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Original Article

Expression and clinical significance of PDK family in breast cancer based on data mining

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Abstract: The pyruvate dehydrogenase kinase (PDK) family, including PDK1, PDK2, PDK3, and PDK4, is involved in tumor progression. However, its role in breast cancer (BC) remains unknown. This study aims to mine the expression, clinical significance, and downstream pathways of PDK family in BC. By analyzing data downloaded from The Cancer Genome Atlas (TCGA) database, we found an enhanced level of PDK3 and decreased expression of PDK2 and PDK4 in BC tissues compared to normal tissues. Also, the expression of PDK3 mRNA is negatively related to that of PDK2 and PDK4, while there is a positive relation between PDK2 mRNA expression and PDK3 mRNA expression. Moreover, we found that PDK2 expression is related to lymph node metastasis, and PDK4 is associated with T stage and stage using analysis of data obtained from TCGA database. Finally, we identified several gene sets related to cancer initiation and progression regulated by PDK2-4 after performing Gene set enrichment analysis (GSEA). In conclusion, PDK2-4 possess potential as targets for BC treatment.

Keywords: PDK, The cancer genome atlas, breast cancer, gene set enrichment analysis

Introduction

Breast cancer (BC) is the most common type of cancer in women, causing nearly half a million women's deaths annually [1]. Adjuvant and targeted therapy provide opportunities for patients with BC [2, 3]. However, as a consequence of poor prognosis due to the unique biologic characteristics of BC and the lack of therapeutic targets, BC remains a threat to the health of women [4]. Therefore, clarifying the pathogenesis of BC and mining new therapeutic targets are urgent.

Tumor cells prefer glycolysis in inadequate and even oxygen-rich environments while shifting energy derivation away from mitochondrial oxidative phosphorylation (OXPHOS) [5, 6], and this metabolic reprogramming is referred to as the "Warburg effect" [7]. This phenomenon is reported to be related to the enhancement of drug resistance and aggressiveness in tumor cells [8]. Pyruvate dehydrogenase kinase family (PDKs) enzymes are responsible for this metabolic switch by phosphorylating pyruvate dehydrogenase [9, 10]. PDK1, PDK2, PDK3 and PDK4 are different isoforms in the bPDK family, involved in various cancers, including ovarian cancer [10], pancreatic cancer [11], hepatocellular carcinoma [12], acute myeloid leukemia [13], diffuse large B-cell lymphoma [14], and breast cancer [6, 15]. The study of Du et al. [15] indicated that in animal models, PDK1 is highly expressed in breast lumps and would further regulate tumor invasion and metastasis, and it is also noted in vitro that inhibition of PDK1 can suppress cancer cell growth. Guda et al. [6] found that in BC, the expression level of PDK4 is considerably high, which can be one of the reasons for the poor prognosis of BC. Nevertheless, due to the limited research about the PDK family, their exact function in BC is still under controversy, and there still is a lack of study how PDK2 and PDK3 are expressed in BC and how their expression affects BC prognosis. To answer the questions, this research targeted BC patients, and studied the expression level of the PDK family in BC and normal tissues as well as investigated the clinical significance of PDK2-4 for the first time. Furthermore,

we also discussed the correlations of the PDK family in BC and conducted GSEA enrichment analysis for PDK2-4.

Materials and methods

Obtaining and processing PDK1-4 expression data

Transcriptome profiling of PDK1-4 in 113 normal and 1109 BC tissues was downloaded from TCGA database (https://tcga-data.nci.nih.gov/tcga/). Expression data of PDK1-4 in these unpaired samples was further extracted by limma packages [16] (version 3.8, http://bioconductor.org/packages/release/bioc/html/limma.html) with marked cut-off values of false discovery rate (FDR) < 0.05 and |log2 fold change (FC)| > 0.1. Expression data of PDK1-4 in 112 paired samples was obtained with The Perl Programming Language 5.30.0 (https://www.perl.org/).

Obtaining and processing clinical data of BC

Clinical data of 1097 cases were downloaded from TCGA database (https://tcga-data.nci.nih.gov/tcga/). Outcomes including age, gender, stage, T stage, distant metastasis and lymph node metastasis, were further obtained using The Perl Programming Language version 5.30.0 (https://www.perl.org/). Incomplete data were deleted based on the analyzed outcomes.

