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Assessment of Anticancer Effect of Rhodium Nanoparticle-based Photodynamic Therapy via Protein-Protein Interaction Network Analysis

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Abstract

Introduction: Photodynamic therapy, as an efficient and safe method, has attracted the attention of experts. This therapeutic method is based on the application of photosensitizers and light radiation. This study was designed to assess the possible molecular mechanism of rhodium nanoparticle-based photodynamic therapy through protein-protein interaction (PPI) network analysis of proteomic data from the literature.

Methods: Proteomic data about rhodium nanoparticle-based photodynamic therapy effect on the HeLa cell line proteome were retrieved from the literature and were included in the CluePedia application of Cytoscape software to create a directed PPI network**.** The network was analyzed, and the crucial targeted proteins were identified and compared with genes in GeneCards for "HeLa cell line" and "cervical cancer". The common gene and protein were selected and discussed.

Results: A directed PPI network of 105 proteins was formed. Six sub-networks were selected for further investigation. Comparison of the PPI data and the genes from the GeneCards database led to the introduction of HLA-B, CYCS, CD44, HSPB1, and RBBP4 as the critical targeted proteins by the applied treatment.

Conclusion: In conclusion, a sub-network including HLA-B, CYCS, CD44, and HSPB1 and another sub-network containing RBBP4 and its neighbors were highlighted as the core of molecular effects of the applied rhodium nanoparticle-based photodynamic therapy.

Keywords: HeLa cell line, Rhodium, Network analysis, Photodynamic therapy, Cancer

Introduction

Photodynamic therapy, as an attractive method against cancers, is known as a safe and efficient therapeutic protocol. This method is organized based on photosensitizers and light radiation. The development of photosensitizers plays a significant role in the progression of photodynamic therapy.[1-3] Nanoparticle-based photodynamic therapy is applied as an advanced method against cancers. The molecular mechanism of rhodium nanoparticle-based photodynamic therapy is studied via metabolomics and proteomics to find the targeted molecules.[4-6] The HeLa cell line is the first immortalized cancer cell line, derived from cervical cancer. This cell line is used widely to detect photodynamic therapy effects on cancers.[7, 8] The effect of 5-aminoketovalerate photodynamic therapy (ALA-PDT) on HeLa cells revealed that this method has a strong killing effect via the induction of apoptosis in the decrement of cell survival.[8]

Proteomics is a high-throughput method for quantifying proteins in a certain biological sample. Proteomics enables researchers to detect protein expression changes in response to interventions or different induced

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conditions. This method is applied in biomarker discovery and exploring drug targets. The study of the molecular mechanism of diseases and the efficacy of therapeutic methods are the fields that are highly affected by proteomics. Proteomics is known as a suitable tool for cancer detection and research.[9-11] Considering the production of large datasets in proteomics, the application of bioinformatics for the interpretation of findings is essential. Similar to proteomics, other omics such as genomics and metabolomics have been integrated with bioinformatics to study the biological and clinical samples.[12, 13]

PPI network analysis is a method for screening many genes or proteins to determine possible interactions between them and to discriminate a few of them as the key individuals. The regulatory relationship between genes can be discovered via the application of directed PPI networks. In such analyses, possible actions, including activation, inhibition, expression, reaction, binding, catalysis, and post-translational modification, bind the nodes of a network to form a directed interactome. The actor nodes and the most controlled individuals appear as the central nodes of the directed PPI network. In the present study, proteomic data about the effect of rhodium nanoparticle-based photodynamic therapy (by using NIR laser radiation) on HeLa cells are extracted from Machuca et al's report[14] and are analyzed via a directed PPI network to find the key core of molecular events.

Methods

Data Extraction

Results of a proteomic study about administration rhodium nanoparticle-based photodynamic therapy on HeLa cell line was extracted from literature. The treated cells with RhNPs were exposed to an 800-nm NIR laser operating at 2.5 W cm^{-2} for 10 minutes.[14] This document is published by Machuca et al in 2024. Since cellular assessment provides useful information about diseases, the data were considered for further analysis. The significant proteins were selected for further analysis via PPI network assessment.

