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Original Article

Dihydrofolate reductase as a predictor for poor response to platinum-based chemotherapy in epithelial ovarian cancer

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Abstract: Background: Platinum-based chemotherapy is the first line chemotherapy regimen for ovarian cancer patients. However, chemotherapy resistance is observed in a large proportion of patients. It is urgently needed to investigate prognostic biomarkers for chemo-sensitivity in ovarian cancer. Methods: Dihydrofolate reductase (DHFR) expression was measured by immunohistochemical staining in 108 specimens, as well as DHFR mRNA variants with qRT-PCR assays. The correlation between DHFR expression and platinum-based chemotherapy response was analyzed. The prognostic significance of DHFR expression was evaluated in ovarian cancer. Results: Positive DHFR expression was observed in 48 specimens, which was correlated to chemotherapy resistance in ovarian cancer patients. Elevated DHFR2 mRNA expression, rather than DHFR1, was observed in chemotherapy resistant tumors. Positive DHFR expression was correlated with higher histologic grade in ovarian cancer (P = 0.014). Kaplan-Meier analysis indicated that DHFR positive expression predicted poor disease-free survival (DFS) (P = 0.040), but not overall survival (OS) of ovarian cancer patients (P = 0.706). The prognostic value was further supported by TCGA data analysis. Cox regression analysis indicated that positive DHFR expression was an independent detrimental factor for disease progression for ovarian cancer patients (P = 0.016). Conclusion: DHFR level measurement was a valuable prognostic biomarker for chemo-sensitivity of ovarian cancer. Molecular analysis for DHFR variants will provide important evidence for chemotherapy regimen options.

Keywords: Dihydrofolate reductase, chemotherapy resistance, ovarian cancer

Introduction

Epithelial ovarian cancer is a common gynecologic malignancy, characterized as late-detected advanced stage disease and poor prognosis [1]. Platinum-based chemotherapy, such as cisplatin or carboplatin, is the predominant regimen after cytoreductive surgery for advanced stage ovarian cancer [2]. However, most patients suffer recurrence within 2 years from the diagnosis in spite of initial objective response [3]. Limited chemotherapy options aer available for the patients with platinum resistant ovarian cancer [4]. Thus, effective prognostic strategies for chemoresistance are needed to improve survival.

A multifactorial procession is involved in the development of platinum-based chemotherapy resistance [5]. The resistant cells show the typical trait of increased DNA synthesis and repair [6]. Dihydrofolate reductase (DHFR) participates in dihydrofolate recycling to maintain DNA synthesis and cell proliferation [7, 8]. The exploitation of inhibitors of DHFR leads to a halt of cell cycle, including methotrexate and pemetrexed for breast cancer [9, 10]. Previous data highlight elevated DHFR expression in cisplatin resistant cells of different cancers [11], but the clinical significance of DHFR in epithelial ovarian cancer has not been explored, especially its role in platinum-based chemotherapy resistance.

In present study, the expression of DHFR in ovarian cancer tissues were analyzed with molecular assays. The therapeutic efficiency was evaluated for the platinum-based chemotherapy. We explored the promising value of

DHFR expression for prognosis of the response to first-line chemotherapy.

Materials and methods

Patients and chemotherapy administration

We collected 108 epithelial ovarian cancer tissues for this retrospective study. All the patients were diagnosed in our hospital from 2008 to 2014. Platinum-based chemotherapy was administered followed by cytoreductive surgery. Clinical information was collected form clinical documents, including age, tumor size, histologic grade, lymph node metastasis, and CA125 levels. All the patients were followed up to evaluate the chemotherapy response. Platinum-resistance was defined as suffering tumor recurrence within six months, whereas tumor recurrence aftersix months of chemotherapy was defined as platinum-sensitive. Our study was approved by the Institutional Review Board of Second People's Hospital of Chengdu.

Immunohistochemical staining (IHC)

Tumor specimens were collected in Pathology Department. Tumor sections were prepared from each specimen, then dewaxed and hydrated as recommendation. Antigen retrieval with citrate buffer (pH 6.0) was performed in boiled water. Endogenous peroxidase blockage was performed with 0.3% H_2O_2 solution. Ventana Discovery XT automated staining system (Ventana Medical Systems, Inc., Tucson, AZ, USA) was used for IHC staining. The primary antibody for DHFR (ab82171, Abcam, Cambridge, MA) and control IgG was used in this study.

