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Hypofractionated Radiation Versus Conventional Fractionated Radiation: A Network Analysis

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Abstract

Introduction: Conventional fractionation (CF) and hypofractionation (HF) are two radiotherapy methods against cancer, which are applied in medicine. Understanding the efficacy and molecular mechanism of the two methods implies more investigations. In the present study, proteomic findings about the mentioned methods relative to the controls were analyzed via network analysis.

Methods: The significant differentially expressed proteins (DEPs) of prostate cancer (PCa) cell line DU145 in response to CF and HF radiation therapy versus controls were extracted from the literature. The protein-protein interaction (PPI) networks were constructed via the STRING database via Cytoscape software. The networks were analyzed by "NetworkAnalyzer" to determine hub DEPs.

Results: 126 and 63 significant DEPs were identified for treated DU145 with CF and HF radiation respectively. The PPI networks were constructed by the queried DEPs plus 100 first neighbors. ALB, CD44, THBS1, EPCAM, F2, KRT19, and MCAM were highlighted as common hubs. VTM, OCLN, HSPB1, FLNA, AHSG, and SERPINC1 appeared as the discriminator hub between the studied cells.

Conclusion: 70% of the hubs were common between CF and HF conditions, and they induced radio-resistance activity in the survived cells. Six central proteins which discriminate the function of the two groups of the irradiated cells were introduced. On the basis of these findings, it seems that DU145-CF cells, relative to the DU145-UF cells, are more radio-resistant.

Keywords: Radioresistant; Protein expression; DU145 cell line; Network analysis; Hub node.

Introduction

Radiation therapy is an effective method against cancer development, and it also inhibits various types of cancers.[1] There are optimized protocols for the doses and processes of radiation application. High dose radiation and hypofractionation (HF) are known as effective radiotherapy methods. The lower dose and elongated fractionation are characterized by lower values of effectiveness. In the case of resistant cancers, higher dose radiation and a few repetitions is recommended.[2]

Utilizing an optimized method of radiation therapy is a goal in the treatment of cancers. Therefore, the understanding of body response to different types of radiation therapy is investigated widely by researchers.[3] The efforts via studying the cellular response to radiation indicate that different methods of radiation are accompanied by various patterns of gene expression and proteome changes.[4,5] Different types of targeted proteins and protein level changes may imply a gross alteration in biological functions. Since such alterations are associated with the dysregulation of large numbers of proteins, proteomics is a suitable method for exploring molecular events. Many proteomic investigations about the mechanism of radiation therapy are administrated, and the findings have led to approving radiation therapy.[6,7]

Since the output of proteomics includes large numbers of dysregulated proteins, bioinformatics can analyze the finding to explore the core of alterations.[8,9] In protein-protein interaction (PPI) network analysis, the studied proteins connect to each other to form a network.

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Each protein plays an almost unique role in network construction. The central proteins play critical roles in network creation.[10] The proteins that make higher values of connection with the first neighbors in the network are known as hubs. It is accepted that the hub nodes that are involved in the affected biological functions are the key elements of the network. Several investigations have shown that network analysis and interpretation of the related hubs are applied in the radiation field.[11,12]

In the present study, the proteomes of prostate cancer (PCa) cell line DU145 which are radiated by two methods (conventional fractionation [CF] and HF radiation) relative to the parental cells in the absence of radiation published by Kurganovs et al[13] are analyzed via PPI network analysis.

Methods

Conventional fractional (CF) radiation therapy is a method in which patients with PCa receive 1.8-2 Gy per fraction daily over several weeks. In the case of HF radiation therapy, a radiation dose > 2 Gy per fraction is applied. PCa cell line DU145 was selected to find the cell response to CF radiation therapy versus HF radiation therapy. To create HF cells (DU145-HF), the DU145 cells were treated with 10 Gy daily for five fractions over several weeks.[13] The CF cells (DU145-CF) were the parental DU145 cells that were exposed to 2 Gy dose daily for 5 days per week followed by a 7- to 10-day recovery. This process was repeated 59 times.[14] As it is investigated by Kurganovs et al, the proteomes of DU145-CF and DU145-UF cells are different from the proteome of parental cells (DU145-PAR).[13]

In the present analysis, the proteomic findings of Kurganovs et al were used to analyze differences between the proteomes of DU145-CF and DU145-UF cells relative to DU145-PAR cells. Among the dysregulated proteins, the individuals which were characterized by $1.5 \leq$ Fold change (FC) $\leq (-1.5)$ were selected as significant differentially expressed proteins (DEPs).

The significant DEPs were included in the STRING database from "protein query" via Cytoscape software. The PPI network was created via undirected edges. Due to poor connections between the nodes of the network, the proper number of first neighbors from the STRING database was added to the queried DEPs and the networks were reconstructed. The networks were analyzed by the "NetworkAnalyzer" application of Cytoscape software to explore the central nodes. 10 top nodes of queried DEPs based on the degree value for each network were identified as hubs. The hubs of the networks were compared and discussed.

