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Research Article

The Impact of Olaparib on Metabolic Pathways in Triple Negative Breast Cancer: A Bioinformatics Approach

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Abstract

Aim: Triple-negative breast cancer (TNBC) is a highly aggressive subtype of breast cancer (BC) characterized by the lacking estrogen receptors, progesterone receptors, and HER2 expression, making it challenging to treat with targeted therapies. Olaparib, a PARP inhibitor, has shown promise in treating TNBC, particularly in patients with BRCA1 or BRCA2 mutations. This study aims to elucidate the metabolic pathways affected by olaparib in TNBC using bioinformatics analysis.

Material and Method: For bioinformatics analysis, mRNA microarray data of control MDA-MB-468 cells (non-treated) and OlaR MDA-MB-468 (3μM olaparib-treated MDA-MB-468 cells) with the study numbered GSE165914 were downloaded from Gene Expression Omnibus (GEO) database. GEO2R was used to analyze and identify differentially expressed genes (DEGs). Gene ontology (GO) and Kyoto gene and genome encyclopedia (KEGG) analysis were carried out for DEGs to determine significant genes and the biological pathways influenced by olaparib treatment. Protein-protein interaction (PPI) network analysis further identified key proteins and interactions within these pathways.

Results: For GEO2R analysis adjusted P-value<0.05 and |log2FC|>1.0 were selected. The results revealed the upregulation of 2277 genes and downregulation of 2298 genes in olaparib-treated cells compared to the controls. It was reported that DEGs enriched in pathways including, metabolic pathways, pathways in cancer, chemical carcinogenesis - reactive oxygen species, cell cycle, autophagy - animal, Efferocytosis and TNF signaling pathway. Both upregulated and downregulated DEGs were associated with metabolic pathways. Moreover, NDUFA5, NDUFA6, NDUFS6, NDUFB3, NDUFB10, NDUFB7, NDUFA7, NDUFA9, H2AC8, H2AC13, H2AC17, H4C11, H4C12, H2BC12, H2BC21 and H2BC4 were identified as the most significant candidate genes.

Conclusion: This comprehensive bioinformatics approach provides insights into the molecular mechanisms of olaparib's action and identifies potential targets for combination therapies to enhance treatment efficacy in breast cancer.

Keywords: Triple-negative breast cancer, olaparib, bioinformatics, gene expression omnibus, gene expression

INTRODUCTION

Breast cancer (BC) is the most prevalent malignant tumor and cause of cancer-related mortality among women all around the world. It is a heterogeneous disorder with various molecular subtypes representing distinct biological, histological and clinical evidences (1,2).

Based on the presence of molecular biomarkers, BC can be classified into 3 subtypes including, BC expressing hormone receptor (progesterone receptors (PR+), estrogen receptors (ER+)), BC expressing human epidermal receptors 2 (HER2+) and Triple-negative breast cancer (TNBC) (ER−, PR−, HER2−) (2,3). TNBC is a very complex malignancy with a wide variety of molecular subgroups (4). It is one of the most aggressive type of BC, with early metastasis, a quick recurrence, and a dismal prognosis (5). The past few decades have seen little advancement in therapy due to the TNBC's high heterogeneity (5). Roughly 20-30% of TNBC patients are reported to have a proven BRCA1/2 mutation (5).

The therapeutic strategies should be based on the molecular properties of BC. It is a challenge to clinicians to manage TNBCs due to the limited availability of targeted therapies and unfavorable results (6).

Many clinical trials, such as a phase 3 trial of veliparib, phase 1/2 trials of niraparib and talazoparib, and phase 2/3 trials of olaparib, are investigating the use of PARP

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inhibitors (PARPi) in the treatment of early-stage BC (7).

Olaparib, an oral PARPi, suppresses both PARP catalytic activity and traps the PARP enzymes at damaged DNA (8). Olaparib also represents promising anti-tumor effects in patients with germline BRCA-positive HER-negative metastatic BC (9). The cytotoxic and apoptotic effects of olaparib in combination with chemotherapy, immunotherapy, and targeted therapies were reported in the recent studies (10-12). Sinha and colleagues reported that olaparib increased the Resveratrol-mediated apoptosis in BC cells by suppressing the homologous recombination repair pathway (11).

Despite the therapeutic potential of olaparib, the number of studies investigating its molecular mechanisms and effects in BC is relatively limited. Therefore, studies to analyze the underlying mechanisms in olaparib therapy for BC are ongoing.

