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HPD overexpression predicts poor prognosis in breast cancer

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ABSTRACT

Background: The enzyme, 4-hydroxyphenylpyruvate dioxygenase (HPD), is critical to tyrosine metabolism; its deficiency can cause tyrosinemia. However, its precise contribution to tumorigenesis is unclear. Here, we investigated the correlation between HPD expression and prognosis in patients with breast cancer.

Methods: 145 breast cancer specimens were selected to analyze HPD protein expression by immunohistochemistry and evaluate its relationship to patients' clinicopathological features. HPD localization was confirmed in MCF-7 and MDA-MB-231 breast cancer cells, using immunofluorescence staining. The expression of HPD protein was detected in breast cancer and cancer-adjacent normal tissues using Western blot analysis. Survival rates were calculated by the Kaplan–Meier method.

Results: We found that HPD protein was mainly located in the cytoplasm/nucleoli/perinucleus in breast cancer cells, as shown by immunofluorescence staining in MCF-7 and MDA-MB-231 cells, and immunohistochemistry in breast cancer and adjacent normal tissues (HPD protein expression—breast cancer: 46.9% [68/145], ductal carcinoma in situ [DCIS]: 22.6% [12/53], and normal tissues: only 4.8% [2/42]). Similarly, the Western blot results further confirmed the increased expression of HPD in breast cancer compared with cancer-adjacent normal tissues ($P < 0.05$). HPD expression level was positively correlated with histological grade and clinical stage, and inversely correlated with 10-year overall survival (OS) rates, in patients with breast cancer. Among patients with breast cancer, those with high HPD expression had worse OS rates than those with low HPD expression. Additionally, when patients were subgrouped by disease stage or grade, those with high HPD expression had worse OS rates than those with low HPD expression for each respective stage or grade.

Conclusions: Our findings indicate that HPD may be a useful prognostic predictor, and a potential therapeutic target for patients with breast cancer.

1. Introduction

Breast cancer is the most common malignant tumor among women worldwide [1,2]; in 2012, it was fifth leading cause of cancer death, and accounted for 25% of all new cancer diagnoses (at about 1.67 million new cases) in the world [3,4]. Because of earlier detection and improved treatment, the mortality rate has decreased in recent years in most Western countries [5]. However, conventional histopathological classification of breast cancer fails to provide sufficient prognosis and predictive power, and thus biomarkers indicative of intrinsic characteristics of tumors at molecular levels have been urgently needed in medical researches [6].

As a homodimer, HPD is composed of 392 amino acids and its molecular weight is 43KD [7]. The human HPD gene is over 30 kb and has 14 exons located at 12q24.31 [8,9]. It is reported that HPD is a soluble cytoplasmic protein with strong expression in the liver [10]. There are also studies showing that HPD is expressed in cortical neurons, cerebellum, hippocampus and corneocytes [9]. What's more, HPD is also expressed on the endoplasmic reticulum, Golgi and transport vesicles, as shown by immuno-electron microscopy and confocal laser

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scanning microscopy [11,12]. HPD is an iron-dependent non-heme oxygenase that plays a key role in the tyrosine catabolic pathway [13]. Studies have shown that Nuclear factor (erythroid-derived 2)-like 2, also known as Nrf2, can promotes cell proliferation by altering metabolism of cellular glucose and glutamine in cells into anabolic processes [14]. In lung cancer cell line A549, cells with knocked-down Nrf2 gene expressed significantly less HPD than did controls. Nrf2 is inextricably linked with cancer, and high Nrf2 expression has been shown to promote occurrence and proliferation of lung cancer [15], which suggests that HPD might also affect tumor occurrence and development; however, this hypothesis needs further study. The level of HPD expression in breast cancer and its association with clinical outcomes are unclear.

In this study, we investigated the location of HPD in breast cancer cells by immunofluorescence staining and expression of HPD in breast cancer tissues and adjacent normal tissues by immunohistochemistry and Western blot, analyzed the correlation between HPD expression and clinicopathological characteristics, and further analyzed the prognostic value of HPD expression for OS, hoping to clarify the prognostic value of HPD in breast cancer patients and give more clues for the treatment and prevention of breast cancer.