GSEA enrichment analysis

GSEA software version 3.0 (software.broadinstitute.org/gsea/index.jsp) was employed for analysis [17]. High vs. low phenotype was used, and the top 50 changed sets were exported. The normalized enrichment score (|NES| ≥ 1.0), the nominal *P* value (Nom *p*-val) < 0.05 and the false discovery rate (FDR q-val) ≤ 0.25 were used as standards. The ggplot2 package (https://cran.r-project.org/web/packages/ggplot2/index.html) was employed to merge the selected graphs.

Statistical analysis

The expression of the PDK family in paired and unpaired samples was analyzed by the beeswarm package (https://cran.r-project.org/web/packages/beeswarm/index.html) using R and Mann-Whitney U test (also known as Wilcoxon rank-sum test). Also, the differential expression of the PDK family in paired samples was analyzed by GraphPad Prism 5 and t test. The Perl Programming Language 5.30.0. was utilized to analyze the clinical correlation, and complementarily, Wilcoxon test and Kruskal test were used when there was more than one group. $P < 0.05$ was considered to display statistical significance. *P* values obtained by the R Project for Statistical Computing version 3.5.1 or by the Perl Programming Language 5.30.0. were expressed by scientific notation if they were < 0.001.

Results

Expression of PDK1-4 mRNA in BC and normal tissues

By comparing PDK1-4 expression levels in 113 normal and 1109 BC samples, a higher level of PDK1 mRNA (P=0.04) (**Figure 1A**) and PDK3 mRNA (P < 0.001) (**Figure 1G**) and lower expression of PDK2 mRNA (P < 0.001) (**Figure 1D**) and PDK4 mRNA (P < 0.001) (**Figure 1J**) were observed in BC tissues. Furthermore, we extracted paired samples of normal and tumor tissues and compared the expression of PDK1-4 genes by Wlicoxon test and t test. For PDK2 mRNA (**Figure 1E**, **1F**), PDK3 mRNA (**Figure 1H**, **1I**) and PDK4 mRNA (**Figure 1K**, **1L**), the results agreed with the above ones (all P < 0.001). However, for PDK1 mRNA expression, no significant difference was observed between normal and tumor samples (**Figure 1B**, **1C**). In addition, we analyzed the correlation between mRNA expression of PDK2, PDK3, and PDK4 in BC. We found that PDK3 mRNA expression is negatively related to PDK2 mRNA (r=0.1366, P < 0.0001) (**Figure 1M**) while PDK4 mRNA expression (r=0.1076, P=0.0003) (**Figure 1O**) and PDK2 mRNA expression are positively related to PDK3 mRNA expression (r=0.02699, P=0.3693) (**Figure 1N**).

Overall, lower PDK2 and PDK4 levels and higher PDK3 level were detected in cancer tissues compared to normal ones.

Clinical significance of PDK2-4 in BC

To explore the clinical significance of the PDK family in BC, we analyzed the relationship between PDK family expression and clinicopathologic data including age, gender, stage, T stage,