In this study, we focused on analyzing genes expression profiles in BC cell lines (MDA-MB-468 cells). Recently, bioinformatics analysis has become a prevalent tool for investigating the potential molecular mechanisms and therapeutic targets of various diseases. The identification of novel biomarkers that can be used for diagnostic and prognostic purposes is very important. In this study, we aimed to investigate the genes and mechanisms which are involved in olaparib therapy. For this purpose, we used bioinformatics tools to investigate target genes and interaction networks between these genes to get deeper insight into the mechanisms underlying olaparib treatment in BC.

MATERIAL AND METHOD

Microarray Data

For genome-wide gene expression profiling between control MDA-MB-468 cells (non-treated) and OlaR MDA-MB-468 (3μM olaparib-treated MDA-MB-468 cells), we conducted a microarray analysis using the study numbered GSE165914. The high-throughput gene expression microarray datasets (GSE165914) were obtained and downloaded from the freely accessible GEO database (https://www.ncbi.nlm.nih.gov/geo/).

Screening and Identification of Differentially Expressed Genes (DEGs)

DEGs were screened and identified by comparing OlaR MDA-MB-468 cells with control MDA-MB-468 cells. The DEGs from the datasets were analyzed by using the GEO2R online statistical tool (https://www.ncbi.nlm.nih.gov/geo/geo2r). The adjusted P-value and |log2FC| were calculated, and genes meeting the criteria of an adjusted P-value<0.05 and |log2FC|>1.0 were considered DEGs.

Gene Ontology (GO) and KEGG Pathway Enrichment Analysis

For functional annotation of GO and analysis of KEGG pathway enrichment, we utilized the web-based DAVID 6.8 tool (DAVID; https://david.ncifcrf.gov). To observe the functional enrichment of the DEGs, the results obtained from DAVID were imported into GO. The DEGs were enriched with 3 categories including, molecular function (MF), cell components (CC) and biological processes (BP) (13).

Construction of Protein-Protein Interaction (PPI) Network

To predict the constructed PPI network and the connections between the DEGs, STRING was utilized. The PPI network were extracted using a total interaction score greater than 0.9. The PPI interactions between the statistically significant DEGs were then interpreted using the Cytoscape software (www.cytoscape.org) (14).

Screening of Hub Gene and Module Analysis

Based on the PPI network of DEGs, five topological algorithms (Maximal Clique Centrality, Radiality Centrality, Edge Percolated Component, and Stress Centrality and Density of Maximum Neighborhood Component) in the cytoHubba plugin of Cytoscape were used to identify hub genes.

RESULTS

Identification of Up-Regulated and 2298 Down-Regulated Genes

We carried out gene expression profile analysis of OlaR MDA-MB-468 cells in comparison with the control MDA-MB-468 cells. We used GSE165914 GEO datastes. In the comparison of olaparib treated cells to the control group, olaparib enhanced the expression of 2277 genes and reduced the expression of 2298 genes (Figure 1).

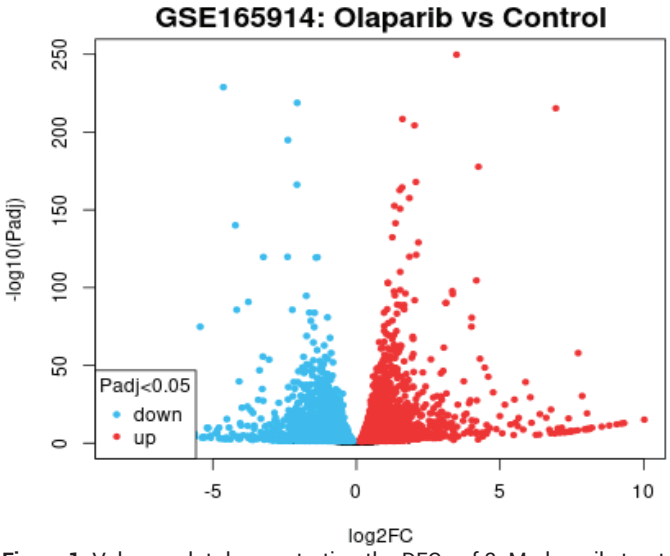

Figure 1. Volcano plot demonstrating the DEGs of 3μM olaparib-treated MDA-MB-468 vs.control, each dot in the volcano map shows a gene; (red = upregulated, blue = downregulated)

GO Functional and KEGG Pathway Enrichment Analysis of the DEGs

Terms related to molecular activities, biological processes, and signaling pathways were used to identify KEGG pathway-enriched genes and potential Gene Ontology categorization.