2. Patients and methods

2.1. Ethics statement

This research complied with the Helsinki Declaration. It has been approved by the Human Ethics Committee and the Research Ethics Committee of Dalian University and Jilin University. Patients were informed that hospital would store the resect specimens and potentially for scientific research, and their privacy would be protected. Follow-up survival data were collected retrospectively through medical record analyses.

2.2. Clinical samples

We collected 140 breast cancer tissue samples, 53 DCIS and 42 adjacent normal breast samples from Xinhua Hospital of Dalian University and 5 breast cancer tissue samples from the Second Hospital of Jilin University. These specimens were collected and stored from 2012 to 2017. All samples were routinely fixed in 10% buffered formalin and embedded in paraffin blocks. The fresh clinical specimens for Western blot were obtained from the surgical patients of Xinhua Hospital affiliated to Dalian University in May 2019. All fresh specimens were confirmed by pathological examination and snapfrozen in liquid nitrogen until utilization. The study protocol was approved by the Review Committee of Dalian University and Jilin University. Pathological parameters were carefully reviewed in all of the 145 breast cancer patients, including age, tumor size, nodal metastasis, histological grade, clinical stage, and survival data. Among the breast cancer patients, 86 cases were older than 55 years old and 59 patients were younger than 55 years old. For tumor sizes, 79 cases were smaller than 3 cm, and 66 cases were equal or larger than 3 cm. Considering of lymph node metastasis, 69 cases were positive and 76 cases were negative. In terms of grading of breast cancer, 12 cases were G1, 80 cases were G2, and 53 cases were G3. Additionally, 25 cases were TNM stage 0-I, 82 cases were TNM stage II, and 38 cases were TNM stage III. TNM assessment referred to the staging system established by American Joint Committee on Cancer (AJCC) [16]. Breast cancer patients received surgical treatment with a curative intent and no adjuvant chemotherapy during data collection. The average follow-up time was 120 months.

2.3. Immunofluorescence staining

Breast cancer cell line MCF-7 cells and MDA-MB-231 cells were grown on coverslips to 70%–80% confluence and then fixed with 4% paraformaldehyde for 10 min. After 24 h, the cells were permeabilized with 0.5% TritonX-100 for 10 min. After Blocking with 3% Albumin Bovine V (Solarbio, Beijing, China) for 1 h at room temperature and washing with PBS, cells were incubated primary HPD antibody (1:100, Abcam, UK) at 4 °C overnight and incubated with Alexa Fluor 488 Goat Anti-Rabbit IgG (H + C) (1:1000, Invitrogen, USA) for 1 h at room temperature. After washing with PBS, the cells were counterstained with 49-6-diamidino-2-phenylindole (DAPI) (Beyotime, Shanghai, China). The last step was mounting the coverslips with Antifade Mounting Medium (Beyotime, Shanghai, China). Immunofluorescence signals were visualized and recorded by using a BX53 OLYMPUS microscope [17].

2.4. Immunohistochemical staining

The Dako LSAB kit (Dako, Glostrup, Denmark) was used for Immunohistochemical analysis. Serial 4 μm-thick tissue sections were prepared on silane-coated slides (Sigma, St. Louis, MO, USA). To eliminate endogenous peroxidase activity, the tissue sections were dewaxed with xylene and rehydrated through a graded series of ethanol and incubated with 3% H2O2 in methanol for 10 min at room temperature. The antigen was retrieved at 95 °C for 20 min by placing the slides in 10 mM sodium citrate buffer (pH 6.0). The slides were then incubated with the primary antibody HPD (1:100, Abcam, UK) overnight at 4 °C in a humidified chamber. After 30 min's incubation at room temperature with biotinylated secondary antibody, the slides were incubated with streptavidin-peroxidase complex at room temperature for another 30 min. And then, the slides were immunostained with 3,3′-diaminobenzidine chromogen and counterstained with Mayer's hematoxylin. Rabbit IgG isotope was used as the negative control. Positive tissue sections processed without the primary antibody to serve as another negative control.

