Radiation Therapy in Patients With Brain Cancer: Post-proteomics Interpretation

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Abstract
Introduction: Radiation therapy (RT) as a common method for cancer treatment could result in some side effects. The molecular investigation is one of the approaches that could assist in decrypting the molecular mechanisms of this incident. For this aim, protein-protein interaction (PPI) network analysis as a complementary study of the proteome is conducted to explore the RT effect on brain cancer after the early stage of exposure prior to the appearance of the skin lesion.
Methods: Cytoscape 3.7.2 and its plug-ins were used to analyze the network of differential expression of proteins (DEPs) in the treatment condition, and the centrality and pathway enrichment was conducted by the use of NetworkAnalyzer and ClueGO+CluePedia.
Results: A network of 15 DEPs indicated that 6 nodes were key players in the network stability and SERPINC1 and F5 were from the query proteins. The pathways of post-translational protein phosphorylation, platelet degranulation, and complement and coagulation cascades were the most highlighted ones for the central nodes that could be affected in RT.
Conclusion: The central proteins of the network of early-stage treatments could have additional importance in the mechanisms of radiotherapy response prior to skin lesions. Introduced biomarkers can be used for the patients' follow-up. These candidates are worth precise attention for this type of therapy after approving by validation studies.
Keywords: Radiation therapy; Protein-protein interaction network analysis; Gene ontology

Introduction

The estimation for the application of radiation therapy (RT) to cancer patients in the field of oncology is about 50% to 60%. This approach is used either alone or with the combination of other methods including surgery, chemotherapy, and immunotherapy.[1,2] This popular line of therapy possesses some accompanied undesirable consequences known as side effects aside from the therapeutic effects. The common one is the irradiation of neighbor normal tissues that could result in injuries.[2] For instance, in breast cancer treatment with radiation, there is a risk of ischemic heart disease at the doses of 1 to 5 Gy.[3] In addition, the skin lesion is another adverse common consequence in cancers cured with RT.[3] Molecular mechanisms by which these changes occur and develop into skin lesions are still required to be studied. The serum is one of the appropriate sources to investigate the molecular level changes.[4] In this way, the potential biomarkers of any condition could be revealed by promising large-scale studies.[5] Moreover, proteomics is a novel molecular investigation for discovering the biomarkers of a specific state. Proteins are the functional parts of our body that their expression modifications could grow abnormal performance in an organism [6]. One of the candidate conditions for proteomics researches is treatment outcome predictions. Radiotherapy is a type of treatment in cancer management, the effect of which could be detected by the assessments of serum. Depending on therapy intensity and duration, the proteome quality could change.[4] Additional information about biomarkers introduced by a proteomics study of these conditions is feasible by bioinformatics such as

protein-protein interaction (PPI) network analysis.[7] A DEP that has centrality values in a PPI network could be more promising as a biomarker since its changes could cause a vast range of malfunctions in a system of protein interactions. Centrality analysis of DEPs can be handled by designating important parameters including degree and betweenness centrality (BC).[8-10] In this sense, we here explore the early stage of RT by the PPI network analysis of the serum proteome profile of brain cancer patients. The purpose of this study is to identify the molecular triggers of RT influence in the human body.

Methods

The serum proteome profile of early-stage treatment with radiation of brain cancer subjects was assessed for the bioinformatics.[4] The list of DE proteins one week after treatment of RT in serum is listed in Table 1. The bioinformatics approach is the PPI network analysis of differentially expressed proteins of patients' serum with the early stage (one week after therapy) prior to the skin lesion. The dosage of RT was 10 GY of cumulated radiation for the first week of treatment. The patients (male and female) were a mixture of different tumor types to reduce the chance of background interference.[4] Fifteen significant proteins in RT-treated serums were identified by the main study 1 week after exposure.[4] These proteins were chosen for PPI network analysis by the application of Cytoscape 3.7.2 and its integrated plug-ins.[11] A network was constructed via the protein query from the STING database source in Cytoscape.[12] A confidence score (edge weight) ranges from 0-1 for physical interaction and here a score of 0.6 was considered for this network building to get a high-quality pattern. The study continued by the addition of some surrounding nodes to get a better understanding of the DEPs role in an interactome scale.

