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ORIGINAL ARTICLE

Differential gene screening and functional analysis in docetaxel-resistant prostate cancer cell lines*

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Abstract

Objective Docetaxel-based combination chemotherapy has traditionally been the standard treatment for metastatic castration-resistant prostate cancer (PCa). However, most patients eventually develop resistance to this treatment, which further reduces their survival. This study aimed to determine key molecular genes in docetaxel-resistant PCa cell lines using bioinformatic approaches.

Methods The analysis of microarray data GSE33455 (including DU-145/DU-145R and PC-3/PC-3R cell lines) obtained from the Gene Expression Omnibus (GEO) database was performed using GEO2R. Differentially expressed genes (DEGs) of DU-145/DU-145R and PC-3/PC-3R cell lines were selected, and the intersection of DEGs between the two groups was obtained. DEGs were annotated with the Gene Ontology (GO) function and enriched with the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway using an online platform (https://cloud.oebiotech.cn/task/detail/array_enrichment/). The online tool Search Tool for the Retrieval of Interacting Genes (https://string-db.org/) was used to obtain the DEG network graph and matrix list, which was imported into Cytoscape 3.6.1 and analyzed using the Molecular Complex Detection plug-in to detect potential functional modules in the network.

Results A total of 131 intersection DEGs were identified between non-treated and docetaxel-resistant PCa cell lines. GO functional annotation showed that the main genes involved were present in the plasma membrane and were involved in positive regulation of ubiquitin-protein transferase activity, positive regulation of pseudopodium assembly, centriolar subdistal appendage, and heterophilic cell–cell adhesion via plasma membrane cell adhesion molecules. KEGG pathway enrichment analysis revealed that DEGs were mainly involved in IL-17 signaling pathway, cytokine-cytokine receptor interaction, rheumatoid arthritis, legionellosis, and folate biosynthesis. We identified two distinct hubs of DEGs: (1) CD274, C-X-C motif chemokine ligand (CXCL)1, DExD/H-box helicase 58, CXCL2, CXCL8, colony-stimulating factor 2, C-X-C motif chemokine receptor 4 (CXCR4), CXCL5, and CXCL6 and (2) argininosuccinate lyase, argininosuccinate synthase 1, and asparagine synthetase. Except for the CXCR4 gene that was downregulated, the other 11 genes showed upregulated expression.

Conclusion Certain differential genes may be potential targets for predicting and treating metastatic docetaxel-resistant PCa.

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Key words: docetaxel-resistant; prostate cancer; differentially expressed genes; bioinformatics; hub genes

The GLOBOCAN 2020 reports that prostate cancer (PCa) accounts for 7.3% of the 19.3 million new cancer cases worldwide [1], which is the second leading cause of cancer-related deaths in men in the USA [2], and its

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incidence in China is rapidly increasing [3]. Between 10% and 15% of patients present with advanced disease and receive hormone therapy as their initial treatment. However, most cases acquire therapy resistance within 2 years and progress to castration-resistant prostate cancer (CRPC) [4]. Docetaxel-based chemotherapy is the standard treatment for metastatic CRPC (mCRPC) [5]. Unfortunately, there is no effective treatment strategy for docetaxel-resistant patients with mCRPC. Moreover, the molecular mechanisms underlying docetaxel resistance remain unclear. In this study, the differentially expressed genes (DEGs) from the microarray data in the Gene Expression Omnibus (GEO) database were identified between non-treated and docetaxel-resistant PCa cell lines. We aimed to explore certain differential hub genes in docetaxel-resistant PCa using bioinformatic approaches.

Materials and methods

Microarray data filtering eligible dataset

We searched and downloaded microarray data GSE33455 (including DU-145, DU-145R, PC-3, and PC-3R cell lines) from the GEO database (www.ncbi.nlm.nih.gov/geo/). The four cell lines in GSE33455 were generated in androgen-independent cell lines. Microarray data were used to analyze the eligible dataset and identify the intersection of DEGs in docetaxel-resistant cell lines (DU-145/DU-145R and PC-3/PC-3R).