2.5. Analysis of immunohistochemical results

All slides were independently scored by two investigators (Liu S and Shan C) who were blind to all clinical data. Briefly, the immunostaining for HPD was mainly semi-quantitatively scored as '−' (negative, no or less than 5% positive cells), '+' (5–25% positive cells), '+ +' (26–50% positive cells) and '+ + +' (more than 50% positive cells). cytoplasm/nucleoli/perinucleus expression patterns were considered as positive staining. In addition, '+ +' and'+ + +' positive cells indicated strongly positive. For survival data analysis, HPD expression including high level (+ + &+ + +) and low level (− &+).

2.6. Western blot analysis

Total protein from two pairs of fresh tumor and cancer-adjacent normal samples were separated by SDS-PAGE and then transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). The membranes were blocked in 5% fat free milk/TBST solution and incubated with primary HPD antibody (1:1000, Abcam, UK) overnight at 4 °C and horseradish peroxidase (HRP)-conjugated secondary antibodies (1:2000, Cell Signaling Technology, USA) for 1 h at 37 °C. Signals were detected using luminol substrate solution and β-actin was used as a loading control.

2.7. Statistical analysis

Statistical analysis was performed by SPSS 20.0 (Chicago, IL, USA). Chi-square tests ($\chi 2$) and Fisher's exact tests were used to assessing the correlations between the HPD expression and clinicopathological characteristics. Survival rates were calculated by the Kaplan-Meier method, and the differences of survival curves were analyzed by log-rank tests. $P < 0.05$ was considered statistically significant.

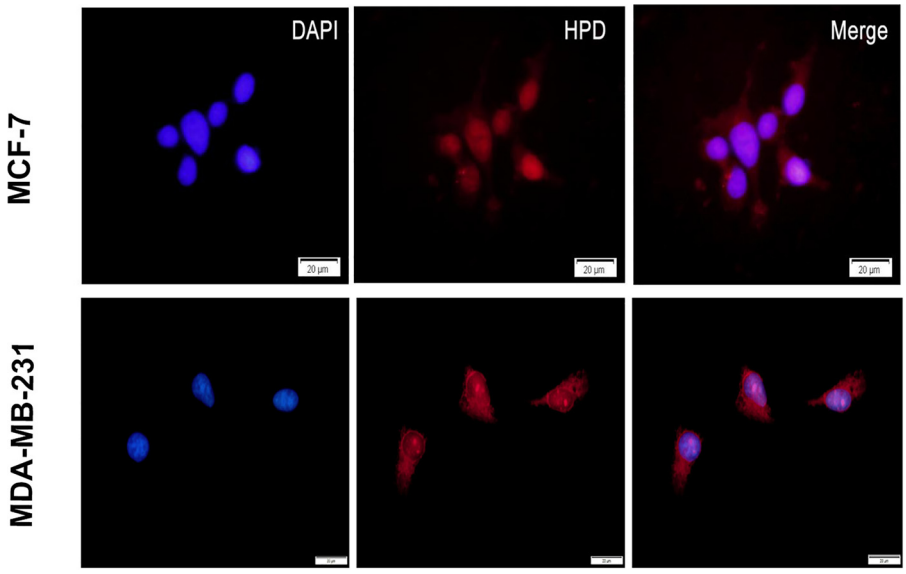

Fig. 1. Immunofluorescence staining for HPD protein in breast cancer cells.
MCF-7 cells and MDA-MB-231 cells were immunostained for HPD (red). Nuclei were visualized by DAPI staining (blue). HPD protein is mainly located in the cytoplasm/nucleoli/perinucleus of MCF-7 cells and MDA-MB-231 cells.

3. Results

3.1. HPD protein expression in breast cancer and normal breast tissue

Immunofluorescence staining indicated that the HPD protein was mainly located in the cytoplasm/nucleoli/perinucleus of MCF-7 cells and MDA-MB-231 cells (Fig. 1). Immunohistochemical staining confirmed that HPD protein was mainly located in the cytoplasm/nucleoli/perinucleus in breast cancer cells. Expression of HPD protein was 77.9% (113/145; $P < 0.001$) in breast cancer, which was significantly higher than in adjacent normal breast tissues (23.8%, 10/42); and strong expression of HPD protein was 46.9% (68/145; $P < 0.001$) in breast cancer and 22.6% (12/53; $P = 0.015$) in DCIS, which was also significantly higher than in adjacent normal breast tissues (4.8%, 2/42; Fig. 2, Table 1). Moreover, the Western blot results for two pairs of fresh tumor and cancer-adjacent normal tissues from breast cancer patients demonstrated significantly upregulated HPD expression in breast cancer samples compared with cancer-adjacent normal tissues from the same patient ($P < 0.05$, Fig. 3).