Two well-known topological parameters, namely degree (K) and BC, in a network of protein interactions were computed for centrality analysis. The nodes with high values of degree are called hubs and those with high amounts of betweenness are known as bottlenecks. The nodes that have both the 2 high values are called hub-bottlenecks. In a network of protein interactions, the nodes that possess both values are recognized as the most central nodes for that network strength.[13] The removal of these important nodes could cause a vast disruption in a network structure.[13] ClueGO+CluePedia applications visualized biological pathways to gain more understanding of molecules properties of hub-bottlenecks in terms of functional groups.[14] In this analysis, we used the sources of KEGG, WikiPathway, and Reactome. The statistical criteria assigned for this analysis were as follows: the kappa score was 0.5 and the gene per term and the gene percentage per term were 2 and 1 respectively.

Results

Through PPI network analysis, it was possible to identify the most promising candidates of differentially expressed proteins in the treatment response. To get a network with high strength quality, a cut-off confidence score of 0.6 was set and a pattern of proteins with their related interaction was obtained as depicted in Figure 1.

As it is shown in Figure 1, there are 15 DEPs, that are connected by 16 edges. Two proteins remained as individual nodes since they did not show any connection with the designated statistical criteria. These 2 proteins are PRDM15 and EEF1A1. There are also 2 sets of pairs of (C1R and FCN3) and (PRDX1 and PRDX2) that are separated from the main network.

The next step is to analyze the centrality values of the DEPs accompanied by their neighbor proteins conducted
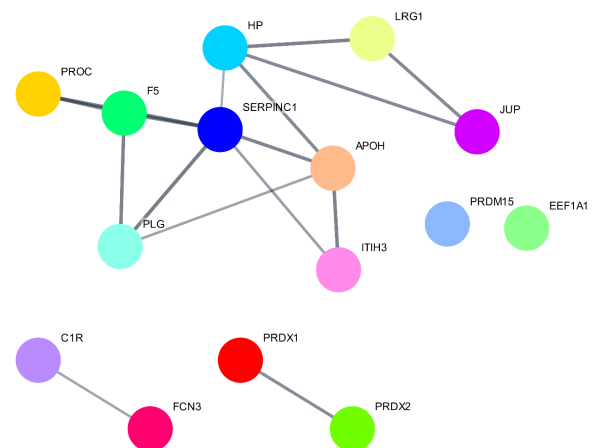
Table 1. The List of DEPs of the Early Stage Exposure to RT in Patients With Brain Cancer

Gene Name	Accession No.
PLG	P00747
APOH	P02749
PRDM15	P57071
EEF1A1	A6NCN2
PROC	B4DPQ7
FCN3	O75636
JUP	F5GWP8
ITIH3	Q06033
HP	P00738
SERPINC1	P01008
LRG1	P02750
PRDX2	P32119
F5	P12259
PRDX1	Q06830
C1R	P00736

Figure 1. The Network of the DEPs of Early-Stage Treatment With RT for Patients With Brain Cancer. The nodes indicate proteins and the links are the interactions between them. The cut-off Confidence score = 0.6. The nodes are shown in different colors.
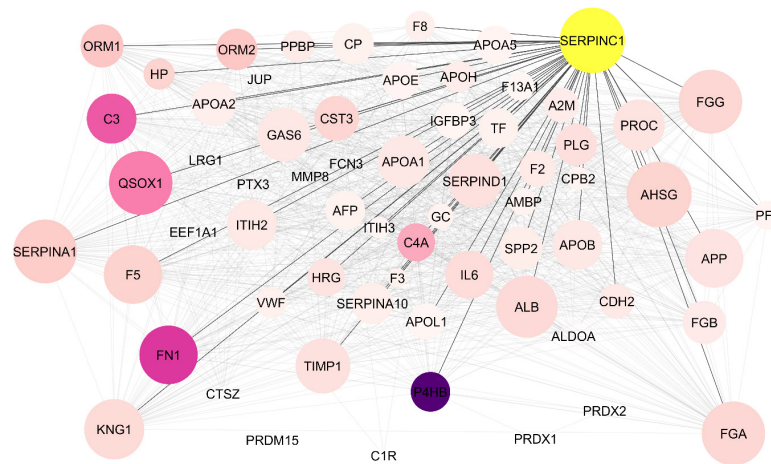
Figure 2. The Centrality Analysis of DEPs and the Neighbor Proteins Via NetworkAnalyzer. The bigger the nodes, the higher the degree value. Similarly, the darker the color, the higher the betweenness value. The first network of the most central hub-bottleneck is highlighted.

by Cytoscape plug-in NetworkAnalyzer (see Figure 2).