DEG screening

DEGs were independently screened using the GEO2R online tool in the GEO database. In our study, DEGs between non-treated and docetaxel-resistant PCa cells (DU-145 and DU-145R and PC-3 and PC-3R) were screened and selected using the cutoff point of adj. P value < 0.05 and |log FC| > 1.0. More accurate intersection DEGs were obtained through the online Venny map (https://bioinfogp.cnb.csic.es/tools/venny/index.html) after deleting duplicate and invalid genes.

Functional enrichment and protein–protein interaction analysis

The online platform (https://cloud.oebiotech.cn/task/detail/array_enrichment/) was used to analyze Gene Ontology (GO) functional annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment. Furthermore, we obtained the protein–protein interaction (PPI) of differential genes using the Search Tool for the Retrieval of Interacting Genes database (STRING, https://string-db.org/), and one big pairing picture was generated. In this study, DEGs with a confidence score of >0.4 were selected to construct the PPI network.

Established DEG network

The DEG network was generated using Cytoscape 3.6.1 software [6]. The Molecular Complex Detection (MCODE) plug-in was used for module clustering analysis to detect potential functional modules in the network. In the MCODE process, the cutoff value of the degree was set to 2, and the cutoff value of the node score was set to 0.2.

Results

DEG screening

A total of 1,311 and 2,027 DEGs were identified in the DU-145/DU-145R and PC-3/PC-3R cell lines, respectively. Using the online Venny map (https://bioinfogp.cnb.csic.es/tools/venny/index.html), we obtained 131 more accurate DEGs.

GO and functional enrichment analysis

Pathway and process enrichment analyses were performed using GO biological processes (BPs), GO cellular components (CCs), GO molecular functions (MFs), KEGG functional sets, and KEGG pathway ontology sources. There were 73 BP annotated genes, 83 CC annotated genes, and 65 MF annotated genes. A bar chart was drawn for items in BP, CC, and MF of the GO enrichment analysis results, and the most significant top 10 GO items in the three categories of GO in the same chart were shown (Fig. 1a).

Through the BP, CC, and MF functional annotations of GO, we found that 131 DEGs were mainly involved in the plasma membrane, positive regulation of ubiquitin-protein transferase activity, positive regulation of pseudopodium assembly, centriolar subdistal appendage, heterophilic cell–cell adhesion via plasma membrane cell adhesion molecules (CAMs), helicase activity, Rho protein signal transduction, phospholipid translocation, integral component of membrane, and bicellular tight junction (Fig. 1a).

The KEGG pathway enrichment analysis showed that DEGs were mainly involved in the IL-17 signaling pathway, cytokine-cytokine receptor interaction, rheumatoid arthritis, legionellosis, folate biosynthesis, CAMs, mitophagy (animal), TNF signaling pathway, alanine, aspartate and glutamate metabolism, and Salmonella infection (Fig. 1b). A bubble chart was drawn for the KEGG pathway enrichment analysis results, and the most significant top 30 GO items in the KEGG database were shown (Fig. 1b).

Construction of DEG network analysis

Considering the selected 131 DEGs, we identified 103 PPI pairs using the STRING database. One big pairing picture mentioned earlier was obtaining a complete DEG network in Cytoscape. The MCODE plug-in in

