Original Article

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TANAFFOS

Introducing Critical Genes Related to X-ray Radiation in Human Bronchial Epithelial BEAS-2B Cells

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Background: X-ray radiation application in radiology is a challenging subject for patients. Understanding the molecular events triggered by X-ray radiation can provide a suitable protocol for maintaining patient health. The primary aim of this study is to explore crucial affected genes in this regard.

Materials and Methods: Extraction and validation of data from the Gene Expression Omnibus (GEO) database is the first step in identifying significantly differentially expressed genes (DEGs). Analysis of gene expression profiles of human bronchial epithelial BEAS-2B cells through a directed protein-protein interaction (PPI) network was used to identify the key targeted genes affected by X-ray radiation.

Results: Results of the analyzed directed PPI networks, based on expression, activation, inhibition, and reaction actions, and considering outdegree values, showed that IL1B, CCND1, RAC2, NDC80, and MIB1 are the crucial genes targeted by X-ray.

Conclusion: In conclusion, upregulation of IL1B, RAC2, and CCND1, which are associated with cellular damages and downregulation of NDC80 and MIB1, which protect the treated cells, were pointed out as the major molecular events in response to X-ray radiation.

Keywords: X-ray radiation, Human, Lung, Gene expression, Network analysis

INTRODUCTION

The rise of X-ray imaging in the late 1800s has been one of the most noteworthy revelations in medical science. The use of X-rays is expanding quickly with the presentation of modern radiation-oriented therapeutic practices. Even though it has significant diagnostic benefits, extensive exposure to X-ray imaging has been appeared to be related with multiple dose-dependent health risks. X-ray is an ionizing electromagnetic radiation. Both low and high doses of ionizing radiation can be harm for living cells

depending on the quantity of the dose, dose rate, sex, age, and the type of the target (1-4). The occurrence of the adverse effects of X-rays had caused scientific research in this field. Awareness of molecular events after X-ray radiation can be effective in early diagnosis of damage and in preventing it. Gene product and proteomic examinations via protein-protein interaction (PPI) network analysis are capable tools which commonly used in clinical research and finding biomarker panels related to diseases (5-8). In this method, big data can be used to map networks of genes or proteins related to disease, based on interactions that depend on their physical or functional association. Hubs, bottlenecks, and hub-bottlenecks are the key features of a PPI network, which are commonly used to identify the molecular aspects of diseases (9, 10). Riego et al. have introduced genes FDXR, CDKN1A, and MDM2 as a candidate biomarker panel for X-ray radiation (11). O'Brien et al. reported an increasing level of FDXR transcript as a biomarker after X-ray exposure (12). Discovering new and additional biomarkers necessitates further research in this field. The primary objective of this study was to identify and determine a warning biomarker through PPI network analysis to prevent cell damage in the human lung following X-ray radiation.

MATERIALS AND METHODS

Data collection

To find the effect of X-ray radiation on the human lung, GSE166340 was mined from the GEO database (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE166340). Data about gene expression profiles of human bronchial epithelial BEAS-2B cells which are treated with 6 MV X-ray photon beam from a Varian linac (Varian Medical Systems, Palo Alto, CA, USA) with a source-to-skin distance of 100 cm, irradiation field of 20 × 20 cm, and irradiation dose of 6 Gy at a dose rate of 300 cGy min-1 and the control individuals were retrieved.

Pre-evaluation analysis

Gene expression profiles of the radiated group were compared with those of the controls via the GEO2R program. A mean-variance trend plot was applied to visualize the final and fitted dispersion values. The significantly up- and downregulated DEGs were visualized via a mean-difference plot. Separation of radiated samples from controls was shown via Uniform Manifold Approximation and Projection (UMAP) plot. The number of dysregulated genes and the significant DEGs were shown by a Venn diagram. The significant DEGs were determined based on adjusted p-value < 0.05.

