Identification and validation of a prognostic index based on a metabolic-genomic landscape analysis of ovarian cancer

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Research

Abstract

Purpose: Tumor metabolism has been a novel driver of personalized cancer medicine, with aggressive efforts to regulate the metabolic system to prolong their life. The aim of this study is to explore the prognostic value of metabolism in ovarian serous cystadenocarcinoma (OSC), which is the most common subtype of ovarian cancer, accounts for 75-80% of reported cases.

Patients and methods: we integrated the expression profiles of metabolism-related genes (MRGs) in survival in 379 OSC patients based on The Cancer Genome Atlas (TCGA) database. Then, several biomedical computational algorithms were employed to identify eight key prognostic MRGs, which were related with overall survival (OS) significantly in OSC. The eight genes represented important clinical significance and prognostic value in OSC. Then a prognostic index was constructed.

Results: A total of 701 differentially expressed metabolism-related genes (MRGs) were identified in OSC patients based on TCGA database. Functional enrichment analyses hinted that metabolism may act in a significant role in the development and progression of OSC. Random walking with restart (RWR) algorithm, Univariate cox and lasso regression analysis indicated a prognostic signature based on MRGs (ENPP1, FH, CYP2E1, HPGDS, ADCY9, NDUFA5, ADH1B and PYGB), which performed moderately in prognostic predictions.

Conclusion: This study provides a latent prognostic feature for predicting the prognosis of OSC patients and the molecular mechanism of OSC metabolism.

Introduction

Ovarian cancer is the deadliest female reproductive system malignancies, and its incidence rate is on the rise. It exhibits the 8th most common cause of lethal malignancies in gynecology and cancer-correlated occurrence rate over the world, leading to a huge social and economical burden[1]. Ovarian serous cystadenocarcinoma(OSC) the most common subtype of ovarian cancer, accounts for 75–80% of reported cases[2]. These types of cancer are either symptomless or show similar symptoms of other benign gynecological diseases, until the neoplasm has invaded on the peritoneal surface and than it will be diagnosed. The most of patients with OSC are often diagnosed at an advanced stage. Existing therapeutic regimens are deficient for patients with locally advanced or distantly metastatic OSC. Hence, OSC patients have awfully poor overall survival rate (OS), and the 5-year relative survival rate would be only 45%[3]. Therefore, development and identification of potential prognostic markers to predict OSC outcomes have a high clinical value.

Metabolism of tumor cells is different to normal cells, which induces them to maintain a great potential ability of proliferation and anti-apoptosis[4]. Tumor metabolism has been a novel driver of personalized cancer medicine, with aggressive efforts to regulate the metabolic system to prolong their life. In recent years, metabolic therapies have entered the therapeutic regimens of multiple types of cancers. At present, The metabolic therapies promise OSC patients another alternative option in cancer treatments. Many

research found that anti-metabolic agents could promote the effective elimination of ovarian cancer cells. Besides, Arun Kanakkanthara and his colleagues found that repression of energy metabolism may be an alternative proposals to selectively target BRCA1-deficient OSC, which is represented by BRCA1 loss and nicotinamide N-methyltransferase overexpression[5]. Zhang J et al. found that the expression of ACTL6A is positively the development and progression of ovarian cancer, which is especially in glucose metabolism of cancer cells[6]. Targeting a metabolizing genes that is specific for ovarian cancer but not the homologous normal cells will be successful in the development of antimetabolites[7]. Accumulation of glycogen is commonly observed in epithelial ovarian cancer, resulting in chemoresistance[8]. Meanwhile, Fatty acid metabolism is also disordered in cancer. Unsaturated lipids are enhanced in ovarian cancer[9, 10], which mediates cancer development and progression, especially in stemness[9]. These studies indicate that specific metabolic phenotypes increase chemoresistance in ovarian cancer. Furthermore, it also suggests that cancer cells ability to quickly switch between metabolic substrates and molecular pathways is correlated with adverse prognosis, such as migration and invasion[11].

Our purpose in this study is to achieve the potential clinical value and important academic significance of metabolism-related genes (MRGs) on prognostic stratification and their implicational latent as markers for OSC targeted treatment. We analyzed MRG expression status with clinical feature, applying computational methods for the determination of progression-free intervals (PFIs) in OSC patients. We systematically integrated the prognostic landscape and expression profiles of MRGs and evolved an individualized prognostic signature for OSC patients. The potential regulatory mechanisms were figured out by Bioinformatics analyses. This study could provide a groundwork for consecutive and intensive metabolism-related research with great progress for individualized treatment of OSC.