IHC score evaluation

DHFR expression was measured with a semi-quantitative system [12]. Final IHC scores were determined by staining intensity and positive proportion of tumor cells. The intensity of IHC staining were ranked in three groups (no staining = 0; weak staining = 1, middle = 2, strong = 3), the percentage of stained cells were determined by the stained tumor cells in five typical high power field. The final score was obtained by multiplying the intensity score with the percentage score of stained cells, ranging from 0 (the minimum score) to 3 (the maximum score).

Reverse transcription-quantitative real-time polymerase chain reaction (qRT-PCR) Trizol® reagent (Invitrogen, CA, USA) and PrimeScript RT Master Perfect Real Time Kit (TaKaRa, Japan) kits were used for total RNA preparation and mRNA reverse transcription. Real-time PCR analysis was performed with SYBR Green PCR master mix (Invitrogen, CA, USA). The sequences of primers are as below: DHFR1: 5'-GTCATGGTTGGTTCGCTAAACTGCA-3', 5'-ATACATACTTTTTTCAGAGGGAGGG-3'; DHFR2: 5'-CAGAGAACTCAAGGAACCTCCACAAG-3', 5'-GAACTGCCACCAACTATCCAGAACCAT-3'; GAPDH: 5'-GTCAGTGGTGGACCTGACCT-3', 5'-TGAGGAGGGGAGATTCAGTG-3'.

Statistical analyses

Statistical analyses were performed with SPSS19.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism version 5.00 (GraphPad Software, CA, USA). Differences between groups were evaluated by one-way ANOVA. Kaplan-Meier analysis and Cox proportional hazards regression model were used for prognostic significance. $P < 0.05$ was considered significant.

Results

Elevated DHFR expression in platinum-based chemotherapy resistant ovarian cancer specimens

IHC analysis was performed with totally 108 epithelial ovarian cancer specimens, and another eight recurrent or metastatic epithelial ovarian cancer specimens were also involved. Positive DHFR staining was predominately localized in the nucleus (**Figure 1A**). More DHFR expression was observed in normal oviduct tissues compared to tumor tissues (**Figure 1A**). Notably, increased IHC scores were observed in recurrent or metastatic ovarian cancer specimens compared to corresponding primary ones, which were significantly lower than normal oviduct tissues (**Figure 1B**). Further analysis also indicated that the tumors resulting in disease progression during chemotherapy showed increased DHFR scores compared to sensitive ones (**Figure 1C**). ROC analysis indicated the cut-off value of IHC scores for DHFR was 1.0 (**Figure 1D**). Notably, the patients with DHFR positive ovarian cancer showed higher percentage of disease progression during chemotherapy than those with DHFR negative tumors (**Figure 1E**).