PPI network analysis

The significant DEGs were included in CluePedia v 1.5.7; the application of Cytoscape software v 3.7.2 was used to form a directed PPI network. The recognized significant DEGs were connected via expression, activation, inhibition, and reaction actions. The directed PPI networks were analyzed via the "Network analyzer" application of Cytoscape software, and the main connected components of the constructed network were laid out based on outdegree value.

RESULTS

As presented in Figure 1, the mean-variance trend plot indicates that the fitted dots (especially the dots with higher values of mean of normalized counts) are characterized by an approximately constant value of dispersion. The dysregulated genes and the significant DEGs are visualized via a mean-difference plot (Figure 2).

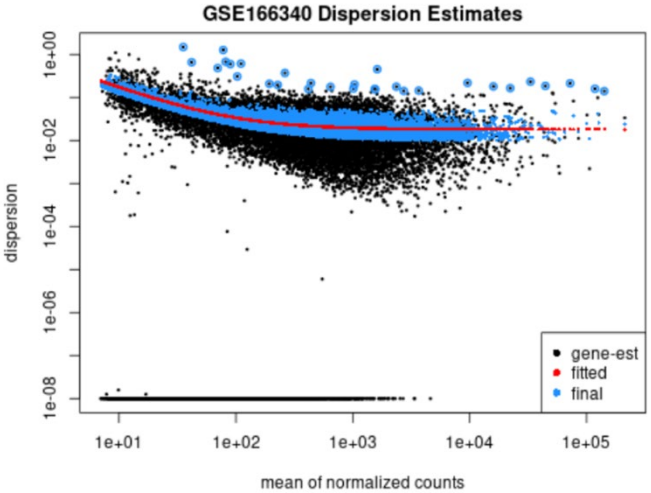

Figure 1. Mean-variance trend plot of compared gene expression profiles of the radiated human bronchial epithelial BEAS-2B cells versus the control cells

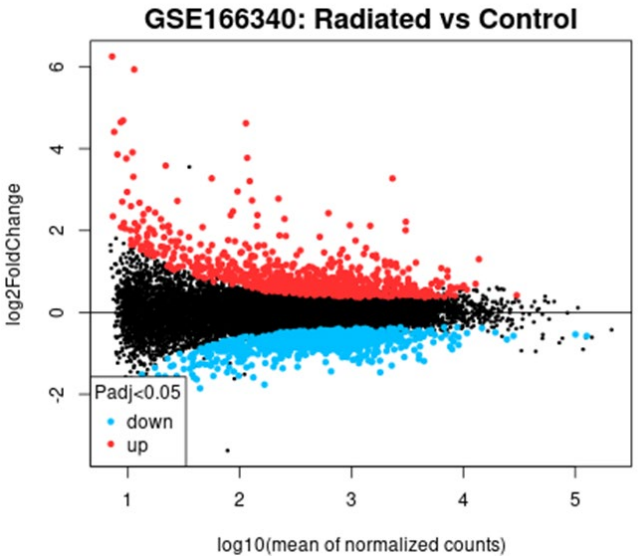

Figure 2. Mean-difference plot of compared gene expression profiles of the radiated human bronchial epithelial BEAS-2B cells versus the control cells

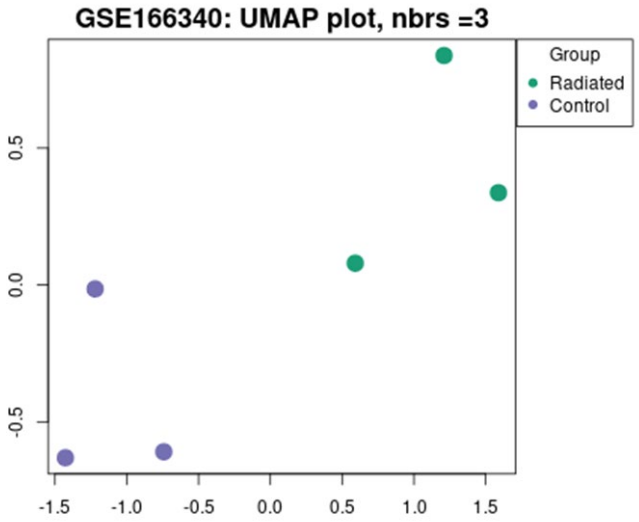

Figure 3. Uniform Manifold Approximation and Projection (UMAP) plot of compared gene expression profiles of the radiated human bronchial epithelial BEAS-2B cells versus the control cells

As depicted in Figure 3 (Uniform Manifold Approximation and Projection (UMAP) plot), the radiated cells are separated from controls. A total of 1629 significant DEGs were identified that are targeted by X-ray radiation (Figure 4).