3.2. Relationship between HPD expression and breast cancer clinicopathological features

We explored the association between HPD expression and the clinicopathological characteristics of breast cancer. HPD expression was significantly correlated with histological grade ($P = 0.012$) and clinical stage ($P = 0.003$), but not to patient age, tumor size or lymph node status ($P > 0.05$; Table 2).

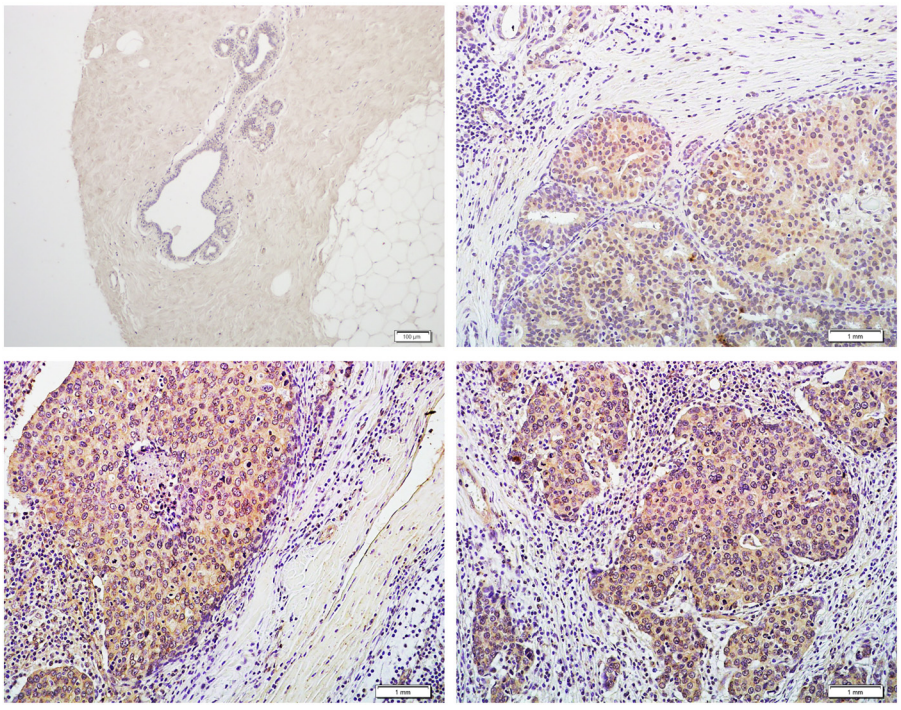

Fig. 2. Immunohistochemical staining for HPD protein in breast cancer tissues.
(A) Normal breast tissues are negative for HPD expression. (B) Positive HPD protein expression was detected in breast cancer tissues. (C)Strongly positive HPD protein was detected in breast cancer tissues. (D) HPD protein is focally strongly positive in breast cancer tissues with lymph node metastasis.

Table 1
Expression of HPD protein in breast cancer.

Diagnosis	No. Of case	HPD				Positive cases rate	Strong positive cases rate
		−	+	++	+++		
Breast cancer	145	32	45	43	25	77.9%**	46.9%**
DCIS	53	33	8	7	5	37.7%	22.6%*
Adjacent normal	42	32	8	2	0	23.8%	4.8%

Compared with adjacent tissues, *P < 0.05, **P < 0.01.
Positive rate: percentage of positive cases with '+', '++', and '+++' staining score.
Strongly positive rate: percentage of positive cases with '++' and '+++' staining score.