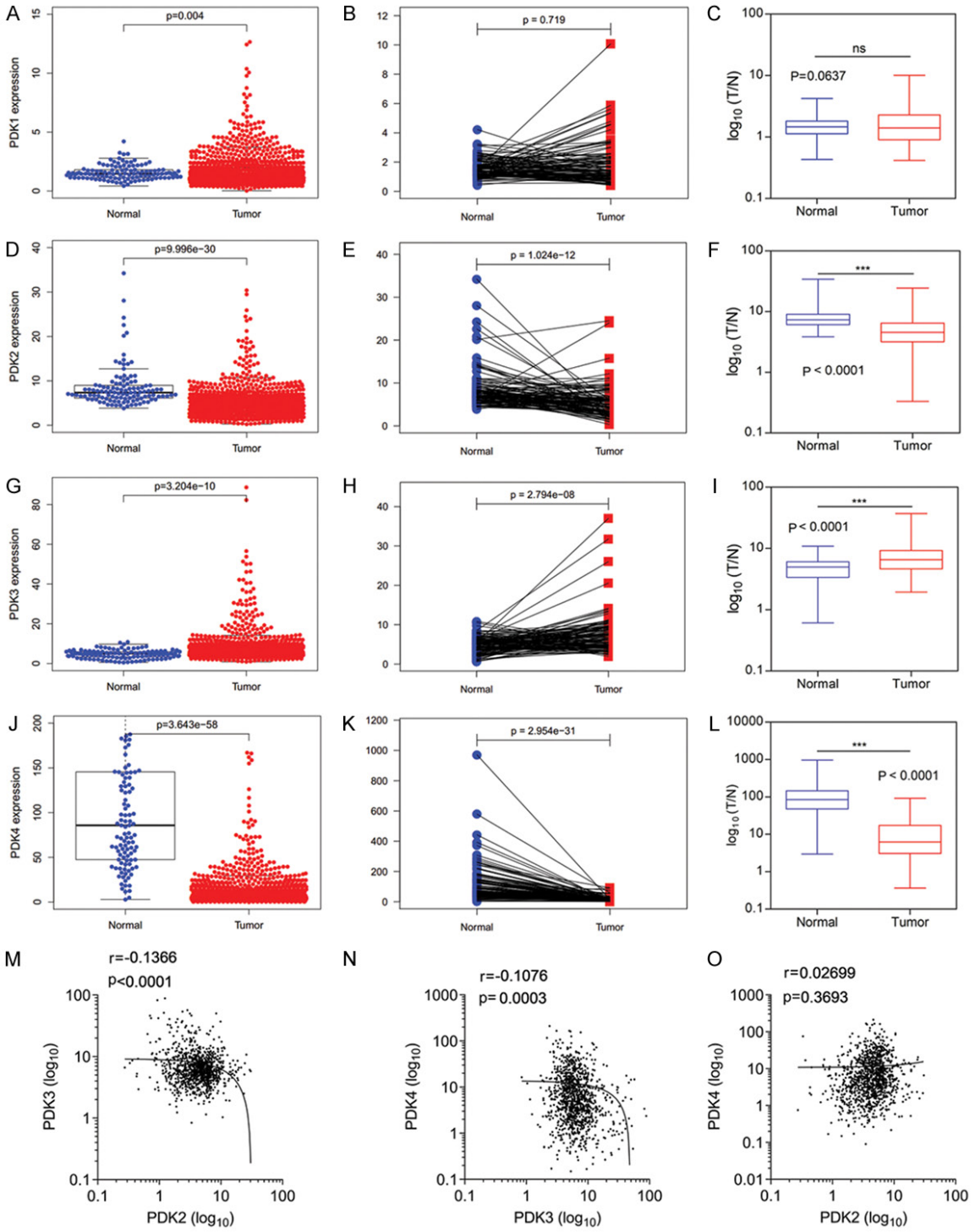

Figure 1. Expression of PDK1, PDK2, PDK3, and PDK4 mRNA in breast cancer and normal tissues. (A) PDK1 mRNA expression was higher in 1109 tumor tissues than that in 113 normal tissues; (B/C) No significant difference of PDK1 mRNA was detected in 112 pairs of BC tissues using Wilcoxon (B) and t test (C). (D) PDK2 mRNA expression was lower in 1109 tumor tissues than that in 113 normal tissues; (E, F) PDK2 mRNA expression was lower in 112 pairs of BC tissues using Wilcoxon (E) and t test (F). (G) PDK3 mRNA expression was higher in 1109 tumor tissues than that in 113 normal tissues; (H/I) PDK3 mRNA expression was higher in 112 pairs of BC tissues using Wilcoxon (H) and t test (I). (J) PDK4 mRNA expression was lower in 1109 tumor tissues than that in 113 normal tissues; (K/L) PDK3 mRNA expression was lower in 112 pairs of BC tissues, examined by Wilcoxon (K) and t test (L). (M) PDK2 and PDK3 mRNA levels were in negative correlation. (N) PDK2 and PDK4 mRNA levels were in negative correlation. (O) PDK3 and PDK4 mRNA levels were in negative correlation.

distant metastasis, and lymph node metastasis of BC patients. Before analysis, we deleted incomplete data based on previous analyzed outcomes. All patients had complete data on age and gender, so 1097 cases were available for analyzing age and gender. 11, 3, 163 and 28 cases failed to exhibited data on stage, T stage, distant metastasis and lymph node metastasis, respectively, and 1086, 1094, 934 and 1069, cases for each group mentioned above were successfully analyzed, respectively. As shown in **Figure 2**, PDK2 expression was associated only with lymph node metastasis ($P < 0.001$) and no significant relation was observed between the expression level of PDK3 and any above data (A-L), whilst PDK4 expression was found to be related to tumor size ($P < 0.001$) and stage ($P=0.005$) (M-R).