The significant DEGs were included in CluePedia, and the recognized individuals were connected via expression action. As shown in Figure 5, IL1B, CCND1, and RAC2 are the top three genes that act as expression regulators. The action map, including activation for the recognized DEGs, is presented in Figure 6. IL1B, CCND1, RAC2, MIB1, and NDC80 appeared as the top actor genes in the action map. The significant DEGs were assessed via the inhibition relationship between the nodes. Again, CCND1 and RAC2 are highlighted as top genes considering outdegree value (Figure 7). In the other step, connections between the queried DEGs were evaluated via reaction action (Figure 8). As depicted in Figure 8, IL1B, CCND1, RAC2, MIB1, and NDC80 were identified as the top regulators in the action map. Finally, expression, activation, inhibition, and reaction are considered to map complex relationships between the studied DEGs. This analysis led to the introduction of IL1B, CCND1, RAC2, MIB1, and NDC80 as the prominent actor DEGs (Figure 9).

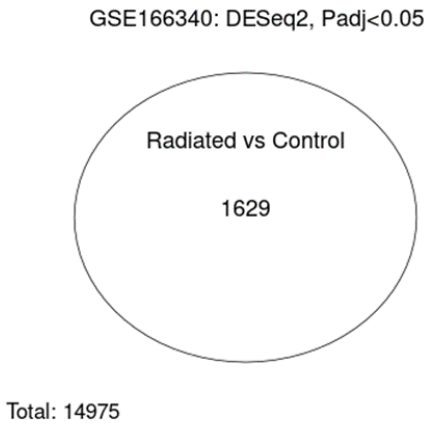

Figure 4. Venn diagram comparing the gene expression profiles of irradiated human bronchial epithelial BEAS-2B cells with those of control cells. A total of 1629 significant DEGs are shown

DISCUSSION

Pre-evaluation analysis showed analyzed data are statistically valid. Suitably fitted data in Figure 1, illustration of significant DEGs in Figure 2, and complete separation of samples in Figure 3 correspond to 1629 significant DEGs (Figure 4). Co-expression relationship determination is a suitable method to study a set of genes (13). The central part of the co-expression map of DEGs is illustrated in Figure 5. IL1B, CCND1, and RAC2 appear as the top nodes of the network. Since the network is laid out

based on outdegree, the mentioned three genes are actors and regulate the expression of many nodes of the studied network. Activation is the other action that is used to form a directed PPI network to explore the critical genes (14). A directed PPI of significant DEGs that are connected by activation action is shown in Figure 6. IL1B, CCND1, RAC2, NDC80, and MIB1are highlighted as top activators of map. Inhibition, like activation and co-expression actions, is applied to analyze the relationship between genes (15). As depicted in Figure 7, CCND1 and RAC2 are actors of the directed PPI network of DEGs, which are connected with inhibition action. The directed PPI network of significant DEGs that are integrated in the interactome via reaction map is presented in Figure 8. IL1B, CCND1, RAC2, NDC80, and MIB1are identified as actors of this map. The final network was constructed from significant DEGs that are linked via expression, activation, inhibition, and reaction (Figure 9). Again, IL1B, CCND1, RAC2, NDC80, and MIB1 appeared as critical DEGs in this PPI network.