As it is shown in Figure 2, the network consists of 65 nodes and 1071 links among them. SERPINC1 is the most central hub-bottleneck that is shown in yellow and the first neighbors in contact with it are highlighted. PRDM15 remains as an individual node after the addition of 50 nodes.

To identify the hub-bottlenecks of the constructed network, NetworkAnalyzer was used and 20% of nodes with the highest degree (hubs) and likewise 20% of nodes with the highest values of betweenness (bottlenecks) were assigned. The common nodes were selected as hub-bottlenecks in Table 2.

As it is shown in Table 2, six nodes were identified as

Table 2. The List of Hub-Bottlenecks[a]

Display Name	Degree	BC
SERPINC1*	52	0.01
AHSG	52	0.01
QSOX1	51	0.03
SERPINA1	51	0.02
FN1	48	0.04
F5*	48	0.01

BC, betweenness centrality.
[a] The ranking is based on the highest degree value.
Note. The query proteins are assigned with an asterisk.

hub-bottlenecks, in which the highest degree belonged to SERPINC1 (with the degree value of 52) and the lowest one was 48 belonging to F5. The most significant bottleneck is FN1 with the BC value of 0.04.

To get a better knowledge of the functional involvement of the hub-bottlenecks in the underlying mechanism of RT treatment, their pathway analysis was handled with ClueGO+ CluePedia in Figure 3.

Three pathway groups including post-translational protein phosphorylation, platelet degranulation, and complement and coagulation cascades were identified.

In Figure 4, three types of actions are present between the hub-bottlenecks. Almost all the hub-bottlenecks have similar action roles except between SERPINC1 and F5 which is the inhibition type.

Discussion

Molecular studies could help in facilitating revealing the mechanisms by which a specific treatment effects on the intervened exposed tissue.[4] One way is to scan the proteome changes of the serum via bioinformatics; which is called PPI network analysis. In this search, 15 differentially expressed proteins in the serum of patients with brain cancer treated with the early stage (first week) of radiotherapy were studied. A network of these DEPs was without any additional neighbor proteins as indicated in Figure 1, in which 2 nodes of PRDM15 and EEF1A1
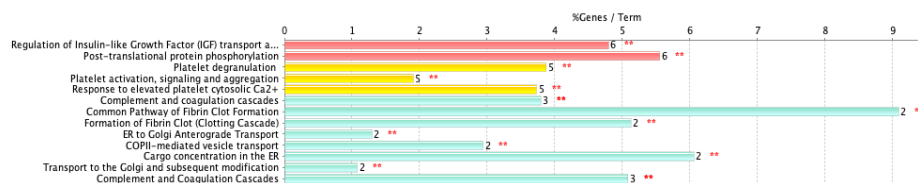

Figure 3. Pathway analysis of the Hub-bottlenecks indicated three groups in different colors, including post-translational protein phosphorylation, response to elevated platelet cytosolic ca2+, and the common pathway of fibrin clot formation. The percentage of hub-bottleneck contribution and its numbers in each term are indicated. Two stars indicate the statistically significant term, $P < 0.01$, kappa score = 0.5.
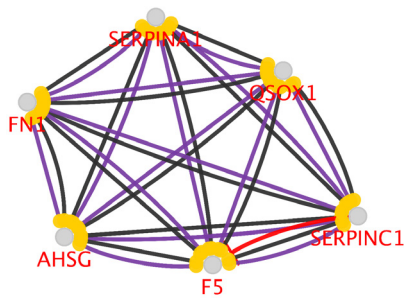
Figure 4. The action map of 6 hub-bottlenecks; red, black, and purple refer to inhibition, reaction, and catalysis respectively. The kappa score = 0.5 was considered. Round and bar tips indicate inhibition and associations respectively. Inhibition and activation actions did not appear.

remained as individuals since they were not involved in condensing interactions with other proteins. Regulation differentiation between the control group and the treated group with RT of these 2 nodes did not show very high values as well.[4] On the other hand, most of these DEPs were in condensed interactions with a high significance. As it is shown in Figure 2, after the addition of surrounding nodes to the query ones, PRDM15 did not show any connections with other nodes yet. Therefore, this DEP might not be playing an important interaction role similar to the others in this network. Centrality analysis indicated that the existence of central proteins in the constructed network implied the scale-free pattern of this network. In this light, SERPINC1 was the most noteworthy hub-bottleneck that showed interactions with other central nodes as well. In addition, this protein was highly altered in expression in the serum of the patients after treatment.[4] More analysis of central proteins in terms of high degree and betweenness values explained that 6 nodes were with the highest amounts of these parameters and 2 of them were from DEPs. As mentioned before, SERPINC1 was the highest-ranked hub-bottleneck and was from DEPs. The next protein was F5 which was the sixth key hub-bottleneck and the previous study also showed high expression changes in the treated subjects.