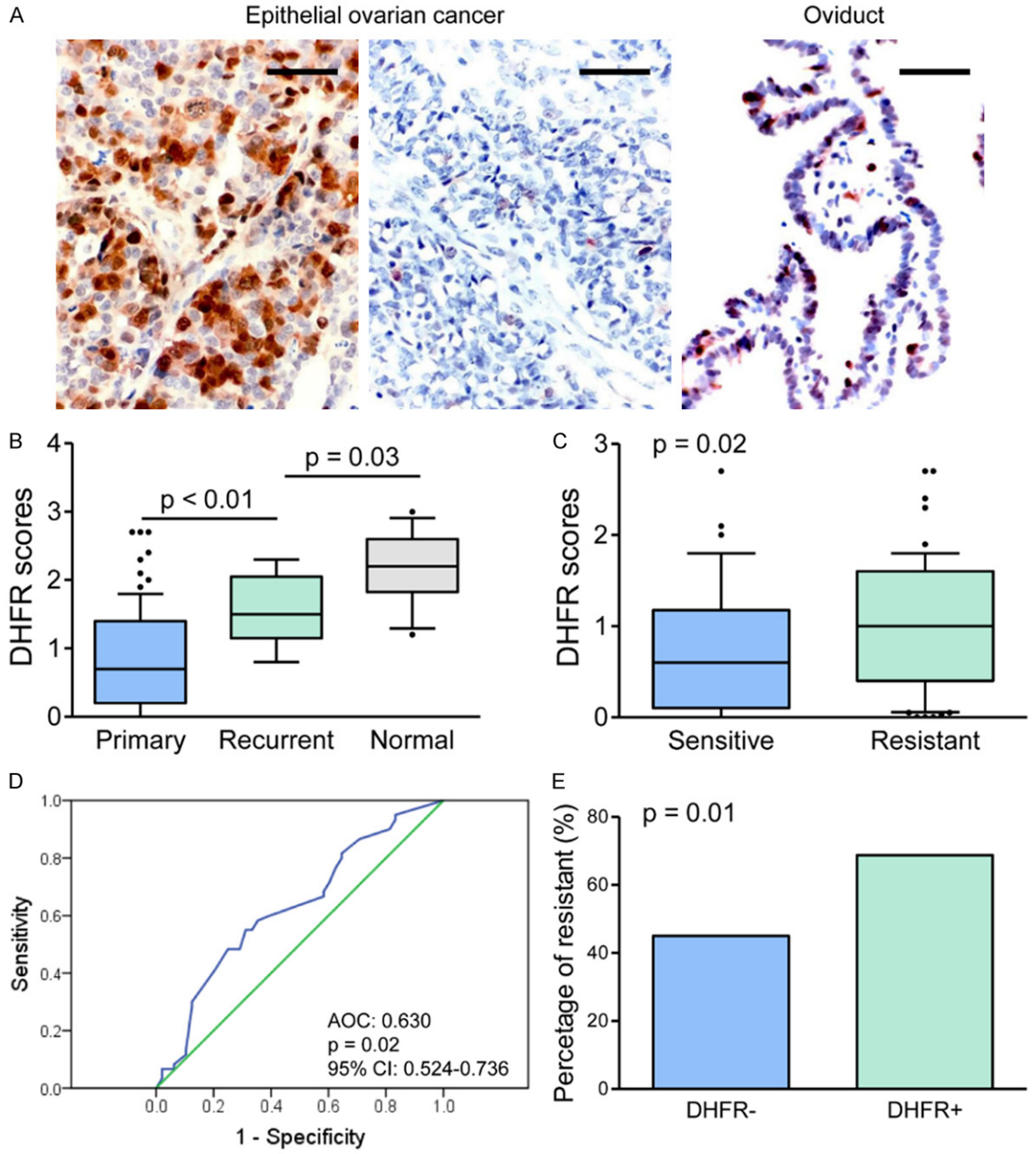

Figure 1. DHFR expression in ovarian cancer specimens. A. IHC staining for DHFR was performed on ovarian cancer specimens and oviduct tissues. Representative images of DHFR IHC staining are shown. Bar, 100 μm. B. IHC scores were compared among groups, primary and recurrent ovarian cancer specimens, as well as oviduct tissues. C. IHC scores are compared between chemotherapy sensitive and resistant ovarian cancer specimens. D. Cut-off value of DHFR scores was determined by ROC analysis. The cut-off score = 1.0, AUC = 0.630, 95% CI: 0.524-0.736, P = 0.002. E. The percentage of chemotherapy resistance was compared between the patients with DHFR positive and negative ovarian tumors. P = 0.01.

Increased transcription of DHFR2 but not DHFR1 is involved in chemotherapy resistance

To further investigate the functional role of different DHFR variants in ovarian cancer chemotherapy resistance, the mRNA levels of DHFR1/2 were evaluated with tumors tissues. The qT-PCR assays were performed with 8 chemotherapy resistant tumors and 8 sensitive tumors. Our results showed significantly