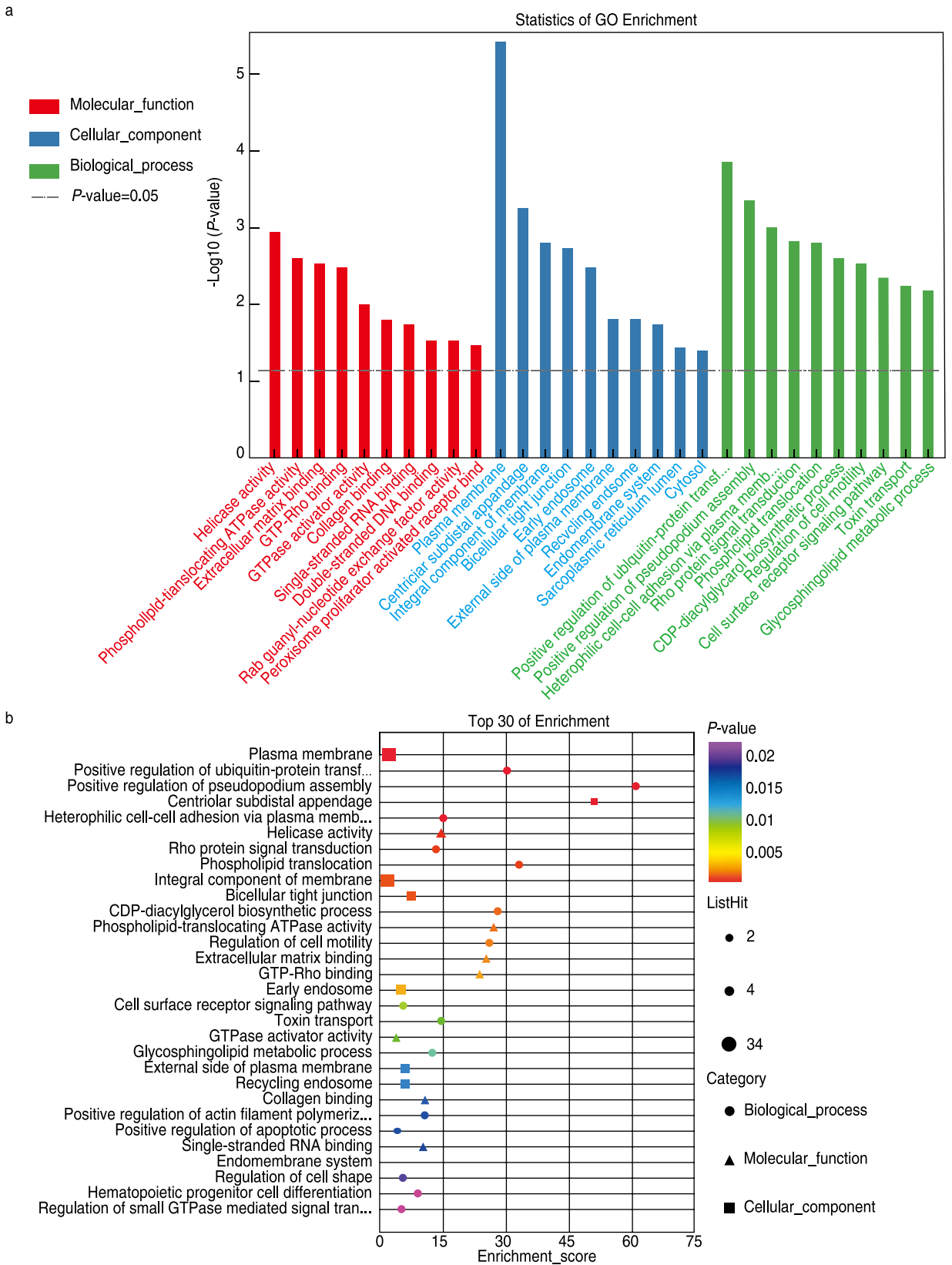

Fig. 1 (a) Gene Ontology bar graph. The y-axis is −log10 (*P* value). The higher the bar graph height, the greater the significance; (b) Kyoto Encyclopedia of Genes and Genomes bubble chart. The y-axis corresponds to the KEGG entries, the x-axis corresponds to the enrichment score, the size of the point corresponds to the number of intersection genes in the KEGG entries, and the smaller the *P* value of KEGG enrichment, the greater the significance

Cytoscape was used to perform module clustering of the DEG network mentioned earlier, after which two key functional modules of the network were evaluated (Fig. 2a). One was CD274 (CD274 molecule), C-X-C motif chemokine ligand (CXCL)1, DDX58 (DExD/H-box helicase 58), CXCL2, CXCL8, colony-stimulating factor 2 (CSF2), C-X-C motif chemokine receptor 4 (CXCR4), CXCL5, and CXCL6. The other was argininosuccinate lyase (ASL), argininosuccinate synthase 1 (ASS1), and asparagine synthetase (ASNS). These modules also occurred in the GO and KEGG enriched earlier, which were associated with the plasma membrane and signaling pathways.

