Results

Network analysis of 45 initial proteins from proteomic evaluation of obesity (9) by the

application of Cytoscape 3.4.0 and String db is presented in figure1.

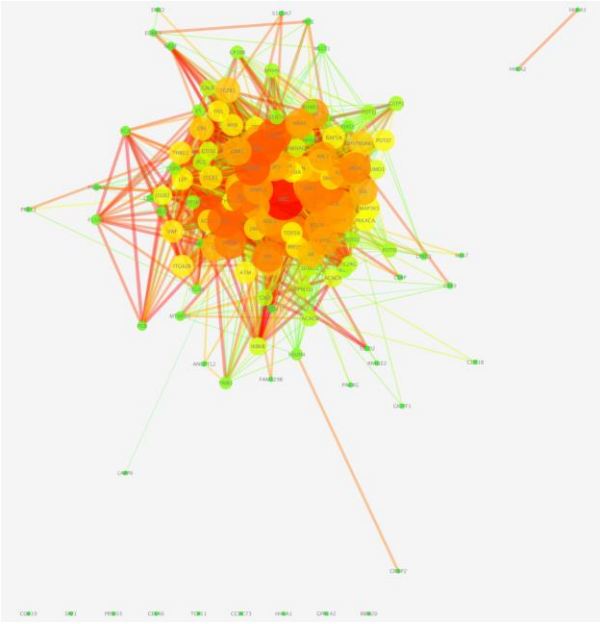

Figure 1. The network pattern of 45 initial proteins comprises of 145 nodes and 2708 edges. Different colors indicate included central parameters. For nodes, changing colors from red to green shows degree with high score to low and edges with high score to low, respectively. In addition, the bigger the node size, the higher degree values. The confidence cut off for network construction = 0.4.

The centrality evaluation is an important part of network analysis. Degree and betweenness centrality are key parameters in this regard. Proteins with the highest degree and betweenness are called hubs and bottlenecks, respectively. The hubs and bottlenecks proteins are presented in table 1.

Enrichment analysis of the candidate proteins was performed by the use of ClueGO. The biological processes and cell compartment related to obesity are described in figures 2 and 3, respectively.

Table1. Central indicators of the obesity network (Degree and Betweenness). Proteins are ordered based on highest degree values. The asterisk sign indicate the only initial protein in the top 20 list of the central proteins.

Gene name	Protein name	Degree	Betweenness
UBC	Ubiquitin C	109	0.12
AKT1	V-akt murine thymoma viral oncogene homolog 1	88	0.02
T53	Tumor protein p53	87	0.03
PRDM10	PR domain containing 10	87	0.01
SRC	V-src sarcoma	86	0.02
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	86	0.01
JUN	Jun proto-oncogene	84	0.01
TSPO	Translocator protein	83	0.01
ALB	Albumin	81	0.02
HSP90AA1	Heat shock protein 90kDa alpha (cytosolic), class A member 1	80	0.01
BCL2	B-cell CLL/lymphoma 2	79	0.01
EGFR	Epidermal growth factor receptor	77	0.02
JAK2*	Janus kinase 2	74	0.01
MTOR	Mechanistic target of rapamycin (serine/threonine kinase)	74	0.01
INS	Insulin	73	0.01
MAPK1	Mitogen-activated protein kinase 1	72	0.007
MYC	V-myc myelocytomatosis viral oncogene homolog	72	0.01
HRAS	V-Ha-ras Harvey rat sarcoma viral oncogene homolog	71	0.007
NFKB1	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1	70	0.01
PTEN	Phosphatase and tensin homolog	69	0.004

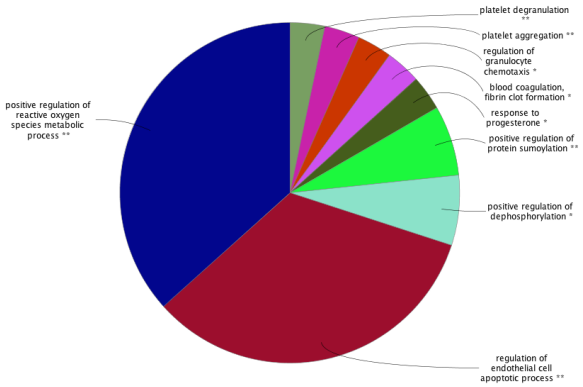

Figure 2. Biological process analysis of 45 initial proteins by the use of ClueGO. P<0.05 and min gene per term= 3 Asterisks indicate significant relations.