Base on the GO analysis, the molecular function (MF) mediated by upregulated DEGs were mostly concentrated in ATP binding, ATP hydrolysis activity, unfolded protein binding, nuclear receptor activity, DNA helicase activity and ATP-dependent protein folding chaperone ($p<0.05$). The results of cellular component (CC) were mainly concentrated in proteasome complex, U2-type precatalytic spliceosome, vesicle membrane, U4/U6 x U5 tri-snRNP complex and ciliary tip ($p<0.05$). Moreover, the results of the biological processes (BP) represented that cell division, DNA repair, cilium assembly, ubiquitin-dependent protein catabolic process, protein folding, protein polyubiquitination, proton transmembrane transport, chromosome segregation, DNA duplex unwinding, protein K48-linked ubiquitination, cellular respiration, regulation of macroautophagy, proton motive force-driven mitochondrial ATP synthesis, DNA-templated DNA replication, mitochondrial electron transport, NADH to ubiquinone and DNA unwinding involved in DNA replication were significant enrichment items (Figure 2A) ($p<0.05$).

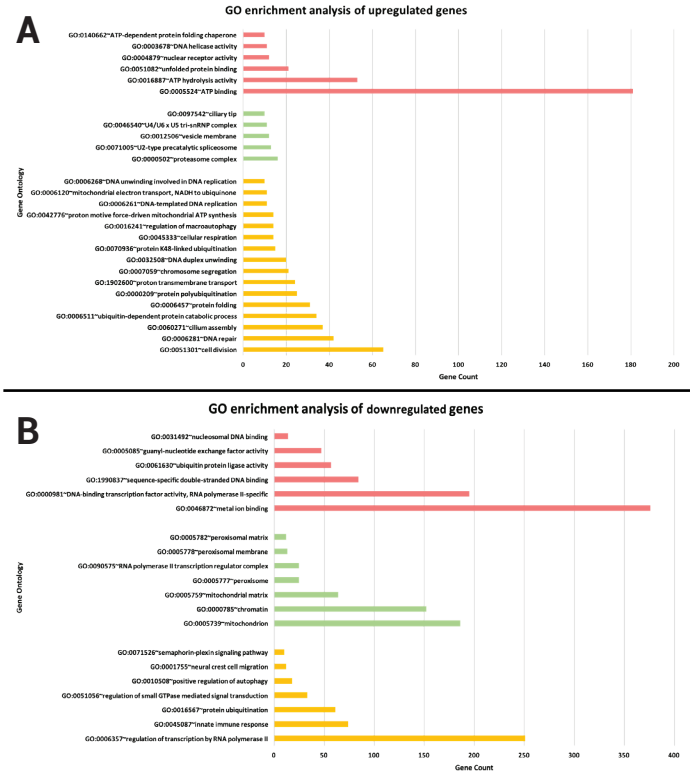

Figure 2. GO enrichment analysis of upregulated and downregulated genes; **A.** OlaR MDA-MB-468 cells compared to the control MDA-MB-468 cells upregulated DEGs; **B.** OlaR MDA-MB-468 cells compared to the control MDA-MB-468 cells downregulated DEGs; Pink: MF, Green: CC and orange: BP

Furthermore, the results of MF mediated by downregulated DEGs were mainly concentrated in ion binding, DNA-binding transcription factor activity, RNA polymerase II-specific, sequence-specific double-stranded DNA binding, ubiquitin protein ligase activity, guanyl, nucleotide exchange factor activity and nucleosomal DNA binding ($p<0.05$). The results of CC showed that mitochondrion, chromatin, mitochondrial matrix, peroxisome, RNA polymerase II transcription regulator complex, peroxisomal membrane, peroxisomal matrix were important enrichment items ($p<0.05$). Moreover, the results of BP were enriched in regulation of transcription by RNA polymerase II, innate immune response, protein ubiquitination, regulation of small GTPase mediated signal transduction, positive regulation of autophagy, neural crest cell migration and semaphorin-plexin signaling pathway (Figure 2B) ($p<0.05$).

KEGG pathway analysis represented that upregulated DEGs were correlated to pathways including metabolic pathways, pathways in cancer, chemical carcinogenesis - reactive oxygen species, cell cycle. Moreover, downregulated DEGs were involved in metabolic pathways, autophagy - animal, Efferocytosis and TNF signaling pathway. Both upregulated and downregulated DEGs were associated with metabolic pathways (Table 1).