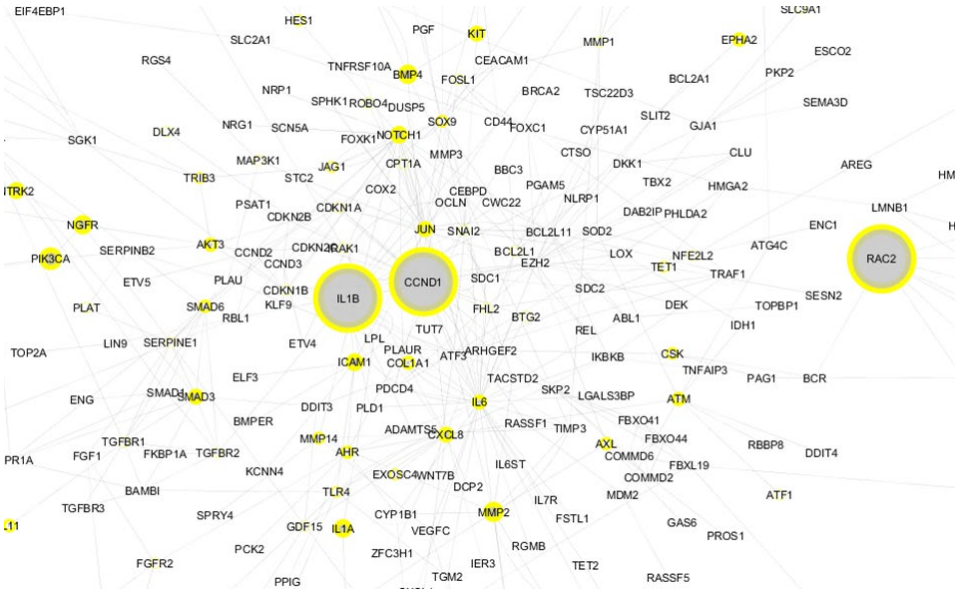

Figure 5. Expression relationship between the elements of the main connected component of the directed PPI network. The recognized DEGs are linked via expression edges. The nodes are laid out based on their outdegree value; the larger size corresponds to a higher outdegree value

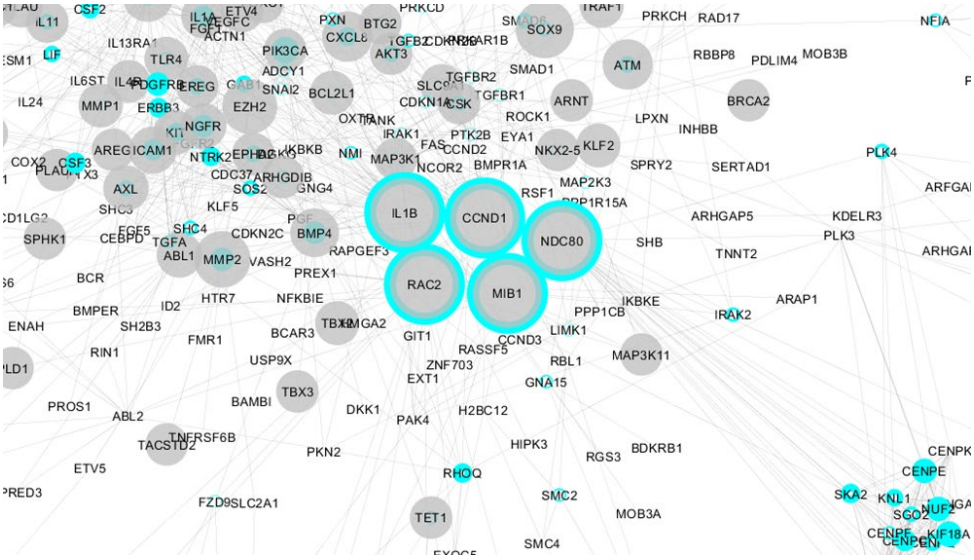

Figure 6. Activation relationship between the elements of the main connected component of the directed PPI network. The recognized DEGs are linked via activation edges. The nodes are laid out based on their outdegree value; the larger size corresponds to a higher outdegree value