Results

One hundred twenty-six and 63 significant DEPs were identified for DU145-CF versus DU145-PAR and DU145-HF versus DU145-PAR analyses respectively (see Figure 1).

Among the 126 significant DEPs of DU145-CF cells, 122 ones were recognized by the STRING database. To decrease the number of isolated nodes and to maximize interactions, 100 first neighbors were added to the queried proteins. The network including 17 isolated proteins, 2 paired nodes, and a main connected component counting 201 individuals (101 queried proteins and 100 added first neighbors) was formed.

Of the 63 queried proteins related to the analysis of DU145-HF, 61 individuals were recognized by STRING. After adding 100 first neighbors from the STRING database, a network including 11 isolated proteins, 1 paired nodes, and a main connected component of 148 nodes (100 first neighbors plus 48 queried DEPs) was constructed.

The hub nodes of the constructed networks were determined. As it is shown in Table 1, 70% of the hubs of the two networks are similar. The similar hubs are ALB, CD44, THBS1, EPCAM, F2, KRT19, and MCAM. VTM, OCLN, HSPB1, FLNA, AHSG, and SERPINC1 discriminate the two networks. Fold changes of the hub nodes are shown in Table 2.

Discussion

Kurganovs et al focused on the role of CD44 as a radioresistant agent in an original investigation.[13] Here, seven common dysregulated central proteins that are involved in radiation resistance activity, including ALB, CD44, THBS1, EPCAM, F2, and KRT19, are highlighted. Albumin as the powerful hub node is a housekeeping protein that is involved in many critical activities in cell function. Considering the role of albumin in optimizing body hemostasis,[15] it seems that its upregulation may be a detoxification role against a non-hemostatic condition after radiation therapy.

Thrombospondin 1 (THBS1) is another hub protein that is highlighted in our analysis. This up-regulated protein plays a role as an angiogenesis inhibitor. It is reported that the decreased level of THBS1 is associated with the progress of cancer.[16] It can be concluded that the upregulation of THBS1 is a part of anticancer activity in the radiated cells.

The epithelial cellular adhesion molecule (EPCAM) is the fourth common hub that was introduced in the present analysis. Mal A et al. have published a document about the overexpression of EPCAM which is associated with a higher degree of cellular plasticity and heterogeneity that encourages the radioresistant performance of cancer cells.[17] As it is depicted in Table 2, EPCAM is down-regulated in the two methods of radiation. Therefore, the expression change of EPCAM cannot be considered a radioresistant activity in the radiated cells.

Another common hub is prothrombin or coagulation factor II (F2) which is up-regulated in both irradiated cells.

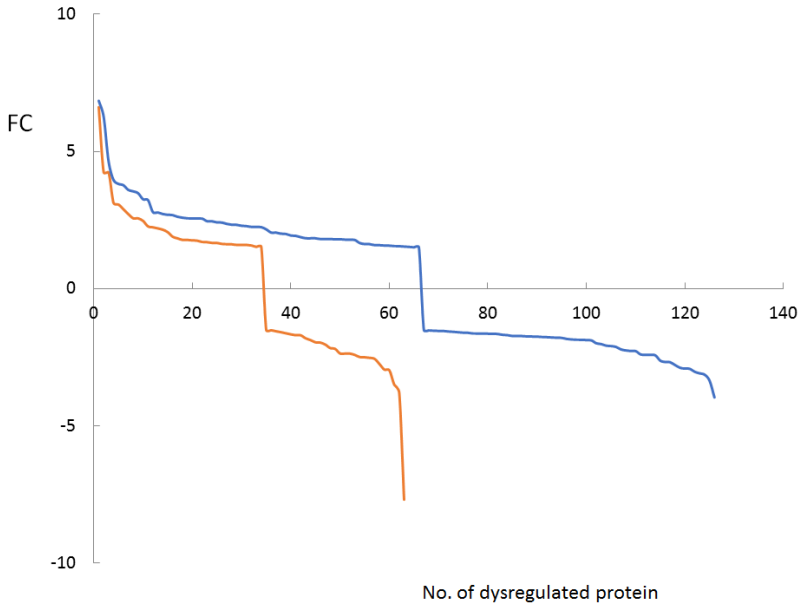

Figure 1. Fold Change Alteration for Significant Dysregulated Proteins ($1.5 \leq FC \leq (-1.5)$) of DU145-HF Cells Relative to the DU145-PAR Cells (Orange Curve) and DU145-CF Cells Versus DU145-PAR Cells (Blue Curve). The analyzed protein is presented as a number based on its ranked FC

Table 1. List of Hub Nodes of the Two Networks (Networks of DU145-CF and DU145-HF Cells)