Further analysis of the online volcano map (sangerbox.com/AllTools? tool_id=9699135) revealed 11 upregulated DEGs, namely CD274, CXCL1, DDX58, CXCL2, CXCL8, CSF2, CXCL5, CXCL6, ASL, ASS1, and ASNS, and one downregulated CXCR4 (Fig. 2b).

Discussion

Docetaxel is the standard first-line chemotherapy for metastatic castration-resistant PCa. However, the occurrence of docetaxel resistance is one of the main reasons for poor chemotherapy response in patients with PCa who fail androgen deprivation therapy. In our study, 1,311 DEGs were identified between the DU-145 and DU-145R cell lines and 2,027 DEGs were identified between the PC-3 and PC-3R cell lines. A total of 131 more accurate common intersection DEGs were identified between 1,311 DEGs and 2,027 DEGs by analyzing the GSE33455 dataset.

The 131 common intersection DEGs included 85 upregulated and 46 downregulated genes. The interactions among these DEGs were investigated in the KEGG and GO enrichment analyses. In the BP category, the DEGs were predominantly enriched in "positive regulation of ubiquitin-protein transferase activity," "positive regulation of pseudopodium assembly," "heterophilic cell–cell adhesion via plasma membrane CAMs," and "Rho protein signal transduction," all of which are closely associated with drug resistance and tumor metastasis. In the MF category, the DEGs were associated with "helicase activity," "phospholipid-translocating ATPase activity," "extracellular matrix binding," "GTP-Rho binding," and "GTPase activator activity"; these data suggested that the DEGs affect the binding of cadherin, proteins, and actin, as well as GTPase activity. In addition, in the CC category, the DEGs were mainly enriched in "plasma membrane," "centriolar subdistal appendage," "integral component of membrane," and "bicellular tight junction"; these data suggest that the DEGs were mainly involved in the transport and transfer of intracellular substances.

The KEGG analysis showed that the DEGs were mainly enriched in "folate biosynthesis," "viral protein interaction with cytokine and cytokine receptor," "legionellosis," "rheumatoid arthritis," "IL-17 signaling pathway," "TNF signaling pathway," and "CAMs." Wu *et al.* [7] demonstrated a novel IL-17–mediated cascade via the IL-17R-Act1-TRAF4-MEKK3-ERK5-positive circuit that directly stimulates keratinocyte proliferation and tumor formation. A previous study indicated that high