3.3. Association between HPD expression and prognosis of breast cancer patients

To substantiate the significance of HPD expression level in breast cancer progression, we analyzed OS over 10 years, using the Kaplan–Meier method for patients whose tumors expressed high vs low HPD levels. We found that the high HPD group had worse OS rates than the low HPD group (log-rank = 16.026, $P < 0.00$; Fig. 4A). Similarly, survival of patients with Grade-1 ((log-rank = 7.572, $P =$ 0.006), Grade-2 (log-rank = 7.676, $P =$ 0.006), and Grade-3 ((log-rank = 4.974, $P =$ 0.026) breast cancer were significantly lower in patients with tumors exhibiting high versus low HPD expression (Fig. 4B–D). Moreover, breast cancer patients with high HPD expression had decreased OS compared to those with low HPD expression whether in stage 0–I (log-rank = 4.485, P = 0.034), stage 2 (log-rank = 4.311, P = 0.038) or stage 3 (log-rank = 6.139, P = 0.013) (Fig. 4E, F).

4. Discussion

Early-stage breast cancer is very subtle and not easily detected by the patient. When advanced, it can easily spread to other tissues and organs, which will seriously threaten patients' physical and psychological health. Approximately 12.4% of women will be diagnosed with breast cancer in their lifetime; the malignancy is diagnosed at an early stage in 90% of patients, and most tumors will progress to advanced or metastatic disease [18]. Early detection and diagnosis is helpful to reduce breast cancer mortality and improve patient outcomes [19]. Conventional diagnosis of breast cancer metastasis depends mainly on the patient's clinical manifestations and imaging diagnosis. However, these methods are relatively poor in sensitivity and effectiveness. Detection of tumor markers is of great importance for clinicians in judging whether metastasis occurs after surgery. Tumor markers for breast cancer include TPS, CA153, CEA, CA125, VEGF, TSGF, and SF [20]. Although great progress has been made in this regard, great potential for exploration still exists. Therefore, discovering a protein with an important function in breast cancer occurrence and expansion, along with its molecular mechanism, can provide a new marker and therapeutic target for breast cancer prediction, diagnosis and treatment.

Table 2
Relationship between HPD protein overexpression and the clinicopathological features of breast cancer.

Variables	No. Of case (*n*)	HPD Strong positive rate (%)	χ^2	*P* value
Age (years)				
≥55	86	40 (46.5%)	0.013	0.911
< 55	59	28 (47.5%)		
Tumor size				
< 3cm	79	34 (43.0%)	1.038	0.308
≥3cm	66	34 (51.5%)		
Lymph node status				
N0	76	39 (51.3%)	1.251	0.263
N+	69	29 (42.0%)		
Histological grade				
Grade-1	12	3 (25.0%)	8.864	0.012*
Grade-2	80	32 (40.0%)		
Grade-3	53	33 (62.3%)		
Clinical stage				
0–I	25	5 (20.0%)	11.312	0.003**
II	82	39 (47.6%)		
III	38	24 (63.2%)		

* $p < 0.05$.
** $p < 0.01$.

Although HPD is mainly expressed in the liver [10], we found that HPD is also expressed in breast cancer. It is located in the cytoplasm/nucleus/perinucleus of breast cancer cells, which needs further investigation to elucidate the mechanism of HPD localization. In addition, we also found that HPD expression was significantly correlated with histological grade and clinical stage in breast cancer, which indicates that HPD may be an effective marker for breast cancer prognosis. Survival analysis showed that HPD expression level was significantly associated with survival over a 10-year period in breast cancer patients, which implies that high HPD expression predicts poor survival in breast cancer patients. We did a multivariate analysis but found no statistical significance. We think it may be related to the number of our samples. In the future, we can expand the number of cases and continue to study in depth. Studies have shown that the ability of HPD-regulated cells to proliferate and migrate in lung cancer does not depend on its metabolic enzyme activity [21]. Furthermore, HPD can activate the NFκB signaling pathway, by enhancing NFκB reporter activity, promoting degradation of IκBα and P65 into the nucleus, and upregulating target genes downstream of the NFκB pathway [21]. Further studies are needed to understand the regulatory mechanism of HPD in breast cancer.