Directed PPI Network Analysis

The extracted proteins were included in a directed PPI network to form an interactome. The nodes were connected via activation, inhibition, expression, binding, catalysis, reaction, and post-translational modification (PTM) actions to explore the regulatory relationship between the studied proteins. The nodes of the sub-networks were assessed to find the major connected components of the PPI network.

GeneCards Analysis

"HeLa cell line" was searched in the GeneCards database to find the related genes. The retrieved genes were grouped based on the relevance score. The data were grouped using Sturges' Rule.[15] The nodes of the six components of the PPI network were searched in the top groups of GeneCards analysis as the critical proteins. Since the HeLa cell line is derived from cervical cancer, the genes related to "cervical cancer" were retrieved from GeneCards, and using a similar method for the genes of "HeLa cell line", they were clustered and the suitable groups of genes were compared with the proteins of the six sub-networks.

Results

Data Extract and PPI Analysis

A total of 108 proteins were extracted from Machuca et al's report.[14] One uncharacterized individual was excluded from more investigations. Among the remaining 107 proteins, 105 individuals were recognized, and they formed a directed PPI network via the CleuPedia application of Cytoscape software (Figure 1). As depicted in Figure 1, 49 proteins remained isolated; a pair of AKR1C2-AKR1C3,

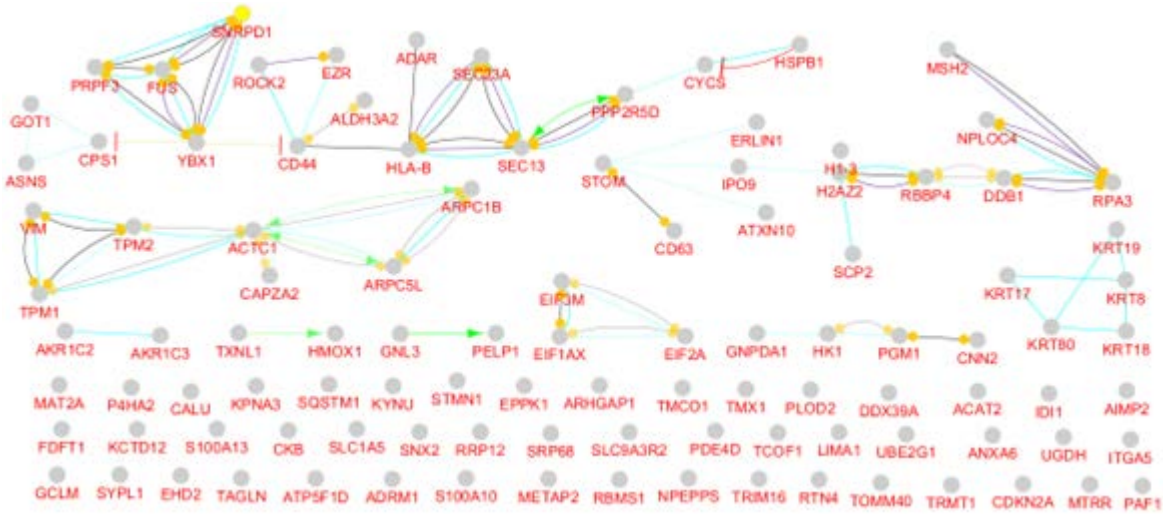

Figure 1. Directed PPI network of 105 recognized proteins in response to phototherapy. Blue, green, red, yellow, purple, pink, and black colors refer to binding, activation, inhibition, expression, catalysis, post-translational modification, and reaction, respectively

a triple of EIF1AX-EIF2A-EIF3M, and a pentad of KRT8, KRT17, KRT18, KRT19, and KRT80, besides others (six components), are constructed. The six subnetworks that possess informative interactions were selected for further investigation (Figure 2). As demonstrated in Figure 2, the six components include 45 proteins, which are connected with 82 edges.

GeneCards Analysis

A total of 5,117 genes related to "HeLa cell line" were extracted from the GeneCards database. Based on Sturgess equation, data should be arranged in 13 groups. The lower and upper values of the relevance score are 0.21 and 3.3, respectively. Therefore, data should be arranged in groups with a 0.24 interval of the relevance score. The analysis demonstrated that 5,080 genes, which are characterized by the relevance score (which refers to the relationship between genes and subjects) of 0.21-0.45, belong to the first group, and the remaining 37 individuals are distributed in the other 12 groups. Therefore, the common proteins of the six subnetworks and the genes of the 12 groups of the up GeneCards analysis were selected as the critical proteins in response to photothermal treatment. The evaluation revealed that there is no common protein or gene between the two compared sets.