Overall, among PDK2-4, which display different expression levels in normal and tumor tissues, PDK2 and PDK4 were observed to be of clinical significance while no relation between PDK3 and clinicopathologic parameters examined was found.

GSEA enrichment analysis for PDK2-4 in BC

To investigate the gene sets regulated by PDK1, PDK2, PDK3, and PDK4, GSEA enrichment analysis was conducted. A total of 177 gene sets were compared in PDK2-4 high and low groups. In PDKs high groups, 137, 135, 100 gene sets are reported to be upregulated by PDK2, PDK3, PDK4, and 51, 48, 42 of them were markedly enriched at nominal $P < 0.05$, respectively. However, in PDKs low groups, an upregulation is observed in 40, 42, 77 gene sets of PDK2, PDK3, PDK4, and a marked enrichment at nominal $P < 0.05$ is found only in the PDK4 group.

Upregulation of high PDK2 was observed in gene sets related to apoptosis, T cell receptor signaling pathway, P53 signaling pathway, natural killer cell mediated cytotoxicity, ERBB signaling pathway, B cell receptor signaling pathway, Toll-like receptor signaling pathway, JAK-Stat signaling pathway, and Wnt signaling pathway (**Table 1**; **Figure 3A**). In gene sets related to B cell receptor signaling pathway, cell cycle, colorectal cancer, ERBB signaling pathway, natural killer cell mediated cytotoxicity, non-small cell lung cancer, pancreatic cancer, pathway in cancer, renal cell carcinoma, T cell receptor signaling pathway and Toll-like receptor signaling pathway, there was an upregulation in the PDK3 high group (**Table 2**; **Figure 3B**). Gene sets related to ERBB signaling pathway, JAK-Stat signaling pathway, MAPK signaling pathway, and MTOR signaling pathway were upregulated in the PDK4 high group, while gene sets related to DNA replication, cell cycle, and bladder cancer were upregulated in PDK4 low group (**Table 3**; **Figure 3C**).

Discussion

PDK is the isoenzyme of the pyruvate dehydrogenase complex (PDC) [18]. In glycometabolism, PDC transforms pyruvate into acetyl-CoA, and the latter enters the mitochondrion to participate in the tricarboxylic acid cycle [19]. But in cancer tissues, the generation of acetyl-CoA is suppressed, since pyruvate dehydrogenase is phosphorylated. Its activity is lowered as a result of the increased expression level of PDK, leading to a shift from mitochondrial oxidative phosphorylation to glycolysis in the glycometabolism of cancer cells. This conversion of metabolism can promote the production of more energy and lactic acid, thereby promoting the growth and metastasis of cancer cells [20]. Studies have shown that PDK participates in the production of mitochondrial ROS, participates in the antioxidant response of cells, and regulates the proliferation and metastasis of tumor cells through the HIF-1α pathway [21].

Liu et al. [22] detected the level of PDK1 protein in 241 paired tumor and normal samples and found that 213 tumor tissues exhibited positive PDK1 expression. Also, studies reported that overexpression of PDK1 cause the initiation of BC [23], while suppression of PDK1 expression leads to the inhibition of growth and migration of BC cells [24]. Our conclusion in unpaired BC cancer and normal samples is in agreement with previous studies. However, no significant difference had been detected in matched tissues. Hence, we assume that there is a similar PDK1 expression in BC cancer and paracancerous samples.