Protein biomarkers include serum biomarkers, tissue biomarkers, and salivary biomarkers [22]. Potential biomarkers in the blood can be proteins present in biopsied cancerous tissue and circulating protein fragments in the diseased tissue microenvironment [23]. Therefore, blood is a suitable biological material for early non-invasive diagnosis,

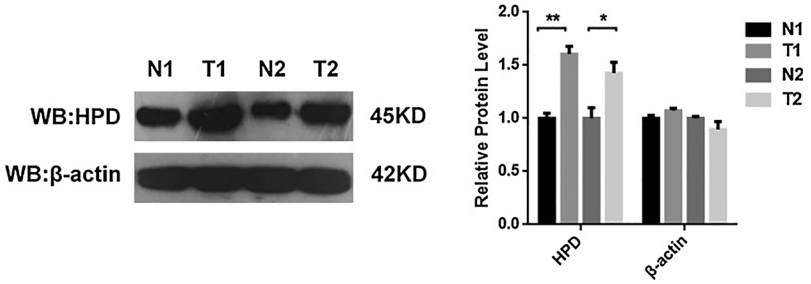

Fig. 3. HPD overexpression in breast cancer tissues.
Two pairs of fresh breast cancer tissues (T) and cancer-adjacent normal tissues (N) were selected and analyzed for HPD protein expression by Western blotting. β-actin was used as a loading control.