Since HeLa cells are derived from cervical cancer, "cervical cancer" was searched in GeneCards, and like the method for "HeLa cell line", the data including 125,299 genes were grouped into 18 clusters. A total of 122,609 genes were arranged in the first group, and the remaining 2,690 genes were distributed in 17 clusters. The first cluster was excluded from further analysis, and the other clusters were considered to be assessed (Figure 3). As depicted in Figure 3, the first group, which contains 2,013 genes, does not have a similar trend to that of the other groups. Therefore, the 45 proteins of the six sub-networks were compared with 677 genes of the 16 clusters. The list of common genes and proteins is shown in Table 1.

Discussion

As depicted in Figure 2, HLA-B, CYCS, CD44, and HSPB1 are located in the main connected component, and RBBP4 belongs to the second major component. Histone-binding protein RBBP4 is an essential element to assemble heterochromatin and prevent the transition of cell destiny from pluripotency to totipotency. RBBP4 plays a role in colon cancer malignancy progression; it has also been introduced as a biomarker for the diagnosis and prognosis of non-small-cell lung cancer.[16-18] It seems that its down-regulation corresponds to the anticancer property of the applied treatment.

Human leukocyte antigen-B (HLA-B) is the upregulated protein in response to the administrated photo-thermodynamic treatment. An investigation indicates that HLA-B is involved in encoding the heavy chain of major histocompatibility class I, which is responsible for innate and adaptive immunity. Based on the literature, HLA-B is associated with promising overall survival in basal-like tumors.[19, 20]

Cytochrome C, somatic (CYCS) is another targeted protein by treatment. Testicular and somatic cytochrome C are two isoforms of this cytochrome. CYCS is a

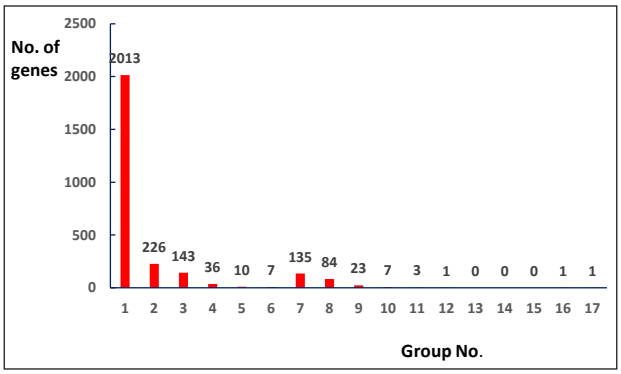

Figure 3. Presentation of 17 groups of related genes of cervical cancer among 122,609 individuals from the GeneCards database. The groups are arranged from left to right based on the increment of the relevance score.