Materials And Methods

Clinical samples and data acquisition

Transcriptome RNA-sequencing data and the clinical information of OSC samples were downloaded and extracted from the TCGA data portal (https://cancergenome.nih.gov/), which contained data from 379 primary OSC. Transcriptome RNA-sequencing data and the clinical information of normal ovarian samples were downloaded and extracted from the GTEX data portal (https://www.gtexportal.org/), which contained data from 88 normal ovarian samples. Seventy MRGs were downloaded from the kegg database of the GSEA website. A total of 1,466 genes related to human metabolism were included. Method of gene set acquisition ibid

Functional enrichment analyses

The R package "ClusterProfiler" was used for functional annotation of differentially expressed metabolic genes to comprehensively explore the functional correlation of these differentially expressed genes. Gene ontology (GO) and the Kyoto encyclopedia of genes and genomes (KEGG) were used to assess the relevant functional categories. The GO and KEGG enrichment pathways with p and q values less than 0.05 were considered to be significant.

Development of the metabolism-related gene-based prognostic index

The String database is used to construct the network map of metabolic genes. Scores as each gene action relationship score. We selected ACOT7, CERK, EHMT2, MTAP and PDE8A as the initial gene in the random walking with restart (RWR) algorithm, which has been determined by the ovarian cancer related pathway in the GSEA database. The random walk model iterated 1e + 5 times. The top 200 metabolic genes were selected for the subsequent model construction. Univariate cox was used to select genes associated with prognosis. was used to further construct the prognostic correlation model. After incorporating the expression values of each specific gene, a risk score formula was constructed for each patient. According to the risk score formula, the patients were divided into low risk group and high risk group with the median risk score as the cut-off point. Survival differences between the two groups were assessed by kaplan-meier and compared using log-rank statistical methods. Finally, ROC curve was used to study the accuracy of model prediction.

Random walking with restart (RWR) method

The RWR was a classical sorting algorithm, which simulated the random walk on a constructed network from one or more seed nodes. In the process of migration, possible new nodes were identified and sorted from high to low probability. The algorithm was often used to find new disease genes or other related problems. In this study, we selected 5 ovarian cancer related genes (ACOT7, CERK, EHMT2, MTAP, PDE8A) as seed nodes, which had been repeatedly verified. The initial probability P^0 of each seed node was set to 1/T (where T was the number of seed nodes), while the initial probability P^0 of the non-seed node was set to 0. The RWR simulated the events when these five genes moved across the network, P^l was a vector representing the probability of each node after the l-th movement was completed: $P^{(l+1)}=(1-b)YP^l+bP^0$. Y was the normalized connection matrix of the moving network. This method could obtain the interaction information between the constructed gene networks and prioritize the candidate nodes according to the distance between the candidate nodes and the known disease seed nodes. The higher the distance to the seed, the higher the score of the candidate node.

Statistical analysis

Survival curves were generated by kaplan-meier method and compared by logarithmic rank test. All statistical analyses were performed using the R Programming Language (version 3.6). All statistical tests were bilateral, and $p < 0.05$ was statistically significant.

Results

Identification of differentially expressed MRGs

The RNA-seq of 379 OSC tissue samples and 88 normal ovarian samples were downloaded from TCGA and GTEX, respectively. Expression values of 701 MRGs were extracted. Considered as the indexes of a P value < 0.05 and |log2(Fold Change)|>1, we obtained 351 up-regulated and 350 down-regulated MRGs

(Fig. 1). As we expected, gene functional enrichment analysis showed that metabolic pathways were most frequently influenced. "small molecule catabolic process," "mitochondrial matrix," and "cofactor binding" were the most common biological terms among biological processes, cellular components, and molecular functions, respectively (Fig. 2A). For the KEGG pathways, thermogenesis were most frequently enriched by differentially expressed MRGs (Fig. 2B).

Construction of the OSC MRGs network

RWR was used to select these metabolic genes that are related to the pathogenesis of ovarian cancer. All MRGs were included in the String database to build a network of interactions among these genes. As the functional relationship score among genes, Scores are used for the network construction of subsequent random walk model. On the basis of the above network, the known ovarian cancer pathogenic genes were selected through KEGG database as the initial genes of the RWR network, respectively ACOT7, CERK, EHMT2, MTAP and PDE8A (Fig. 3). The RWR model iterated for 100,000 times in total. According to the score of the model, the genes with the TOP 200 score value (TOP 200 metabolic genes that can be considered as the most closely related to the pathogenesis of ovarian cancer) were selected as the basis for the subsequent model construction.