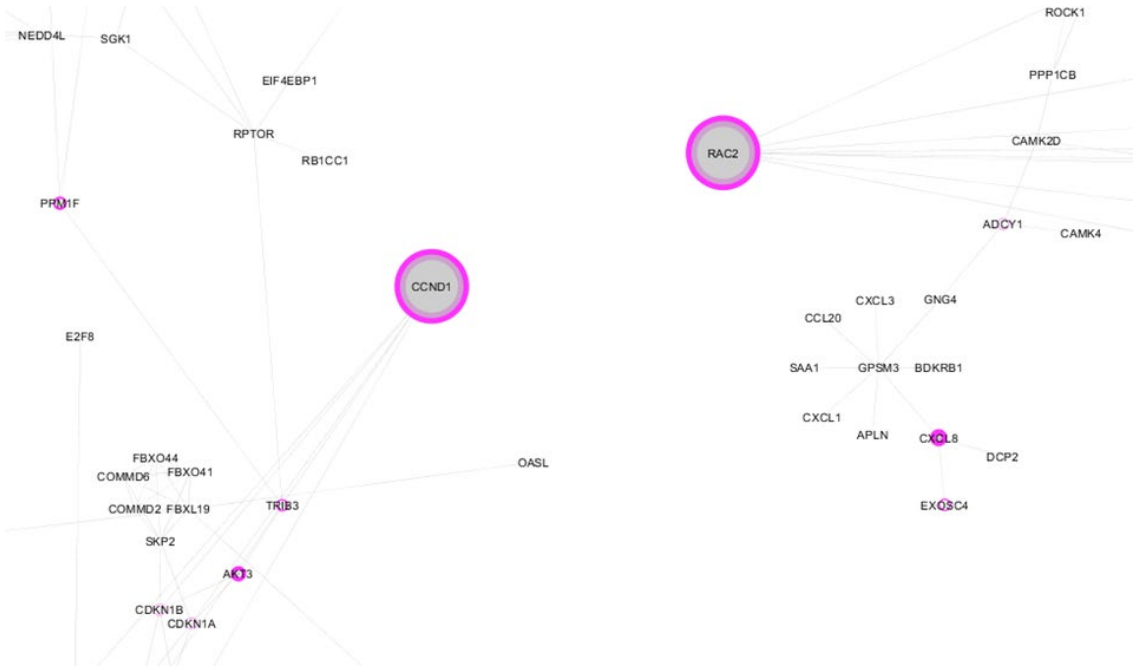

Figure 7. Inhibition of the connection between the elements of the main connected component of the directed PPI network. The recognized DEGs are linked via inhibition links. The nodes are laid out based on their outdegree value; the larger size corresponds to a higher outdegree value