Table 1. Results of KEGG pathway enrichment analysis of common genes by David ($p<0.05$)

Category	Term
Upregulated genes	
KEGG_PATHWAY	hsa01100: Metabolic pathways
KEGG_PATHWAY	hsa05200: Pathways in cancer
KEGG_PATHWAY	hsa05208: Chemical carcinogenesis - reactive oxygen species
KEGG_PATHWAY	hsa04110: Cell cycle
KEGG_PATHWAY	hsa00190: Oxidative phosphorylation
KEGG_PATHWAY	hsa04015: Rap1 signaling pathway
KEGG_PATHWAY	hsa04218: Cellular senescence
KEGG_PATHWAY	hsa04150: mTOR signaling pathway
KEGG_PATHWAY	hsa04120: Ubiquitin mediated proteolysis
KEGG_PATHWAY	hsa01232: Nucleotide metabolism
KEGG_PATHWAY	hsa03013: Nucleocytoplasmic transport
KEGG_PATHWAY	hsa00230: Purine metabolism
KEGG_PATHWAY	hsa04350: TGF-beta signaling pathway
KEGG_PATHWAY	hsa04721: Synaptic vesicle cycle
KEGG_PATHWAY	hsa05222: Small cell lung cancer
Downregulated genes	
Category	**Term**
KEGG_PATHWAY	hsa01100: Metabolic pathways
KEGG_PATHWAY	hsa04140: Autophagy - animal
KEGG_PATHWAY	hsa04148: Efferocytosis
KEGG_PATHWAY	hsa04668: TNF signaling pathway
KEGG_PATHWAY	hsa00230: Purine metabolism
KEGG_PATHWAY	hsa03083: Polycomb repressive complex
KEGG_PATHWAY	hsa04146: Peroxisome
KEGG_PATHWAY	hsa00310: Lysine degradation
KEGG_PATHWAY	hsa01230: Biosynthesis of amino acids
KEGG_PATHWAY	hsa04330: Notch signaling pathway

Construction of the PPI Network and Identification of Hub Genes

To analyze the interactions of DEGs and identify hub genes involved in olaparib therapy for breast cancer, we used the STRING database to create PPI networks. Further analysis was conducted using cytoHubba, plugins of the Cytoscape software. Ten hub genes were ranked based on the MCC score, which demonstrated the number of gene interactions in the PPI network. The hub genes with increased expression, NDUFA5, NDUFA6, NDUFS6, NDUFB3, NDUFB7, NDUFB10, NDUFA7 and NDUFA9 had the highest node degree in olaparib treated cells (Figure 3A). The hub genes with the lowest expression levels were: H2AC8, H2AC13, H2AC17, H4C11, H4C12, H2BC21 and H2BC4 (Figure 3B).