Roh et al. [25] reported that down-regulation of PDK2 can reverse cisplatin resistance in head and neck cancer cell lines. Also, Hu et al. [26] identified PDK2 as the most up-regulated kinase in cisplatin-resistant lung adenocarcinoma and as a factor correlated to the poor

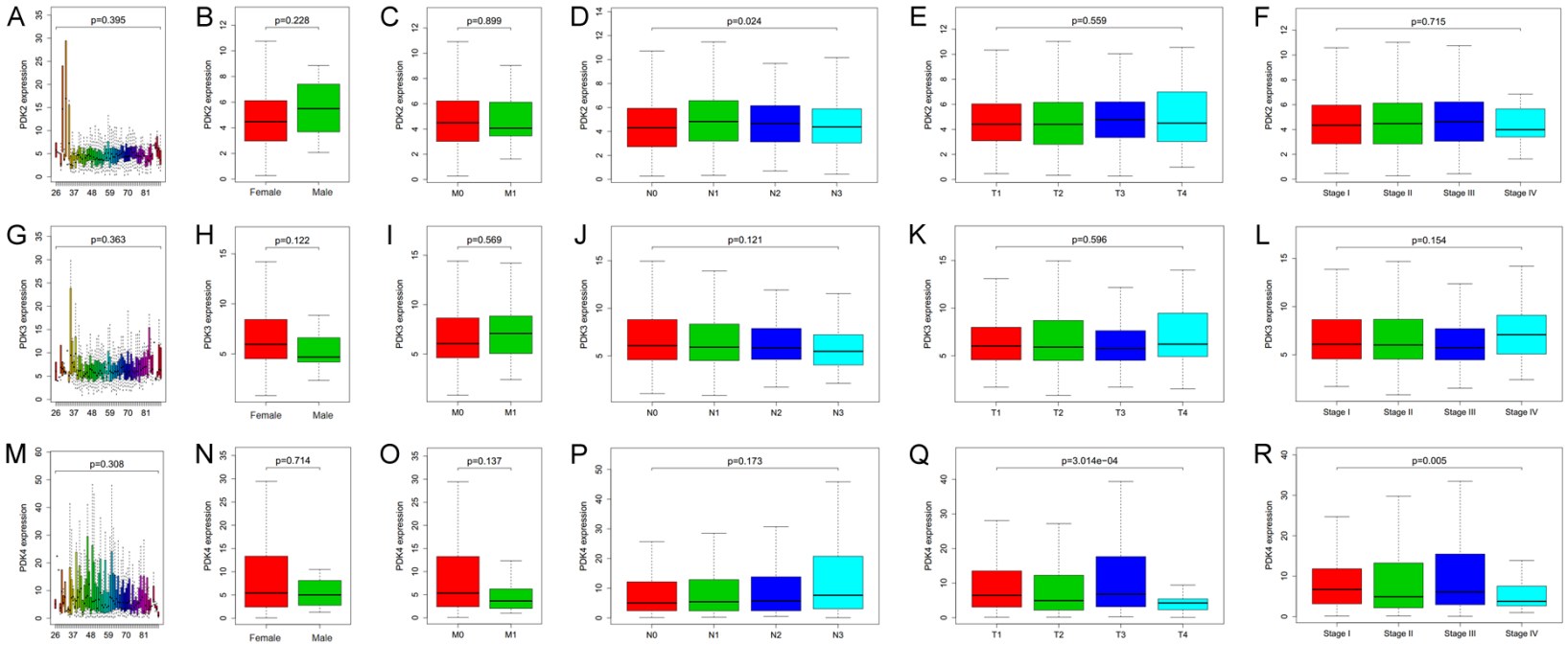

Figure 2. Clinical significance of PDK2-4 expression in breast cancer. (A-F) PDK2 expression was associated with lymph node metastasis (D), but not with age (A), gender (B), distant metastasis (C), T stage (E), and stage (F). (G-L) PDK3 level was not associated with age (G), gender (H), distant metastasis (I), lymph node metastasis (J), T stage (K), and stage (L). (M-R) PDK4 expression was associated with T stage (Q) and stage (R), but not with age (M), gender (N), distant metastasis (O), and lymph node metastasis (P).