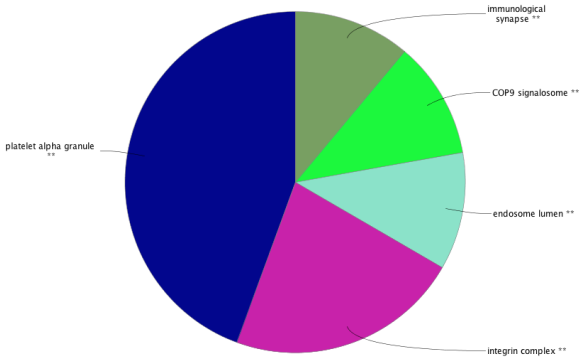

Figure 3. Cellular compartment analysis of 45 investigated proteins by the application of ClueGO. P<0.05 and min gene per term= 3, Asterisks indicate significant relations.

Discussion

Apparently, molecular investigation of any different kind of conditions can provide a better understanding of its underlying mechanisms. Here a PPI network approach is conducted for obesity. This disease as a serious problem in today modern world can be better manageable if all of the correlated factors to be evaluated (2). The PPI network and applied algorithms analysis can provide further insight in any different molecular aspects. Centrality is one of the key evaluating parameters in studying topology of PPI in a specific condition. Central proteins in a network may be a better candidate for drug targeting (16). Top central nodes are derived from the network assessment and nodes with highest degree and betweenness are hub-bottlenecks (17). As indicated in figure 1, constructed network shows that some initial proteins are not presented in the network, when enriching is done with 100 proteins. These excluded proteins are including HHLA2 HHLA3, SPZ1, PRSS55, CEOR6, TCP11, CCDC73, HHLA1, GPR142, and RBM20. As it is shown, only one initial protein was detected in top 20 central proteins and the rest were the added proteins. Proteins with high degree and high confidence of interactions were also recognizable in this pattern. The node with red color that implies on the highest degree value is Ubiquitin C. This protein is one of the enriched proteins. More details about central elements of obesity network are presented in table 1. The list shows that some of the essential elements of the network belongs to initial proteins as JAK2. UBC, AKT1, and T53 are hub-bottlenecks in the obesity network as their centrality are the highest. Ubiquitin C has the highest score and proved to have key role in protein metabolism. This protein is present as a free protein or conjugated to another protein. In a free format, it is responsible for activation of protein kinases and signaling. In the attached format to other proteins, UBC conducts many other functions (18). On the other hand, people with obesity show difference in protein degradation that is due to the ubiquitin-proteasome system

(UPS) dysregulation. In fact, in obese people, the metabolism of the whole-body protein is modified (19). Here, the centrality of UBC in obesity network supports its fundamental role in obesity. Another central protein, AKT1, proofed to have associations with obesity (4). This protein is involved in many regulatory events including metabolism, proliferation, cell survival, growth and angiogenesis. The third hub-bottleneck protein is P53. This protein is a popular protein that is mainly involved in tumor suppressor activities (20). Other roles of P53 are less discussed. An important one is its pathway activation in adipocyte cells to regulates obesity condition (1). Further evaluations based on "GO" analysis identified associated biological processes in obesity profile based on initial proteins' query. Positive regulation of reactive oxygen species metabolic process, regulation of endothelial cell apoptotic process, positive regulation of dephosphorylation, and positive regulation of protein sumoylation are highlighted in obesity (As shown in Figure 2). In addition, the places in the cell that most of these actions take part are studied as shown in figure 3. Platelet alpha granule and integrin complex are the two referred ones. However, all the three important hub-bottlenecks were previously reported for obesity pathogenesis. In this study, network analysis provided another perspective of their contribution. In addition, as protein expression of the initial proteins changed, the related biological processes may be influenced.

Conclusion

In conclusion, UBC, AKT1, and T53 may be involved in obesity mechanism; however, further investigations are required to manipulate in diagnosis and treatment of obesity in the future. It also can be concluded that biomarker panel formation may be possible for obesity.

Conflict of Interest

Not Declared

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