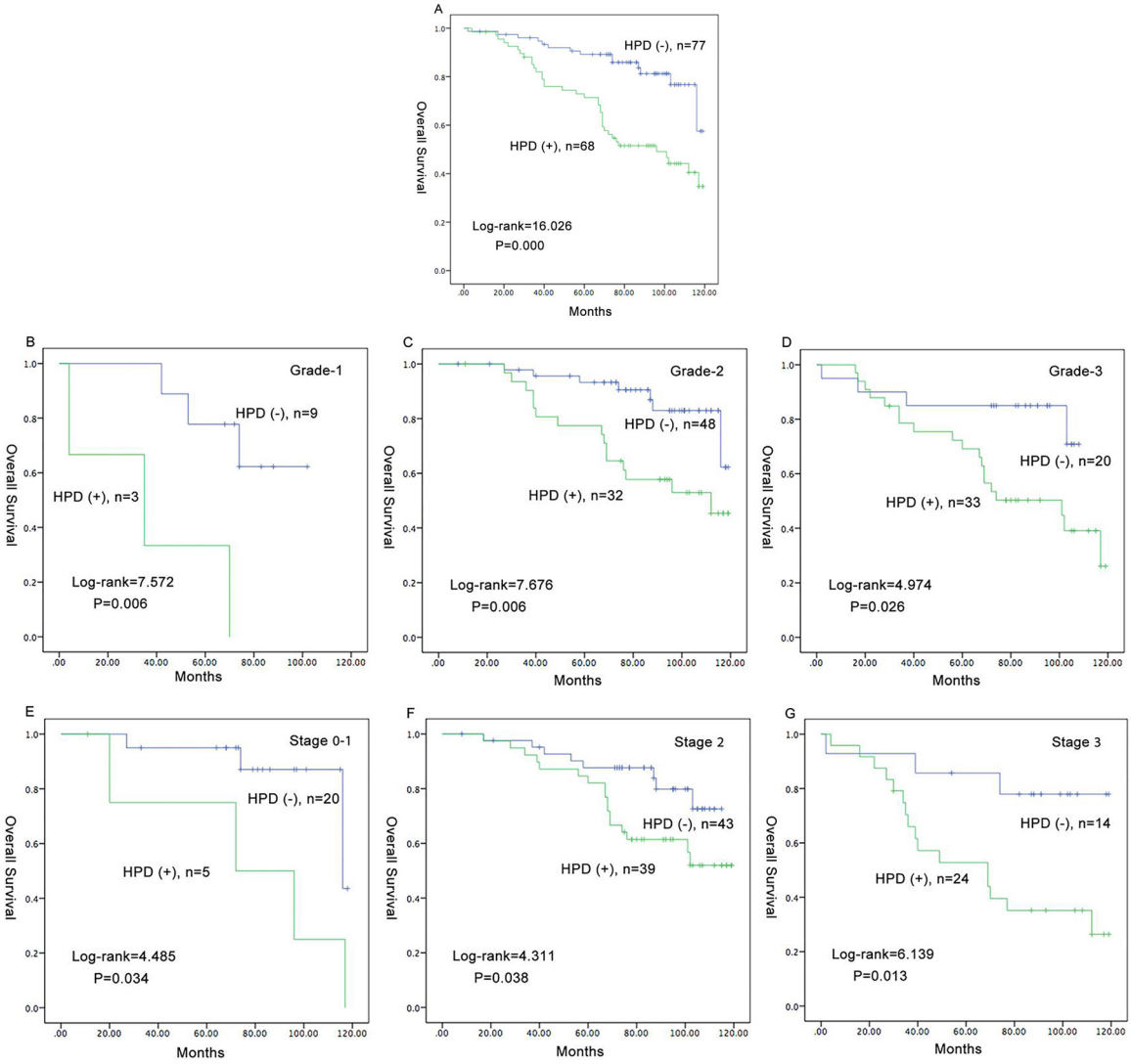

Fig. 4. Kaplan–Meier analyses of survival rates in 145 breast cancer patients in relation to HPD protein overexpression.
(A) shows comparison of OS, respectively, in HPD low-expression (L) and high-expression (H) patients. (B) shows comparison of OS, respectively, in HPD (L) and (H) patients of Grade-1. (C) shows comparison of OS, respectively in HPD (L) and (H) patients of Grade-2. (D) shows comparison of OS, respectively, in HPD (L) and (H) patients of Grade-3. (E) shows comparison of OS, respectively, in HPD (L) and (H) patients of Stage0-1. (F) shows comparison of OS, respectively in HPD (L) and (H) patients of Stage2. (G) shows comparison of OS, respectively, in HPD (L) and (H) patients of Stage3.

and for monitoring treated cancers [24,25]. As a secreted protein that is detectable in serum, HPD potentially has benefits over some other markers [9].

5. Conclusion

HPD plays a key role in breast cancer progression. The high percentage of HPD^+ cells and prognostic value of HPD expression suggests that it may be a significant biomarker and a potential therapeutic target for patients with breast cancer.

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Author's contributions

XY Wang and YJ Shang contributed to this article equally. XY Wang and YJ Shang participated in the study conception, design, case selection and experiments. YL and TX carried out data collection. ZH was responsible for the transportation of specimens. WX, SC and LS performed the data analysis and wrote the manuscript.

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References

[1] M.M. Desouki, I. Doubinskaia, D. Gius, S.A. Abdulkadir, Decreased mitochondrial SIRT3 expression is a potential molecular biomarker associated with poor outcome in breast cancer, Hum. Pathol. 45 (5) (2014) 1071–1077.
[2] C. Desantis, J. Ma, L. Bryan, A. Jemal, Breast cancer statistics, 2013, CA Cancer J. Clin. 64 (1) (2014) 52–62.
[3] S. Mcguire, World Health Organization, International Agency for Research on Cancer, WHO Press, 2015, Adv. Nutr. 7 (2) (2016) 418–419.