Table 1. GSEA enrichment analysis for PDK2 in breast cancer

Gene sets	NS[1]	ENS[2]	Nom *P*-val[3]	FDR q-val[4]
KEGG_T_CELL_RECEPTOR_SIGNALING_PATHWAY	0.57	1.89	0.024	0.096
KEGG_P53_SIGNALING_PATHWAY	0.52	1.87	0.012	0.098
KEGG_NATURAL_KILLER_CELL_MEDIATED_CYTOTOXICITY	0.54	1.84	0.029	0.101
KEGG_ERBB_SIGNALING_PATHWAY	0.47	1.84	0.002	0.091
KEGG_B_CELL_RECEPTOR_SIGNALING_PATHWAY	0.57	1.8	0.038	0.074
KEGG_TOLL_LIKE_RECEPTOR_SIGNALING_PATHWAY	0.48	1.71	0.022	0.081
KEGG_APOPTOSIS	0.45	1.63	0.04	0.097
KEGG_JAK_STAT_SIGNALING_PATHWAY	0.42	1.6	0.048	0.107
KEGG_WNT_SIGNALING_PATHWAY	0.38	1.6	0.014	0.106

Notes: 1. NS: enrichment score; 2. NES: normalized enrichment score; 3. Nom *P*-val: nominal *p* value; 4. FDR q-val: false discovery rate.