Query Term	Degree in DU145- HF Network	Degree in DU145-CF Network	BC in DU145- HF Network	BC in DU145-CF Network
ALB	102	105	0.036	0.022
CD44	87	92	0.008	0.018
THBS1	72	70	0.006	0.002
VTN	-	70	-	0.007
EPCAM	55	56	0.008	0.006
OCLN	-	54	-	0.004
F2	49	47	0.004	0.003
KRT19	48	44	0.001	0.003
HSPB1	-	44	-	0.002
MCAM	43	38	0.001	0.000
FLNA	40	-	0.006	-
AHSG	38	-	0.005	-
SERPINC1	37	-	0.002	-

Note: The common hubs are bold. VTN, OCLN, and HSPB1 are the hubs of DU145-CF and FLNA; AHSG, and SERPINC1 are hub nodes of DU145-HF cells and do not appear as common hubs. BC is the abbreviation of betweenness centrality.

Table 2. Fold Changes of the Hub Node for DU145-CF and DU145-HF Cells

Query Term	FC for DU145-CF Cells	FC for DU145-HF Cells
ALB	2.6	2
CD44	2.3	1.9
THBS1	2.5	1.6
VTN	6.3	-
EPCAM	-2.3	-1.7
OCLN	-1.8	-
F2	4.6	3.1
KRT19	-1.8	-1.6
HSPB1	-2	-2.4
MCAM	2.6	2.3
FLNA	-	-2.7
AHSG	3.8	2.9
SERPINC1	2.8	1.8

Note: If $(-1.5) < FC < 1.5$, the value was presented as (-).

As it is reported, impairment of coagulation hemostatic may happen after preoperative irradiation.[18] Keratin19 (KRT19) is the sixth common hub node. An investigation indicates that the expression of KRT19 is correlated to the poor overall survival of breast cancer patients.[19] The last common hub is the melanoma cell adhesion molecule (MCAM) which is up-regulated in both studied cells. Evidence indicates that MCAM overexpression can promote the tumorigenicity of human osteoblastic PCa cells. The initiation of metastasis in breast cancer is attributed to the MCAM.[20] It seems that the up-regulation of the MCAM can lead to the promotion of cancer and an increase in radiation resistance activity in the treated cells.

Vitronectin (VTN), occludin (OCLN), and heat shock protein B1 (HSPB1) are the hubs of DU145-CF cells that do not appear as hubs in the network of DU145-HF cells. As it is shown in Table 2, VTN is up-regulated with $FC = 6.3$. On the basis of the literature, the amplified level of VTN in breast cancer patients is associated with a poor survival rate relative to the patients without an amplified level of VTN.[21] Evidence indicates that irradiation up-regulates VTN in the treated mice, which is accompanied by increased lung fibrosis.[22] OCLN is a down-regulated hub in the DU145-CF cells network. It is reported

that occludin and claudin-1 as tight junction marker proteins are overexpressed in response to conventional fractionated radiation in the course of 2 weeks of fractionation.[23] In the original research, this hub protein is down-regulated. Different outcomes of experiments may be related to the samples (mice oral mucosa versus cell line). HSPB1 is another downregulated hub whose upregulation was highlighted as a radioresistant agent.[24] Since HSPB1 was down-regulated in the DU145-CF cells, it can be concluded that the mentioned cells are radio sensitive considering the function of HSPB1.

Filamin A (FLNA), alpha 2-HS glycoprotein (AHSG), and alpha-1 antitrypsin (SERPINC1) are the three hubs of the DU145-HF cells network that are not pointed as hubs in the DU145-CF cells network. On the basis of previous investigations, the radio-sensitization effect of curcumin on bladder cancer is mediated by FLNA.[25] Therefore, FLNA down-regulation can be interpreted as an increasing mode in the radio-resistance activity of the treated cells. AHSG, another upregulated hub, is highlighted in the report of Arjmand et al[26] about the deregulation of AHSG and SERPINA1 in response to low-level laser radiation. SERPINC1, the third up-regulated hub protein, was down-regulated in the serum of an irradiated rat by low-level laser radiation.[27] Like FLNA, the expression change of SERPINC1 in the cell line and rat serum follows the opposite direction.

Conclusion

The findings indicate that 70% of central deregulated proteins in the DU145 cells under CF and HF radiation are similar and induce radio-resistance activity in the survived cells. This effect is controlled by ALB, CD44, THBS1, EPCAM, F2, KRT19, and MCAM. The function of six central proteins (MCAM, VTM, OCLN, HSPB1, FLNA, AHSG, and SERPINC1) discriminates the destiny of the two groups of irradiated cells. Based on these findings, it seems that DU145-CF cells are more radio-resistant relative to the DU145-UF cells.

Ethical Considerations

Not applicable.

Conflict of Interests

The authors declare that they have no conflict of interest.

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