[4] L.A. Torre, F. Bray, R.L. Siegel, J. Ferlay, J. Lortet-Tieulent, A. Jemal, Global cancer statistics, 2012, CA Cancer J. Clin. 65 (2) (2015) 87–108.
[5] S. Krennpilko, U. Langsenlehner, E.M. Thurner, T. Stojakovic, M. Pichler, A. Gerger, K.S. Kapp, The elevated preoperative platelet-to-lymphocyte ratio predicts poor prognosis in breast cancer patients, Br. J. Cancer Suppl. 110 (10) (2014) 2524–2530.
[6] G. Li, J. Hu, G. Hu, Biomarker studies in early detection and prognosis of breast cancer, Adv. Exp. Med. Biol. 1026 (2017) 27–39.
[7] F. Endo, H. Awata, A. Tanoue, M. Ishiguro, Y. Eda, K. Titani, I. Matsuda, Primary structure deduced from complementary DNA sequence and expression in cultured cells of mammalian 4-hydroxyphenylpyruvic acid dioxygenase. Evidence that the enzyme is a homodimer of identical subunits homologous to rat liver-specific alloantigen F, J. Biol. Chem. 267 (34) (1992) 24235–24240.
[8] H. Awata, F. Endo, I. Matsuda, Structure of the human 4-hydroxyphenylpyruvic acid dioxygenase gene (HPD), Genomics 23 (3) (1994) 534–539.
[9] L. Aarenstrup, A.M. Falch, K.K. Jakobsen, S. Neve, L.L. Henriksen, N. Tommerup, H. Leffers, Expression and post-translational modification of human 4-hydroxyphenylpyruvate dioxygenase, Cell Biol. Int. 26 (7) (2002) 615–625.
[10] I. Garcia, M. Rodgers, C. Lenne, A. Rolland, A. Sailland, M. Matringe, Subcellular localization and purification of a p-hydroxyphenylpyruvate dioxygenase from cultured carrot cells and characterization of the corresponding cDNA, Biochem. J. 325 (3) (1997) 761–769.
[11] S. Neve, L. Aarenstrup, D. Tornehave, H. Rahbek-Nielsen, T.J. Corydon, P. Roepstorff, K. Kristiansen, Tissue distribution, intracellular localization and proteolytic processing of rat 4-hydroxyphenylpyruvate dioxygenase, Cell Biol. Int. 27 (8) (2003) 611–624.
[12] R. Hummel, P. Norgaard, P.H. Andreasen, S. Neve, K. Skjødt, D. Tornehave, K. Kristiansen, Tetrahymena gene encodes a protein that is homologous with the liver-specific F-antigen and associated with membranes of the Golgi apparatus and transport vesicles, J. Mol. Biol. 228 (3) (1992) 850–861.
[13] G.R. Moran, 4-Hydroxyphenylpyruvate dioxygenase, Arch. Biochem. Biophys. 433 (1) (2005) 117–128.
[14] Y. Mitsuishi, K. Taguchi, Y. Kawatani, T. Shibata, T. Nukiwa, H. Aburatani, M. Yamamoto, H. Motohashi, Nrf2 redirects glucose and glutamine into anabolic pathways in metabolic reprogramming, Cancer Cell 22 (1) (2012) 66.
[15] S. Homma, Y. Ishii, Y. Morishima, T. Yamadori, Y. Matsuno, N. Haraguchi, N. Kikuchi, H. Satoh, Nrf2 enhances cell proliferation and resistance to anticancer drugs in human lung cancer, Clin. Cancer Res. 15 (10) (2009) 3423–3432.
[16] L.G. Frederick, L.P. David, D.F. Irvin, G.F. April, M.B. Charles, G.H. Daniel, M. Monica, AJCC Cancer Staging Manual, (2010), pp. 803–819.
[17] J. Jin, T. Jin, M. Quan, Y. Piao, Z. Lin, Ezrin overexpression predicts the poor prognosis of gastric adenocarcinoma, Diagn. Pathol. 7 (2012) 135.
[18] G.M. Baker, T. Murphy, T. Block, D. Nguyen, F.J. Lynch, Development and validation of an immunohistochemistry assay to assess glucocorticoid receptor expression for clinical trials of mifepristone in breast cancer, Cancer Manag. Res. 7 (2015) 361–368.
[19] Q. Zi, Joint detection of CA15-3, CA125, CEA on the clinical value of breast cancer, China Med. Front. 12 (2013) 99.
[20] Z. Yang, Tumor markers CEA, CA15-3 and CA125 in the evaluation of the efficacy of chemotherapy in breast cancer, China Women Children's Health Res. 28 (2) (2017) 695–696.
[21] X. Kong, The Role of Tyrosine Metabolic Enzyme HPD and its Mechanism in the Development and Progression of Lung Cancer, Tianjin University, 2015.
[22] G.M. Strauss, A.T. Skarin, Use of tumor markers in lung cancer, Hematol. Oncol. Clin. North Am. 8 (3) (1994) 507.
[23] H. Sung, J. Cho, Biomarkers for the lung cancer diagnosis and their advances in proteomics, BMB Rep. 41 (9) (2008) 615.
[24] G.S. Omenn, Strategies for plasma proteomic profiling of cancers, Proteomics 6 (20) (2006) 5662–5673.
[25] N. Rifai, M.A. Gillette, S.A. Carr, Protein biomarker discovery and validation: the long and uncertain path to clinical utility, Nat. Biotechnol. 4 (8) (2006) 971–983.