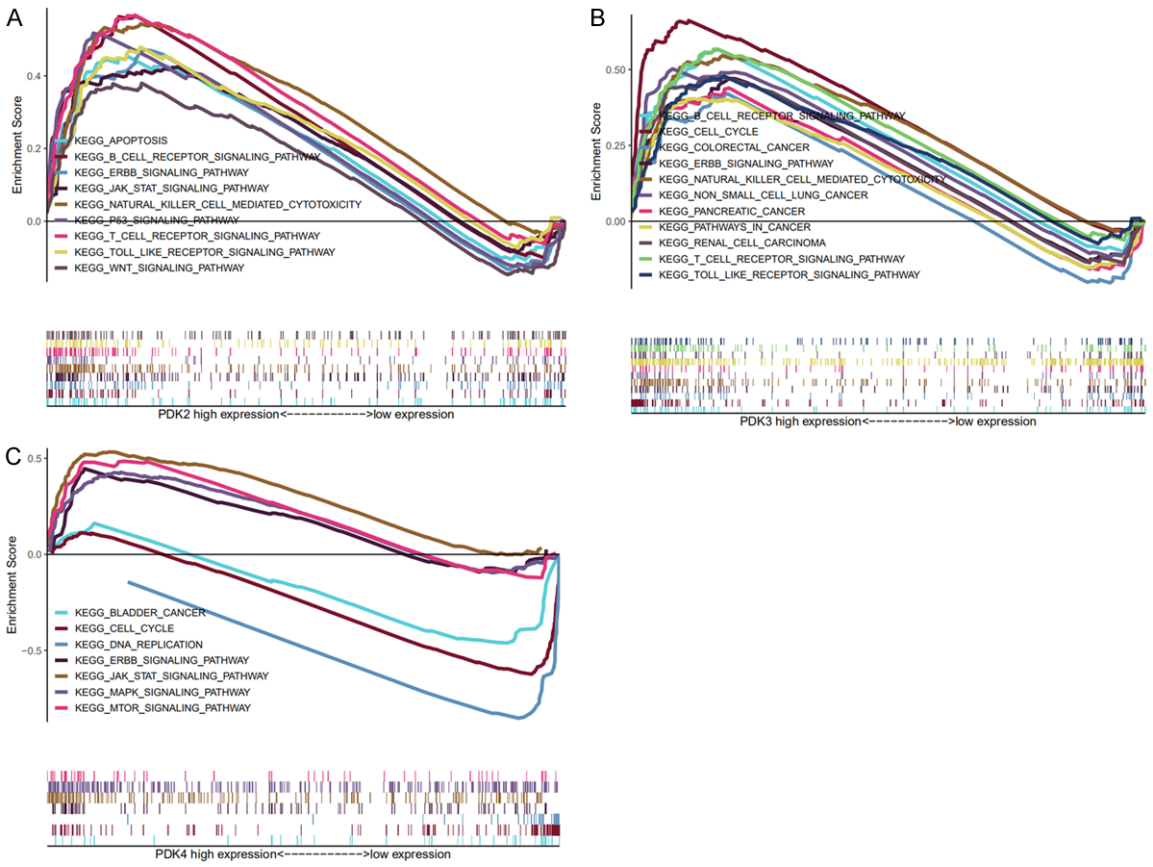

Figure 3. GSEA enrichment analysis of PDK2-4 in breast cancer. A. Gene sets related to apoptosis, T cell receptor signaling pathway, P53 signaling pathway, natural killer cell mediated cytotoxicity, ERBB signaling pathway, B cell receptor signaling pathway, Toll-like receptor signaling pathway, JAK-Stat signaling pathway, and Wnt signaling pathway were up-regulated in the PDK2 high group. B. Gene sets related to B cell receptor signaling pathway, cell cycle, colorectal cancer, ERBB signaling pathway, natural killer cell mediated cytotoxicity, non-small cell lung cancer, pancreatic cancer, pathway in cancer, renal cell carcinoma, T cell receptor signaling pathway, and Toll like receptor signaling pathway were up-regulated in the PDK3 high group. C. Gene sets related to ERBB signaling pathway, JAK-Stat signaling pathway, MAPK signaling pathway, and MTOR signaling pathway were up-regulated in PDK4 high group; while gene sets related to DNA replication, cell cycle, and bladder cancer were up-regulated in the PDK4 low group.

prognosis of patients. At the same time, a promotion of cisplatin-resistance of lung adenocarcinoma through regulation of cyclin and CBS domain divalent metal cation transport media-

Table 2. GSEA enrichment analysis for PDK3 in breast cancer

Gene sets	NS[1]	ENS[2]	Nom *P*-val[3]	FDR q-val[4]
KEGG_CELL_CYCLE	0.66	2.16	0	0.014
KEGG_T_CELL_RECEPTOR_SIGNALING_PATHWAY	0.57	1.88	0.016	0.088
KEGG_ERBB_SIGNALING_PATHWAY	0.47	1.86	0.004	0.058
KEGG_NATURAL_KILLER_CELL_MEDIATED_CYTOTOXICITY	0.55	1.84	0.014	0.06
KEGG_B_CELL_RECEPTOR_SIGNALING_PATHWAY	0.56	1.84	0.017	0.058
KEGG_NON_SMALL_CELL_LUNG_CANCER	0.5	1.83	0.004	0.055
KEGG_RENAL_CELL_CARCINOMA	0.48	1.79	0.015	0.063
KEGG_TOLL_LIKE_RECEPTOR_SIGNALING_PATHWAY	0.48	1.75	0.017	0.067
KEGG_PATHWAYS_IN_CANCER	0.4	1.73	0.006	0.065
KEGG_PANCREATIC_CANCER	0.44	1.69	0.022	0.077
KEGG_COLORECTAL_CANCER	0.42	1.6	0.031	0.105

Notes: 1. NS: enrichment score; 2. NES: normalized enrichment score; 3. Nom *P*-val: nominal *p* value; 4. FDR q-val: false discovery rate.

Table 3. GSEA enrichment analysis for PDK4 in breast cancer

Gene sets	NS[1]	ENS[2]	Nom *P*-val[3]	FDR q-val[4]
KEGG_JAK_STAT_SIGNALING_PATHWAY	0.53	2.09	0.002	0.065
KEGG_MAPK_SIGNALING_PATHWAY	0.43	1.86	0.004	0.069
KEGG_MTOR_SIGNALING_PATHWAY	0.48	1.78	0.016	0.068
KEGG_ERBB_SIGNALING_PATHWAY	0.45	1.76	0.008	0.061
KEGG_RENAL_CELL_CARCINOMA	0.46	1.7	0.018	0.081
KEGG_PROSTATE_CANCER	0.41	1.6	0.018	0.108
KEGG_SMALL_CELL_LUNG_CANCER	0.4	1.51	0.056	0.121
KEGG_DNA_REPLICATION	-0.87	-2.22	0	0.001
KEGG_CELL_CYCLE	-0.63	-2.1	0.002	0.002
KEGG_BLADDER_CANCER	-0.48	-1.68	0.022	0.045

Notes: 1. NS: enrichment score; 2. NES: normalized enrichment score; 3. Nom *P*-val: nominal *p* value; 4. FDR q-val: false discovery rate.

tor 3 (CNNM3) by PDK2 was observed. Our study detected a lower expression of PDK2 in BC tissues, and found that PDK2 expression is related to lymph node metastasis.