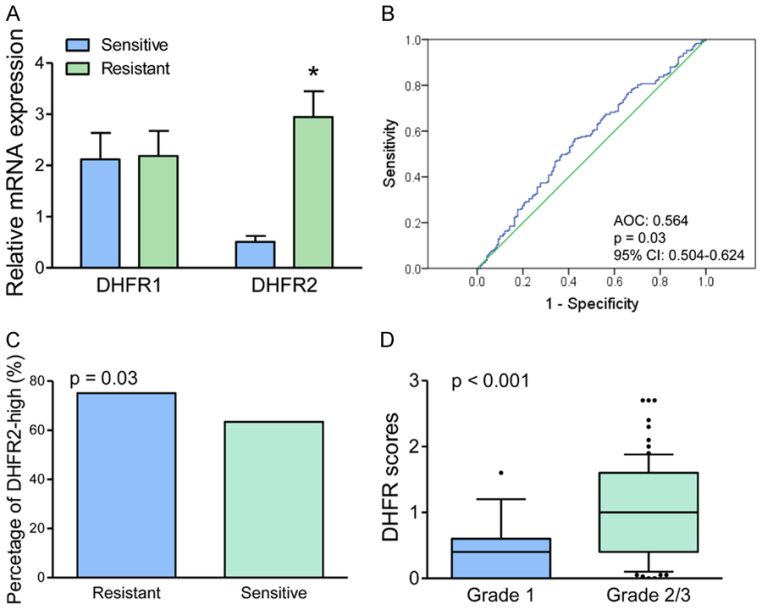

Figure 2. Increased transcription of DHFR2 but not DHFR1 was involved in chemotherapy resistance. A. qT-PCR assays for the mRNA levels of DHFR1/2, which were analyzed with chemotherapy resistant/sensitive tumors. *P < 0.01. B. ROC analysis with TCGA data of epithelial ovarian cancer for the prognostic value of DHFR2 in disease progression (AOC: 0.564, P = 0.03, 95% CI: 0.504-0.624). C. The percentage of DHFR2 high expression was compared between chemo-resistant tumor and sensitive tumor. P = 0.03. D. Histologic grade 2/3 specimens showed significantly higher DHFR scores than grade-1 ones. P < 0.001.

Table 1. Relationship between clinical characteristics and DHFR expression

Characteristic	Number (%)	DHFR expression		*p* value
		Negative	Positive	
Total	108	60, 55.56%	48, 44.44%	
Age, years				
< 50	45 (41.67)	22 (20.37)	23 (21.30)	0.239
≥ 50	63 (58.33)	38 (35.19)	25 (23.15)	
Tumor size, cm				
≤ 10	66 (61.11)	38 (35.19)	28 (25.93)	0.596
> 10	42 (38.89)	22 (20.37)	20 (18.52)	
Clinical stage				
I-II	34 (31.48)	19 (17.59)	15 (13.89)	0.963
III-IV	74 (68.52)	41 (37.96)	33 (30.56)	
Histologic grade				
I	36 (33.33)	26 (24.07)	10 (9.26)	0.014
II-III	72 (66.67)	34 (31.48)	38 (35.19)	
CA125				
< 400	58 (53.70)	31 (28.70)	27 (25.00)	0.635
≥ 400	50 (46.30)	29 (26.85)	21 (19.44)	
Lymph node status				
0	44 (40.74)	25 (23.15)	19 (17.59)	0.827
≥ 1	64 (59.26)	35 (32.41)	29 (26.85)	

increased DHFR2 mRNA expression in chemo-resistant tumors versus sensitive tumors, whereas no significant change was observed in DHFR1 (**Figure 2A**). Further analysis with TCGA data of epithelial ovarian cancer also indicated the prognostic value of DHFR2 in disease progression (**Figure 2B**). A higher percentage of DHFR2 high expression was found in chemo-resistant tumor than in the sensitive tumor (**Figure 2C**), whereas no significant difference was observed in DHFR1 expression.

The correlation between DHFR expression and clinicopathologic features

The correlation was evaluated between the IHC results of DHFR expression and clinicopathologic parameters. Our results indicated that DHFR positive expression was associated with histologic grade (P = 0.014). The poorly differentiated tumors showed an increased percentage of positive DHFR expression, as shown in **Table 1**. Notably, histologic grade 2/3 specimens showed significantly higher DHFR scores than grade-1 ones (P < 0.001, **Figure 2D**). However, we did not observe significant correlation in DHFR positive expression with age, tumor size, clinical stage, CA125 levels, and lymph node metastasis (**Table 1**).

Positive DHFR expression suggests progression of ovarian cancer

Survival estivation value was analyzed for the expression status of DHFR in ovarian cancer patients. Kaplan-Meier analysis showed poorer progression-free survival (DFS) in