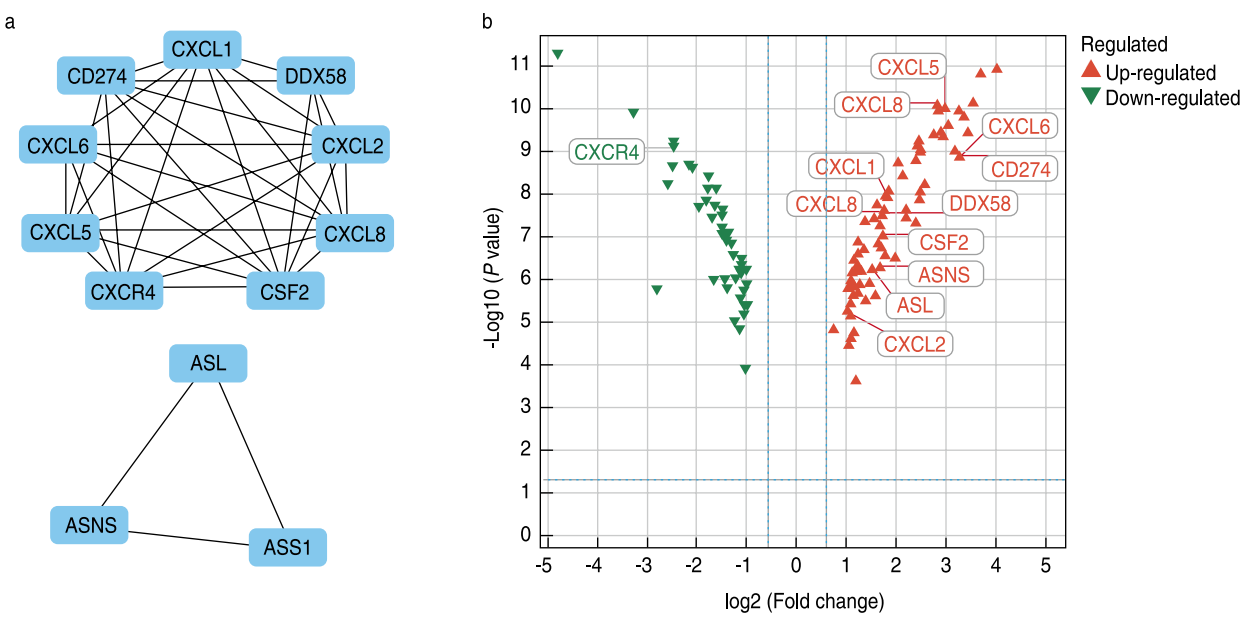

Fig. 2 (a) Two outstanding hubs of differentially expressed genes (DEGs) in metastatic docetaxel-resistant prostate cancer cell lines; (b) Volcanic map of two outstanding hubs of DEGs

serum folate levels increased cancer cell proliferation in PCa and were involved in cellular development, cell cycle, cell death, and molecular transport [8]. There is a growing body of evidence suggesting that changes in the expression or function of CAMs have been implicated in all steps of tumor progression, including detachment of tumor cells from the primary site, intravasation into the bloodstream, extravasation into distant target organs, and formation of secondary lesions [9].

In this study, a PPI network with 130 nodes and 103 edges was constructed using DEGs, and two significant modules, termed modules one and two, were obtained through the MCODE function in the Cytoscape software. Module one comprised nine genes, including the CD274 molecule, CXCL1, DDX58, CXCL2, CXCL8, CSF2, CXCR4, CXCL5, and CXCL6, which were enriched in "chemokine-mediated signaling pathway," "neutrophil chemotaxis," "antimicrobial humoral immune response mediated by antimicrobial peptide," and "cellular response to lipopolysaccharide." This enrichment results in the interaction of multiple signal transduction pathways in effector cells and the expression of related stimulating genes, which have many biological functions, including inflammation-mediated signaling pathways and humoral immune regulation [10]. Module two consisted of three genes, including ASL, ASS1, and ASNS, which were mainly associated with "arginine biosynthetic process," "urea cycle," "aspartate and glutamine family amino acid metabolic process," and "alpha-amino acid biosynthetic process." Several studies have shown that these enrichment results are mainly involved in the processes of tumor adhesion, invasion, metastasis, and drug resistance [11-12].

These 12 DEGs were considered to be hub genes, including 11 upregulated genes, namely CD274, CXCL1, DDX58, CXCL2, CXCL8, CSF2, CXCL5, CXCL6, ASL, ASS1, and ASNS, and one downregulated CXCR4, which may play a key role in docetaxel-resistant PCa [13-15]. It has been reported that CXCL8 is related to the migration and proliferation of various types of cancer cells, including PCa cells [16-17]. CXCL8 promotes the proliferation, growth, and development of cancer cells by regulating the expression of the tumor growth factor, which increases the drug resistance of androgen-independent PCa to cytotoxic chemotherapeutic drugs [18]. The data from this study suggest that these DEGs are closely related to the carcinogenesis, progression, prognosis, and drug resistance of PCa.

Conclusion

In summary, the bioinformatic analysis of the intersection gene data between DEGs in different docetaxel-resistant PCa cell lines revealed several hub genes, which may assist in the understanding of the potential molecular mechanism of docetaxel resistance. Subsequent RT-qPCR validation and in vivo experiments are required for further confirmation in the future.

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Conflicts of interest

The authors indicated no potential conflicts of interest.

Author contributions

Not applicable.

Data availability statement

The raw data analysis in this study were downloaded from GEO database (GSE33455).

Ethical approval

Not applicable.

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