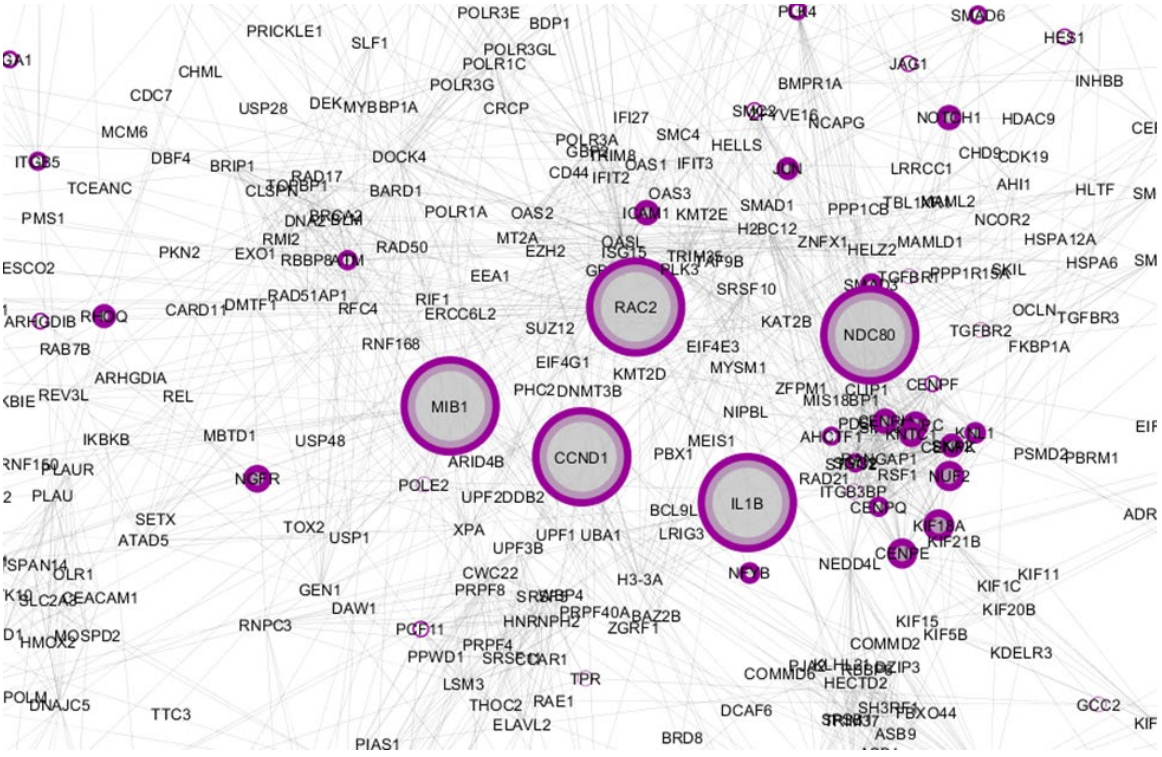

Figure 8. Reaction action between the elements of the main connected component of the directed PPI network. The recognized DEGs are linked via reaction links. The nodes are laid out based on their outdegree value; the larger size corresponds to a higher outdegree value