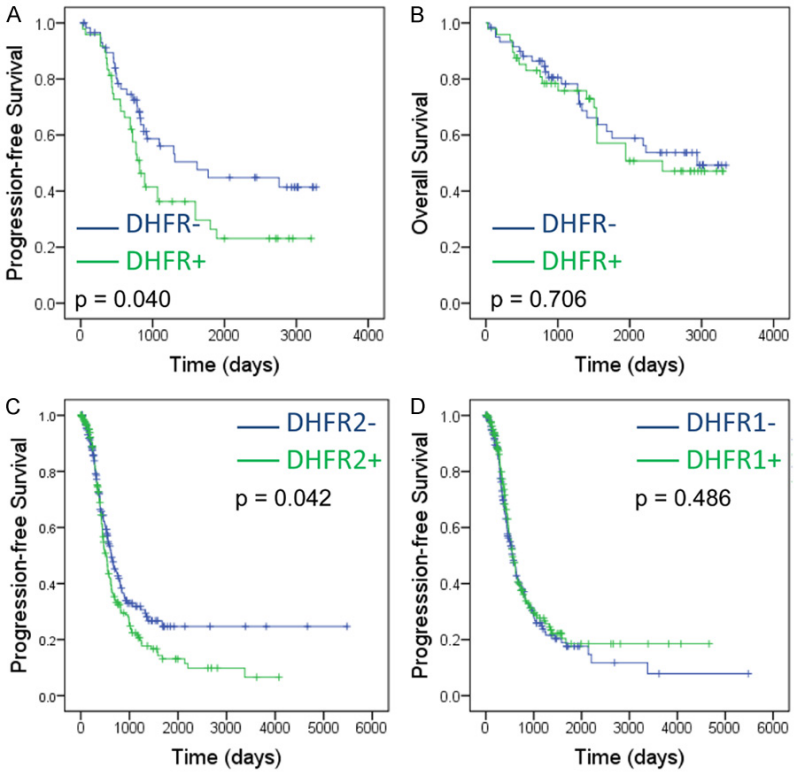

Figure 3. Positive DHFR expression suggests disease progression of ovarian cancer. (A, B) Kaplan-Meier analysis of Disease-Free Survival (A) and Overall Survival (B) for ovarian cancer patients with DHFR positive or negative expressing tumors in our center. (C, D) Kaplan-Meier analysis of Disease-Free Survival (C) and Overall Survival (D) for ovarian cancer patients with DHFR2 mRNA high or low expressing tumors with TCGA data.

Table 2. Cox regression analyses of progression-free survival and overall survival for DHFR expression in ovarian cancer

Variable Analysis	PFS			OS		
	HR	95.0% CI	P	HR	95.0% CI	P
Univariate						
DHFR	1.697	1.018-2.828	0.042	1.122	0.616-2.044	0.707
Multivariate						
Age	1.470	0.853-2.535	0.165	1.211	0.635-2.308	0.561
Size	0.740	0.408-1.342	0.322	0.768	0.362-1.629	0.492
Stage	2.648	0.863-8.127	0.089	3.320	0.84-13.129	0.087
Grade	1.797	0.608-5.315	0.289	1.236	0.352-4.342	0.741
Ca125	0.914	0.503-1.663	0.769	0.989	0.467-2.092	0.977
LNM	2.181	0.804-5.912	0.126	2.048	0.688-6.094	0.198
DHFR	1.975	1.134-3.441	0.016	1.122	0.616-2.044	0.707

HR, hazard ratios; CI, confidence interval. The variables were compared in the following ways: Age, > 50 years vs. ≤ 50 years; size, > 10 cm vs. ≤ 10 cm; Grade, G2-3 vs. G1; CA125, < 400 vs. > 400; LNM, metastasis vs. none; DHFR, positive vs. negative.

positive DHFR expression patients than negative ones (P = 0.040. **Figure 3A**), whereas no significant difference of overall survival (OS) was observed between groups (P = 0.706. **Figure 3B**). Further analysis with TCGA data also confirmed that elevated DHFR2 expression predicted poor PFS in ovarian cancer, but not OS (P = 0.042, 0.486, respectively. **Figure 3C**, **3D**). The independent prognostic values of DHFR expression was also analyzed with univariate and multivariate Cox regression models. Positive DHFR expression was a risk factor for PFS (HR, 1.697; 95% CI, 1.018-2.828, P = 0.042), but not for OS (HR, 1.122; 95% CI, 0.616-2.044; P = 0.707, **Table 2**). Multivariate analysis also supported positive DHFR expression was an independent risk factor for PFS (HR, 1.975; 95% CI, 1.134-3.441; P = 0.016), but not for OS (HR, 1.122; 95% CI, 0.616-2.044; P = 0.707, **Table 2**). Therefore, increased DHFR expression was an independent detrimental factor for epithelial ovarian cancer patients.

Discussion

Platinum-based chemotherapy is a first-line regimens for epithelial ovarian cancer after surgery [13], but shows high percentage of recurrence with chemotherapy treatment [14]. It is valuable to exploit a prognostic biomarker for chemotherapy efficacy to optimize chemotherapy options [15]. Our study indicated that DHFR expression, especially DHFR2 mRNA, was an effective biomarker to predict platinum-based chemothera-

py resistance. Enhanced DHFR expression was an independent prognostic biomarker for the disease progression of ovarian cancer.