The next step was the pathway analysis of the retrieved hub-bottlenecks, showing the linkage of 3 highlighted groups of pathways, including post-translational protein phosphorylation, response to elevated platelet cytosolic ca2+ and the common pathway of fibrin clot formation. The analysis showed that the function of these pathways could be influenced by the exposure of RT. Moreover, SERPINC1 and F5 were both present in all 3 retrieved pathways. This shows their essential part in the integration of these biological processes. Furthermore, the action type analysis by the use of CluePedia also marked deeper associations between these central nodes. The common actions between these nodes, which were very apparent, were catalysis and reaction actions. This map demonstrated that SERPINC1and F5 as the 2 DEPs

also had an additional unique action type in comparison with other hub-bottlenecks in a way that SERPINC1 inhibited F5. The relations of SERPINC1 and F5 were evident in each analysis. First of all, they were both from highly differentially expressed proteins of serum in the treatment of RT. Second, F5 was one of the first neighbors of SERPINC1. The third relationship was the centrality values of these 2 proteins, which was very high. The fourth important linkage was their contribution in all of the resulted pathways, and finally, there were exclusive interactions between them, which were inhibition besides the others.

The literature review of the identified hub-bottlenecks could assist to better decode the possible role of hub-bottlenecks in the mechanisms of RT treatments. The first and most central node, SERPINC1 (antithrombin-III) as mentioned earlier, was among the significant differential expressed proteins of early stage treatments. This protein had anti-inflammation and anticoagulation action in the serum.[15] In fact, it is accounted the most inhibitory effector on coagulation.[16] On the other hand, F5 (coagulation factor V) was responsible for the clotting process and it was shown that it had high expressions after one week of treatments with RT in the present study. This process was required to be activated for the healing of damaged tissues caused by RT. The tight relationships between SERPINC1 and F5 pinpointed by our analysis confirmed the concrete important interactions of SERPINC1 and F5 in the process of RT response. Nevertheless, SERPINC1 showed up-regulation after a week and it changed towards down-regulations until the treatment process was completed.[4] What is more, while AHSG (alpha 2-HS glycoprotein) and FN1 (fibronectin 1) as the second and fifth-ranked hub-bottlenecks were not found differentially expressed after one week of treatments, their expressions changed after this duration in a way that FN1 started showing alterations 2 weeks, 3 weeks, and 1 month after the end of RT and conversely, AHSG only showed changes 1 month after the treatment ended.

In other studies of cancer therapy with radiation, the increment of the Alpha 2-HS Glycoprotein level was reported.[17] FN1 was not a queried protein because it was not detected by the original data. The low levels of this protein in serum had been assigned with a connection to the proliferation of tumors in astrocytoma.[18] For fibronectin as a tissue remolding agent, the expression change was down-regulation in the early stage of RT treatment based on the study by Ouerhani et al.[4] The other 2 central nodes including QSOX1 (quiescin sulfhydryl oxidase 1) and SERPINA1 (alpha-1 antitrypsin) could still be important in the underlying mechanism of RT despite the fact that they were not among the differentially expressed protein. SERPINA1 was another factor playing a role in coagulation[19] that could be imperative in the process of healing. The other hub-bottleneck, QSOX1,

showed some linkage in radiotherapy response in terms of sensitivity. It has been reported that targeting this protein could result in the nasopharyngeal carcinoma radiotherapy sensitivity.[20] On the whole, the early stage treatment with the RT network indicated that the central nodes, especially the 2 co-expressed proteins of SERPINC1 and F5, might be important in the mechanisms of the RT effect on serum. Further enrichment analysis in terms of centrality, function, and action approved their relationships in our study. Other central nodes could also be important in this regard, though their relations require more investigations by complementary studies.

Conclusion

It can be concluded that radiotherapy could affect some central differential proteins of serum in an interactome scale. As mentioned in the discussion section, 3 classes of biochemical pathways including post-translational protein phosphorylation, response to elevated platelet cytosolic ca2+ and the common pathway of fibrin clot formation were affected by RT. These findings can be useful for the follow-up and response to treatment in patients going under brain radiotherapy. However, the complementary studies in this regard are required to validate this claim.

Ethical Considerations
Not applicable.

Conflict of Interests
The authors declare no conflict of interest.

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