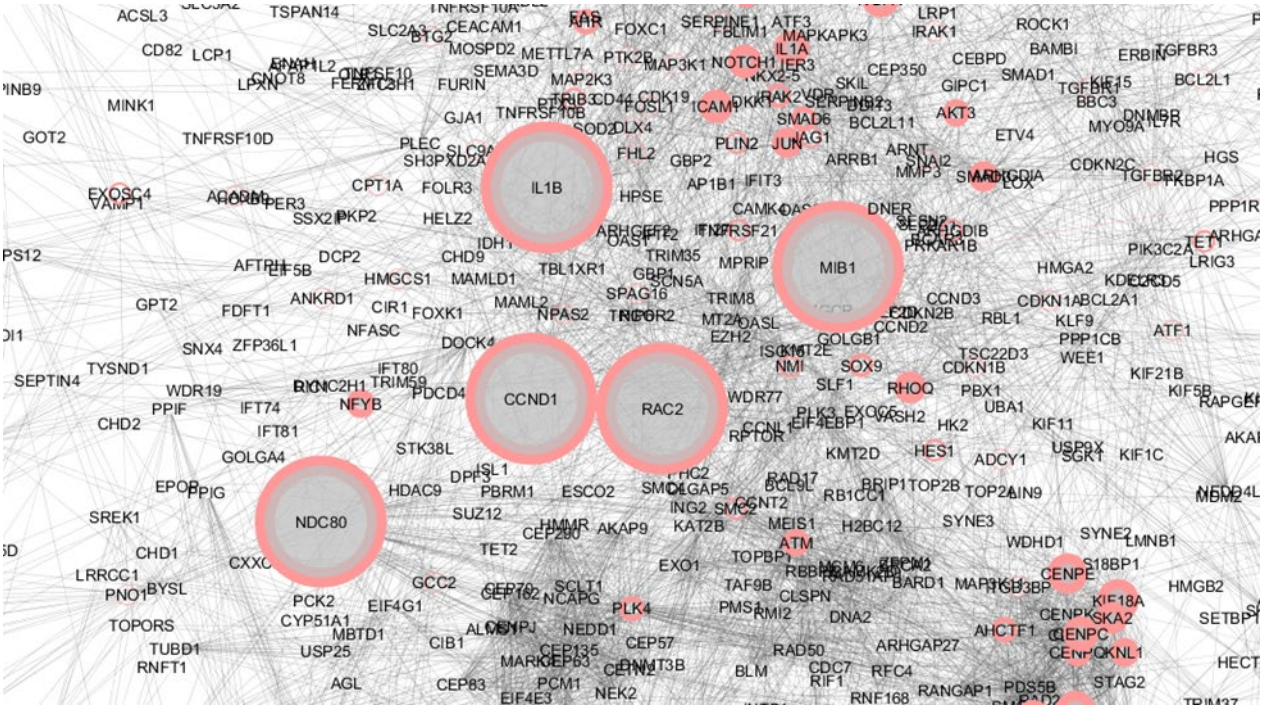

Figure 9. Expression, activation, inhibition, and Reaction actions between the elements of the main connected component of the directed PPI network. The recognized DEGs are linked via reaction links. The nodes are laid out based on their outdegree value; the larger size corresponds to a higher outdegree value

Rac family small GTPase 2 (RAC2) is a common crucial DEG of all studied PPI networks. This gene is upregulated in response to X-ray radiation. RAC2 acts as a GTPase and is expressed entirely in hematopoietic cells. It is suggested that RAC2 plays important roles in the biology of different cell types, such as lymphocytes and neutrophils (16). Investigation indicates that RAC2 was upregulated in clear cell renal cell carcinoma (ccRCC) tissues and cell lines. This malignancy of the urinary system is characterized by progressive metastatic tumor with a high mortality rate (17).

Cyclin D1 (CCND1) is another common gene of all networks. CCND1 is well-known due to its role in tumorigenesis and cell cycle progression (18). Based on the literature, cyclin D1 is alleviated after fractionated radiation of 0.5 Gy of X-rays for 31 d on human tumor cells (19). Cyclin D1 is upregulated in the present study. Interleukin-1 beta (IL1B) is another upregulated DEG that appears as an actor of expression, activation, and reaction maps. Chronic obstructive pulmonary disease (COPD) is known as a chronic and advanced lung disease, including a mixture of lung tissue parenchymal devastation and small airway disease. There is evidence about the upregulation of IL1B in COPD small airway epithelial cells (20).

NDC80 kinetochore complex component (NDC80) is a downregulated DEG that is highlighted as a crucial gene in activation and reaction maps. The positive role of NDC80 in the proliferation and metastasis of cancer cells is confirmed in the literature. Upregulation of NDC80 has been documented in pancreatic and colorectal cancer cells by researchers (21, 22). Wei et al. pointed out the significant role of NDC80 in the development of non-small cell lung cancer (23). It seems downregulation of NDC80 is a cell-protective event after X-ray radiation. The last crucial DEG is MIB E3 ubiquitin protein ligase 1 (MIB1), which is downregulated. Investigation showed MIB1 is overexpressed in a subclass of lung adenocarcinoma and squamous carcinoma samples. Based on this research, overexpression of MIB1 is associated with overall patient survival. The positive role of MIB1 on ferroptosis regulation is highlighted in this report (24). It can be concluded that downregulation of MIB1 by X-ray radiation is a beneficial occasion in the treated cells.

CONCLUSION

In conclusion, the upregulation of IL1B, RAC2, and CCND1, along with the downregulation of NDC80 and MIB1, represents the primary molecular effects observed in human bronchial epithelial BEAS-2B cells in response to X-ray radiation. The upregulation of these critical genes is associated with cellular damage, while the downregulation of certain genes helps protect cell survival. It is suggested that the levels of IL1B, RAC2, and CCND1 in patients exposed to X-ray radiation through diagnostic imaging be investigated as potential biomarkers for assessing damage. These findings could help develop effective protective protocols for patients against X-ray radiation.

Ethical considerations

This project has been approved under the ethical code IR.SBMU.RETECH.REC.1403.105.

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Conflicts of Interest

There is no conflict of interest.

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