The combined regimen of paclitaxel and platinum compounds, such as cisplatin or carboplatin, was the first-line option for epithelial ovarian cancer following surgery [16]. Chemotherapy resistance usually occurs rapidly after platinum-based regimen treatment in epithelial ovarian cancer patients. It is still a focus to develop more effective systemic therapies for improved survival estimation [17]. In this case, it was necessary to exploit biomarkers to predict chemotherapy response, which will favor ovarian cancer patients. We focused on the expression of DHFR in ovarian cancer tissues, which is a valuable biomarker to predict platinum-based chemotherapy resistance. Elevated DHFR expression in ovarian cancer tissues was correlated with platinum-based chemotherapy resistance. Thus, the measurement of DHFR expression level provided important evidence of chemotherapy options for ovarian cancer patients.

Previous studies supported that elevated expression of folate-dependent proteins was involved in chemotherapy resistance, such as thymidylate synthase [18], DHFR [19] and phosphoribosylglycinamide formyltransferase (GART) [20], which is crucial for cell replication. The platinum-based chemotherapy resistant cell lines also showed increased DNA replication and repair activity [21]. The transformation of folate and 7, 8 dihydrofolate (DHF) into 5, 6, 7, 8 tetrahydrofolate (THF) is dependent on the catalytic activity of DHFR, which is an essential step in the synthesis of DNA nucleic acid bases [7]. In this study, we provided further evidence that folate-dependent proteins are involved in chemotherapy resistance and disease progression of ovarian cancer. We identified that elevated mRNA levels of DHFR2, rather than DHFR1, was positively correlated with increased chemotherapy resistance. Furthermore, no significant correlation was observed between disease progression and thymidylate synthase or GART mRNA levels (data not show). Further studies are still needed for the different functional role of DHFR variants, DHFR1 and 2, in disease progression of ovarian cancer, especially in chemotherapy resistance.

Molecular biologic methods paved sensitive and special ways to analyze protein expression status. Immunohistochemical staining detects protein by its specific antigens, which provides evidence of protein expression levels by staining percentage and intensity in cancer tissue [22]. Quantitative mRNA measurements with PCR analysis identifies mRNA transcription by specific mRNA sequence. In our study, DHFR protein expression was determined by IHC staining. DHFR expression was positively correlated with histologic grade, but not with other clinical parameters. Notably, the patients with DHFR positive tumors showed worse prognosis than those negative ones. Further analysis with TCGA data showed that DHFR2 mRNA exhibited prognostic value for ovarian cancer. Our results suggest elevated DHFR2 expression indicates poor prognosis. However, no specific antibody for DHFR2 was validated for IHC staining previously. Further analysis for IHC staining with DHFR2 antibody will further prove the pathologic role of DHFR2 in ovarian cancer development and progression.

Drugs against folate metabolism are being investigated these years, which had been used as potential regimens for platinum chemotherapy-resistant cancer [4]. DHFR inhibitors have exhibited therapeutic value for cancer chemotherapy [23], such as methotrexate and pemetrexed [24], which is already used in a broad spectrum of cancer types, including lung, colon, and pancreatic cancer [25]. Pemetrexed is shown to improve the progression free-survival in resistant ovarian cancer [26, 27]. However, notable side effects were observed in these patients, including myelosuppression and mucositis [28-30], which was caused by folate metabolism inhibition in bone marrow or gastrointestinal tract lining. Limited patients were collected in our center for those treated with DHFR inhibitors as second or third-line therapy after platinum-based chemotherapy resistance. Further analysis is still needed for the therapeutic value of DHFR inhibitors in chemoresistant ovarian cancer.

Conclusion

Increased DHFR expression is positively correlated to platinum-based chemotherapy efficacy in ovarian cancer patients. DHFR positive expression, as well as DHFR2 mRNA expres-

sion, indicate poor prognosis in ovarian cancer. DHFR positive expression is an independent detrimental factor for ovarian cancer patients.

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Disclosure of conflict of interest

None.

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References

[1] Landen CN Jr, Birrer MJ and Sood AK. Early events in the pathogenesis of epithelial ovarian cancer. J Clin Oncol 2008; 26: 995-1005.

[2] Martin L and Schilder R. Novel approaches in advancing the treatment of epithelial ovarian cancer: the role of angiogenesis inhibition. J Clin Oncol 2007; 25: 2894-2901.