Evaluation of clinical outcomes

Univariate cox method was used to analyze the relationship between the above 200 genes and prognosis. A total of 8 genes were closely related to the prognosis of ovarian cancer patients, including ENPP1, FH, CYP2E1, HPGDS, ADCY9, NDUFA5, ADH1B and PYGB (P < 0.05) (Fig. 4A). Furthermore, lasso regression was used to construct the prognostic model, and the risk score formula was as follows: Risk scores = ENPP1*0.0347 + FH*(-0.010) + CYP2E1*0.690 + HPGDS*0.124 + ADCY9*0.068 + NDUFA5*(-0.031) + ADH1B*0.049 + PYGB*0.016. According to the risk score formula, the patients were divided into low risk group and high risk group with the median risk score as the cut-off point (Fig. 4B).

This metabolic-based prognostic index could be an important tool to differentiate OSC patients based on latent discrete clinical outcomes (Fig. 6A). The 1, 3 and 5 year area under curve of the receiver operating characteristic (ROC) curve were 0.653, 0.68 and 0.616 respectively, indicating moderate latent for the prognostic signature on account of MRGs in survival monitoring (Fig. 6B).

Characteristics of hub MRGs

Because these MRGs have important clinical value, we have explored their molecular features comprehensively. We found genetic alterations in these genes, and found that mRNA upregulation and deep deletion were the two most frequent types of mutations (Fig. 7A). Moreover, the Kaplan-Meier curves indicate statistically significant differences in OS (P < 0.05) of cases with alterations and cases without alterations in the eight genes which indicated that alterations group was more closely correlated with poor prognosis compared to another group of without alterations (Fig. 7B).

Gene set enrichment analysis (GSEA)

GO-GSEA indicated that OSC samples were markedly enriched in energy metabolism-related biological processes, such as "regulation of lipid kinase activity" and "positive regulation of GTPase activity". KEGG-GSEA suggested that OSC samples were obviously enriched in cellular metabolic pathways, including adipocytokine signaling pathway and oxidative phosphorylation (Fig. 8A–B).

Discussion

OSC is one of the leading lethal malignancies worldwide. The slow progress of molecular targeted therapy and the absence of effective molecular markers for OSC prognostic monitoring make it necessary to better understand the molecular mechanisms leading to this condition. The exploration of metabolic mechanism opens up and important perspective for OSC[1].

Although the significance of MRGs in cancer development and progression has been well-established, no comprehensive, genome-wide analysis has been conducted to explore its clinical significance and molecular mechanism. Most importantly, a personalized metabolic signature based on the selection, differential expression of MRGs is presented to measure cancer cell development and evaluate potential clinical outcomes. Since the beginning of the "War on Cancer", our understanding of carcinogenesis and clinical management techniques has made remarkable progress, but many aspects of OSC metabolic-related molecular mechanisms still unclear. This comprehensive and complete analysis of MRGs in OSC improve our understanding of its clinical implications and clarifies its underlying molecular features. The large number of OSC samples based on bioinformatics we were exposed to in this study contributed to robust results.

In recent years, with the development of high-throughput sequencing technology, large databases like TCGA, SEER and GEO have emerged, which provide an effective means for the selection of genetic markers. In the current study, we dug into the expression profile of MRGs in TCGA in order to search for molecular markers to detect the prognosis of patients with OSC. We first screened 1466 differentially expressed MRGs in OSC and normal ovarian tissues. Considering that these genes may have a closely association with the development and progression of OSC, we performed GO and KEGG analyses on these genes. Interestingly, functional analysis showed that the KEGG pathway (metabolism-related pathway), the most important of these enriched genes, was reduced. Based on the above results, we speculated that tumor metabolism may play an important role in the process of tumorigenesis. tumor metabolism is of great concern; of particular interest is its multifaceted feature in tumorigenesis. Glutamine, amino acids, glucose and free fatty acids are the basic and significant substances that support the growth and survival of cancer cells. These metabolites are either synthesized in cancer cells or assimilated from the blood circulation[12]. In summary, a better understanding of the relationship between cell origin and its metabolic status, as well as the function of MRGs, will help better map the metabolic profile of OSC.