Previous studies based on the TCGA database or experiments on tissues reported that enhanced PDK3 can be observed in clear cell renal cell carcinoma [27], and colon cancer [28] samples. Further, the clinical significance of PDK3 in colon cancer and acute myeloid leukemia has also been revealed. For instance, by comparing patients with high and low PDK3 level through TCGA database, Cui et al. [13] concluded that a higher PDK3 expression in acute myeloid leukemia leads to a decrease in overall survival rate. Lu et al. [24] found that PDK3 level is adversely associated with a disease-free survival rate and positively related to the severity of colon cancer. The results mentioned above largely agree with ours. Comparing PDK3 levels in paired and unpaired tumor and normal tissues, we found that in tumor tissues the expression of PDK3 was increased, and that enhanced PDK3 expression could result in an up-regulation of gene sets related to B cell and T cell receptor signaling pathway, cell cycle, Toll like receptor signaling pathway, and several cancers. However, the clinicopathologic data, including age, gender, T stage, distance and lymph node metastasis, and tumor stage, in the PDK3 high group were close to those in the PDK3 low group. Previous research proved that PDK3 can promote the drug resistance to chemotherapy of cancer by its activation as a direct target gene for HSF1. The ectopic expression of PDK3 blocks the ubiquitination of HSF1 by FBXW7 as its competitive inhibition binding to

HSF1 in the nucleus suppresses the phosphorylation regulation of GSK315 to HSF1 [29]. In addition, Lu et al. [30] also indicated that the expression of PDK3 is related to HIF. The up-regulation of PDKs induced by HIF will lead to the accumulation of pyruvate, and the accumulated pyruvate will inactivate the enzymatic activity of PDK1, PDK2, and PDK4, but PDK3 will not. Therefore, the up-regulation of PDK3 ensures a continuous inhibition of mitochondrial respiration. Given that PDK3 possesses high enzymatic activity and that high concentrations of pyruvate do not inhibit its activity, these unique characteristics hint at the potential importance of PDK3 in bioinformatics. This may also be the mechanism of PDK3 in breast cancer. Its specific role still needs further research and clarification.

Guda et al. [6] revealed that PDK4 expression in BC is higher than that in normal tissues, which is associated with poor prognosis. Furthermore, they found that silencing PDK4 by miR-211 promotes a phenotype shift towards a pro-glycolytic state in BC cell lines. Walter et al. [31] concluded that PDK4 is highly expressed in tamoxifen-resistant BC cell lines (TamR-MCF-7) relative to their parental cells (MCF-7L), and knocking down PDK4 level in TamR-MCF-7 cells can increase cell stamoxifen and fulvestrant. These two studies indicated that PDK4 is a proto-oncogene associated with higher drug resistance and aggressiveness of BC. Nonetheless, our results are contrary to theirs, since in this study, a lower expression of PDK4 mRNA in BC cancer was reported, and patients with higher PDK4 level tended to present with lower stage and T stage. Further, GSEA enrichment analysis indicated that down-regulated PDK4 causes up-regulation of gene sets related to DNA replication, cell cycle, and bladder cancer.

There exist several limitations in this study. First, we failed to assess the prognostic value of the PDK family in BC. Second, we were lacking the experimental evidence to verify the results obtained from TCGA database. Third, expression data of the PDK family were reported in limited paired samples in TCGA database, so there might exist bias in the results of PDK1 expression.

Although there exist limitations, this study found a lower PDK2 and PDK4 expression and higher PDK3 expression in BC cancer tissues. Furthermore, it identified that PDK2 was negatively related to lymph node metastasis, while PDK4 was positively associated with tumor stage and size. Lastly, the regulation of PDK2, PDK3, and PDK4 in several gene sets related to tumor initiation and progression were also analyzed.

Conclusion

This research reported the expression of the PDK family (PDK1-4) in BC for the first time. Compared with normal samples, in BC tissues the expression levels of PDK2 and PDK4 are lower whilst that of PDK3 is higher, and a negative relation could be observed between PDK2 expression and BC metastasis. PDK4 is found to be related to tumor stage. Meanwhile, by enrichment analysis we found possible gene sets that could be regulated by PDK2-4, respectively. This research can provide a theoretical basis for utilizing the PDK family as treatment targets for BC, and may lead to therapy for curing BC.

Disclosure of conflict of interest

None.

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