[3] Walker JL. Intraperitoneal chemotherapy requires expertise and should be the standard of care for optimally surgically resected epithelial ovarian cancer patients. Ann Oncol 2013; 24 Suppl 10: x41-45.

[4] Li X and Wang X. The emerging roles and therapeutic potential of exosomes in epithelial ovarian cancer. Mol Cancer 2017; 16: 92.

[5] Xu X, Han L, Yang H, Duan L, Zhou B, Zhao Y, Qu J, Ma R, Zhou H and Liu Z. The A/G allele of eIF3a rs3740556 predicts platinum-based chemotherapy resistance in lung cancer patients. Lung Cancer 2013; 79: 65-72.

[6] Helleman J, Smid M, Jansen MP, van der Burg ME and Berns EM. Pathway analysis of gene lists associated with platinum-based chemotherapy resistance in ovarian cancer: the big picture. Gynecol Oncol 2010; 117: 170-176.

[7] Bhabha G, Ekiert DC, Jennewein M, Zmasek CM, Tuttle LM, Kroon G, Dyson HJ, Godzik A, Wilson IA and Wright PE. Divergent evolution of protein conformational dynamics in dihydrofolate reductase. Nat Struct Mol Biol 2013; 20: 1243-1249.

[8] Hammes-Schiffer S. Quantum-classical simulation methods for hydrogen transfer in enzymes: a case study of dihydrofolate reductase. Curr Opin Struct Biol 2004; 14: 192-201.

[9] Llado V, Teres S, Higuera M, Alvarez R, Noguera-Salva MA, Halver JE, Escriba PV and Busquets X. Pivotal role of dihydrofolate reductase knockdown in the anticancer activity of 2-hydroxyoleic acid. Proc Natl Acad Sci U S A 2009; 106: 13754-13758.

[10] Bai F, Yin Y, Chen T, Chen J, Ge M, Lu Y, Xie F, Zhang J, Wu K and Liu Y. Development of liposomal pemetrexed for enhanced therapy against multidrug resistance mediated by ABCC5 in breast cancer. Int J Nanomedicine 2018; 13: 1327-1339.

[11] Marverti G, Ligabue A, Paglietti G, Corona P, Piras S, Vitale G, Guerrieri D, Luciani R, Costi MP, Frassineti C and Moruzzi MS. Collateral sensitivity to novel thymidylate synthase inhibitors correlates with folate cycle enzymes impairment in cisplatin-resistant human ovarian cancer cells. Eur J Pharmacol 2009; 615: 17-26.

[12] Wang Q, Jiang J, Ying G, Xie XQ, Zhang X, Xu W, Zhang X, Song E, Bu H, Ping YF, Yao XH, Wang B, Xu S, Yan ZX, Tai Y, Hu B, Qi X, Wang YX, He ZC, Wang Y, Wang JM, Cui YH, Chen F, Meng K, Wang Z and Bian XW. Tamoxifen enhances stemness and promotes metastasis of ERalpha36(+) breast cancer by upregulating ALDH1A1 in cancer cells. Cell Res 2018; 28: 336-358.

[13] Raja FA, Counsell N, Colombo N, Pfisterer J, du Bois A, Parmar MK, Vergote IB, Gonzalez-Martin A, Alberts DS, Plante M, Torri V and Ledermann JA. Platinum versus platinum-combination chemotherapy in platinum-sensitive recurrent ovarian cancer: a meta-analysis using individual patient data. Ann Oncol 2013; 24: 3028-3034.

[14] Vaughan S, Coward JI, Bast RC Jr, Berchuck A, Berek JS, Brenton JD, Coukos G, Crum CC, Drapkin R, Etemadmoghadam D, Friedlander M, Gabra H, Kaye SB, Lord CJ, Lengyel E, Levine DA, McNeish IA, Menon U, Mills GB, Nephew KP, Oza AM, Sood AK, Stronach EA, Walczak H, Bowtell DD and Balkwill FR. Rethinking ovarian cancer: recommendations for improving outcomes. Nat Rev Cancer 2011; 11: 719-725.

[15] Kipps E, Tan DS and Kaye SB. Meeting the challenge of ascites in ovarian cancer: new avenues for therapy and research. Nat Rev Cancer 2013; 13: 273-282.

[16] Markman M and Walker JL. Intraperitoneal chemotherapy of ovarian cancer: a review, with a focus on practical aspects of treatment. J Clin Oncol 2006; 24: 988-994.