When performed RWR to the network, ACOT7, CERK, EHMT2, MTAP and PDE8A were also identified in association with the pathogenesis of OSC. In the network of these initial genes, it could interact with 200

metabolic genes considered as the most closely related to the pathogenesis of ovarian cancer. On the basis of univariate cox analysis, a total of 8 genes were closely related to the prognosis of ovarian cancer patients, respectively ENPP1, FH, CYP2E1, HPGDS, ADCY9, NDUFA5, ADH1B and PYGB. Further analysis helped us distinguish high-risk and low-risk group to develop the metabolic-based prognostic index, which could be an independent prognostic indicator for OSC patients. Furthermore, we explored its expression profile, prognostic value and mutation status, and found valuable data for future clinical studies. To explore potential molecular mechanisms corresponding to potential clinical value, we constructed a MRGs network to reveal important and hub MRGs that regulate the tumor metabolism and progression.

ENPP1, FH, CYP2E1, HPGDS, ADCY9, NDUFA5, ADH1B and PYGB it characterized in this network. Given the potential molecular mechanisms of the eight MRG, reports on the functions and mechanisms of HPGDS, ADCY9 and NDUFA5 have not been published on OSC[13–17]. However, five of these eight hub MRGs have been studied, namely, ENPP1, FH, CYP2E1, ADH1B and PYGB. ENPP1 is increased in ovarian cancer and may promote the migration ability[13]. High level of FH could promote the aggressive and metastatic behaviors.[18] Increased activity of CYP2E1 was correlated with raised serum levels of IL-6, IL-8, and TNF-α, which mediated drug metabolism, and may have profound effects for drug development and prescribing in oncological settings[19]. High expression of ADH1B was correlated with markedly higher risk of residual disease in OSC[20], which played a significant role in accelerating ovarian cancer cell infiltration and may enhance the possibility of postoperative residual lesions[21]. PYGB obviously promoted ovarian cancer cell proliferation, invasion and migration via wnt pathway[22]. Therefore, previous studies just provided limited information on the mechanism of 8 MRGs in OSC patient survival. Oxidative phosphorylation pathway is the most important pathway in functional enrichment analysis, and it is speculated that oxidative phosphorylation pathway may play an important role in OSC process. Taken together, this study proposed a signature with metabolic-based prognostic index as the endpoint, which was most suitable for the survival monitoring of OSC patients. In addition, metabolic-based prognostic index is not only a prognostic indicator, but also an indicator of metabolic status.

Nowadays, some of the prognostic characteristics of cancer based on expression profiles have been proposed with the help of large public databases. For example, Zhong et al. also indicated a prognostic marker with 6 genes as a potential survival prediction marker for ER-positive breast cancer patients[23]. Bao et al. analyzed RNA-Seq data of 234 BC patients from TCGA and successfully obtained 4-lncRNA signature, which has prognostic value[24]. Nevertheless, these researches only focused on classic tumor biological behavior and ignored tumor metabolic. We attach enormous interests to the classic biological behavior as well as tumor metabolic. Therefore, prognostic characteristics are expected to be translated into clinical applications. However, the limitation of this study lies in its retrospective nature. Due to the lack of sufficient cases, we were unable to detect the expression of ENPP1, FH, CYP2E1, HPGDS, ADCY9, NDUFA5, ADH1B and PYGB in OSC and normal ovarian tissues. As we look to the future, there are still many problems. For instance, the relationship among metabolomics, immune genomics, proteomics and epigenomics should be investigated to further describe global metabolic alterations in OSC. It is important to further explore the potential relationship between metabolomics disorders and precancerous lesions. We anticipate that this prognostic feature may have important clinical significance. We

systematically analyzed the role of MRGs in monitoring the occurrence and prognosis of OSC. Our findings provide new ideas for individual treatment of OSC.

Conclusion

In conclusion, a comprehensive analysis of MRGs expression profile and correspondingprognostic data identified eight prognostic MRGs (ENPP1, FH, CYP2E1, HPGDS, ADCY9, NDUFA5, ADH1B and PYGB). The discovery of genes in the metabolic pathway also opens up new possibilities for the treatment of ovarian cancer. Combined with molecular expression and survival analysis, we constructed a new metabolic-based prognostic index risk score model, which can better predict the survival of OSC patients. In addition, the metabolic-based prognostic index risk score model was validated by a large sample. However, further prospective trials are expected to test clinical efficacy and help in the search for the best personalized targeted therapy.

Declarations

Availability of data and materials

The datasets used and/or analyzed in the present study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable

Author contributions

Conception and design: Zi-yao Wang and Yu-kun Li. Collection and assembly of data: Zi-yao Wang, Yu-kun Li, Gui-fang Luo, and Juan Zou. Data analysis and interpretation: Chang-ye Chen, Juan Wang and Xin Zeng. Manuscript writing: Zi-yao Wang, Yu-kun Li and Jiao Xiao. Paper revision: Yan Ma and Wen-juan Tong. Final approval of manuscript: All authors.

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