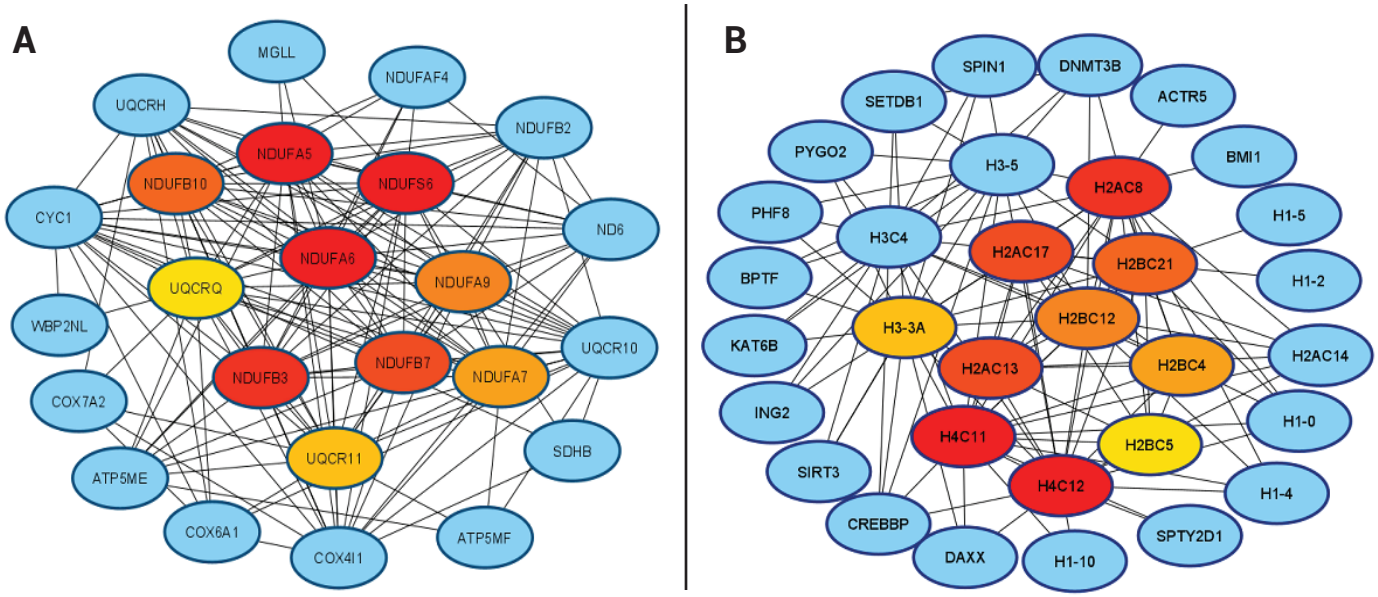

Figure 3. Top 10 hub genes screened by degree according to cytoHubba plug-in; **A.** upregulated genes OlaR MDA-MB-468 cells vs control MDA-MB-468 cells; **B.** downregulated genes OlaR MDA-MB-468 cells vs control MDA-MB-468 cells. The top 10 hub genes ranked by the MCC algorithm and their neighbors in the blue nodes. The red nodes represent genes with a high MCC score, while yellow nodes represent genes with a low MCC score

DISCUSSION

BC is a significant health concern, and scientists looked for novel biomarkers for predicting the disease's prognosis (15). Differential expression of many molecular biomarkers together with genetic, epigenetic, and phenotypic changes describe BC, a complicated disease (15,16). TNBC, a subtype of BC, is negative for PRs, ERs, and HER2 expressions. Due to its strong metastatic character, invasive nature, and high recurrence rate, it is the BC type with the worst prognosis (17,18). Treatment of TNBC is difficult by the lack of these biomarkers, and the disease has a poor survival rate and a high metastasis rate in comparison to other BC types (17). A variety of treatment approaches, such as immunotherapy, chemotherapy and targeted therapy have been used to treat TNBC (17). All these therapeutics have their own side effects.

Thus, it is essential to find out novel, trustworthy diagnostic biomarkers and develop distinctive BC immunotherapeutic targets.

In the current study, we investigated the significant pathways and genes which were upregulated and downregulated in olaparib-treated MDA-MB-468 cells compared to the control MDA-MB-468 cells. According to the KEGG pathway analysis, both upregulated and downregulated DEGs were associated with metabolic pathways. These results represented the importance of metabolic pathways underlying olaparib effect mechanisms.

Based on the bioinformatics analysis, there are upregulated (NDUFA5, NDUFA6, NDUFS6, NDUFB3, NDUFB10, NDUFB7, NDUFA7 and NDUFA9) and downregulated (H2AC8, H2AC13, H2AC17, H4C11, H4C12, H2BC12, H2BC21 and H2BC4) hub genes which showed higher node degree in PPI network.

NADH dehydrogenase 1 alpha subcomplex 5 (NDUFA5) contributes to the development of the electrochemical potential required to produce (19). In the study by Shimada and colleagues, NDUFA5 was significantly overexpressed in HPV-positive cervical cancer, therefore, they suggested the possible role of NDUFA5 in the multi-step carcinogenesis in human cervical cancer (20).

NDUFA6 and NDUFB3 genes which were found to be overexpressed in olaparib-treated cells. The NDUFA6 is a gene connected to mitochondrial translation process (21) and encoded a protein which is localized to the mitochondrial inner membrane (22). Zhao and colleagues reported NDUFA6 as a novel prognostic biomarker in multiple myeloma (MM) and suggested its association with the progression of MM (23). According to their study,

MM patients represented higher expression of NDUFA6 than healthy group, and the expression of NDUFA6 enhanced with the tumor stage (23). Upregulation of NDUFB3 (NADH:ubiquinone oxidoreductase subunit B3) protein lead to activation of NLRP3 inflammasome and cell pyroptosis in Nasopharyngeal Carcinoma (NPC) (24).

In contrast to the upregulated genes, H2AC8 is among the genes which was downregulated in olaparib-treated BC cells compared to the untreated cells.

There are not enough studies to explain the roles of the genes proposed in our study in many cancers, especially breast cancer. Therefore, the roles of these genes in breast cancer have not been clearly explained and needs further studies to determine these genes roles.

CONCLUSION

In conclusion, this study aimed to identify genes and pathways associated with olaparib therapy in TNBC. These genes were identified and could be considered as potential biomarkers for TNBC. Additional experimental and clinical researches are required to validate and confirm the biological roles and clinical relevance of these genes in TNBC prognosis and progression. One possible therapeutic approach for the treatment of TNBC is targetting the elevated genes or inducing the downregulated genes.

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Ethical approval: *Not applicable. This paper does not involve human or animal subjects.*

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