[17] Abdul Razak AR, Li L, Bryant A and Diaz-Padilla I. Chemotherapy for malignant germ cell ovarian cancer in adult patients with early stage, advanced and recurrent disease. Cochrane Database Syst Rev 2011; 16: CD007584.

[18] Ozasa H, Oguri T, Uemura T, Miyazaki M, Maeno K, Sato S and Ueda R. Significance of thymi-

dylate synthase for resistance to pemetrexed in lung cancer. Cancer Sci 2010; 101: 161-166.
[19] Sharma M and Chauhan PM. Dihydrofolate reductase as a therapeutic target for infectious diseases: opportunities and challenges. Future Med Chem 2012; 4: 1335-1365.
[20] Sato Y, Matsuda S, Maruyama A, Nakayama J, Miyashita T, Udagawa H, Umemura S, Yanagihara K, Ochiai A, Tomita M, Soga T, Tsuchihara K and Makinoshima H. Metabolic characterization of antifolate responsiveness and non-responsiveness in malignant pleural mesothelioma cells. Front Pharmacol 2018; 9: 1129.
[21] Hamilton G. Cyclophilin a as a target of cisplatin chemosensitizers. Current Cancer Drug Targets 2014; 14: 46-58.
[22] Jensen K, Krusenstjerna-Hafstrøm R, Lohse J, Petersen KH and Derand H. A novel quantitative immunohistochemistry method for precise protein measurements directly in formalin-fixed, paraffin-embedded specimens: analytical performance measuring HER2. Mod Pathol 2017; 30: 180-193.
[23] Srinivasan B, Tonddast-Navaei S, Roy A, Zhou H and Skolnick J. Chemical space of Escherichia coli dihydrofolate reductase inhibitors: New approaches for discovering novel drugs for old bugs. Med Res Rev 2019; 39: 684-705.
[24] Hopper A, Brockman A, Wise A, Gould J, Barks J, Radke JB, Sibley LD, Zou Y and Thomas S. Discovery of selective toxoplasma gondii dihydrofolate reductase inhibitors for the treatment of toxoplasmosis. J Med Chem 2019; [Epub ahead of print].
[25] Mok TS, Wu YL, Ahn MJ, Garassino MC, Kim HR, Ramalingam SS, Shepherd FA, He Y, Akamatsu H, Theelen WS, Lee CK, Sebastian M, Templeton A, Mann H, Marotti M, Ghiorghiu S, Papadimitrakopoulou VA and Investigators A. Osimertinib or platinum-pemetrexed in EGFR T790M-positive lung cancer. N Engl J Med 2017; 376: 629-640.
[26] Miller DS, Blessing JA, Krasner CN, Mannel RS, Hanjani P, Pearl ML, Waggoner SE and Boardman CH. Phase II evaluation of pemetrexed in the treatment of recurrent or persistent platinum-resistant ovarian or primary peritoneal carcinoma: a study of the gynecologic oncology group. J Clin Oncol 2009; 27: 2686-2691.
[27] Chambers SK, Chow HH, Janicek MF, Cragun JM, Hatch KD, Cui H, Laughren C, Clouser MC, Cohen JL, Wright HM, Abu Shahin N and Alberts DS. Phase i trial of intraperitoneal pemetrexed, cisplatin, and paclitaxel in optimally debulked ovarian cancer. Clin Cancer Res 2012; 18: 2668-2678.
[28] Wei GL, Huang XE, Huo JG, Wang XN and Tang JH. Phase II study on pemetrexed-based chemotherapy in treating patients with metastatic gastric cancer not responding to prior palliative chemotherapy. Asian Pac J Cancer Prev 2013; 14: 2703-2706.
[29] Wu XY, Huang XE, You SX, Lu YY, Cao J, Liu J and Xiang J. Phase II study of pemetrexed as second or third line combined chemotherapy in patients with colorectal cancer. Asian Pac J Cancer Prev 2013; 14: 2019-22.
[30] Tsutani Y, Miyata Y, Masuda T, Fujitaka K, Doi M, Awaya Y, Kuyama S, Kitaguchi S, Ueda K, Hattori N and Okada M. Multicenter phase II study on cisplatin, pemetrexed, and bevacizumab followed by maintenance with pemetrexed and bevacizumab for patients with advanced or recurrent nonsquamous non-small cell lung cancer: MAP study. BMC Cancer 2018; 18: 1231.