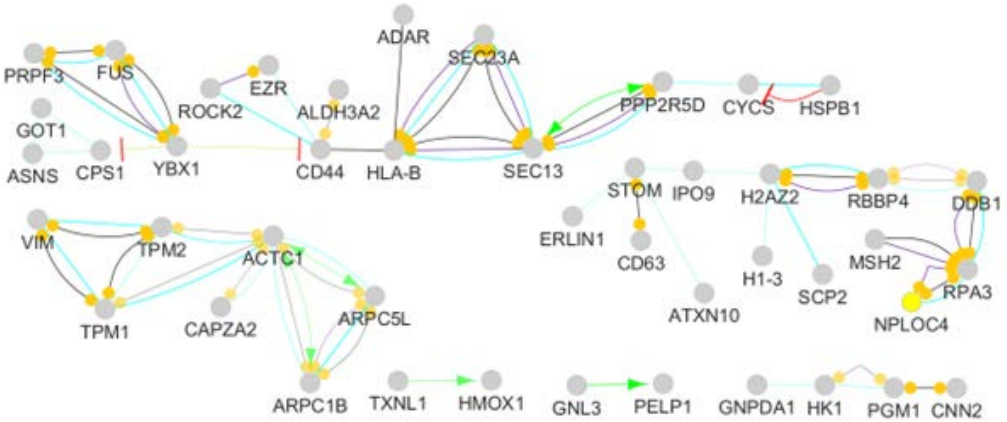

Figure 2. The six selected components of the directed PPI network of 105 recognized proteins in response to phototherapy. Blue, green, red, yellow, purple, pink, and black colors refer to binding, activation, inhibition, expression, catalysis, post-translational modification, and reaction, respectively. The isolated nodes and the components which were formed from the family members of a certain protein were deleted from the PPI network.

Table 1. List of common proteins between the six selected components of PPI network and the top 16 gene groups of cervical cancer related genes from GeneCards

No.	Gene or protein name	Relevance score in GeneCards	Expression in the proteomic study
1	HLA-B	285	Upregulation
2	CYCS	441	Upregulation
3	CD44	487	Upregulation
4	HSPB1	524	downregulation
5	RBBP4	676	downregulation

crucial element of the respiratory chain.[21, 22] This protein is upregulated in the treated cancer cell line. An investigation demonstrated that the upregulation of cytochrome C leads to the enhancement of caspase activation, which accelerates cell death in response to apoptotic stimulation.[23] An experiment revealed that activation of the mitochondrial pathway, accompanied by the release of cytochrome c protein, led to the construction of the apoptosome complex and the depolarization and inhibition of cell proliferation.[24]

CD44 antigen (CD44) is another upregulated targeted protein. CD44 and CD44s are introduced as the standard isoform of this transmembrane glycoprotein. CD44 is involved in multiple physiological processes. Therefore, its unusual expression is accompanied by the initiation and progression of tumors. The promotion of epithelial-mesenchymal transition in cancer stem cells is attributed to CD44. Various essential signaling pathways related to invasion, proliferation, and therapy resistance in cancer are attributed to CD44.[25]

The last critical down-regulated protein is heat shock protein HSPB1. The single inhibition relationship in Figure 2 is located between HSPB1 and CYCs. Since CYCS is up-regulated, the down-regulation of HSPB1 that inhibits CYCS corresponds with more activation of CYCS in response to photodynamic therapy. It has been reported that various essential cellular functions in normal cells are regulated by human small heat shock proteins such as HSPB1. Overexpression of HSPB1 has been confirmed in many cancer cells.[26] As demonstrated in Figure 2, the direct connection between HSPB1 and CYCS and also the direct neighborhood between CD44 and HLA-B refer to the important functional role of the analyzed main component in response to the applied photodynamic therapy method. It seems that this sub-network is the core of the molecular event in the administrated treatment.

Conclusion

In conclusion, HLA-B, CYCS, CD44, HSPB1, and RBBP4 are the major targeted proteins in rhodium nanoparticle-based photodynamic therapy. There was a close relationship between HLA-B, CYCS, CD44, and HSPB1 in the studied PPI network, which corresponds to the importance of this protein core in response to applied treatment. Findings can improve knowledge about the properties of photosensitizers (especially about Rhodium Nanoparticle-based Photodynamic Therapy), which are known as the principal parts of photodynamic therapy. However, access to the patient tissue is problematic. The development of photodynamic therapy is an ongoing progression in medicine, and it can be considered a complementary method in combination with other therapeutic protocols.

Authors' Contribution

Conceptualization: Zahra Razzaghi, Farideh Razi.
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Methodology: Zahra Razzaghi.
Project administration: Zahra Razzaghi, Nikoo Hossein-Khannazer, Fatemeh Bandarian.
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Software: Alireza Ahmadzadeh.
Supervision: Zahra Razzaghi, Babak Arjmand, Farideh Razi.
Validation: Alireza Ahmadzadeh.
Visualization: Nikoo Hossein-Khannazer.
Writing–original draft: Nikoo Hossein-Khannazer, Babak Arjmand.
Writing–review & editing: Zahra Razzaghi, Fatemeh Bandarian, Fatemeh Bandarian.

Competing Interests

There is no conflict of interest.

Ethical Approval

This project is approved via IR.SBMU.LASER.REC.1403.044 Ethical code.

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