Research Article

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Mining expression and prognosis of FOLR1 in ovarian cancer by using Oncomine and Kaplan-Meier plotter

<https://doi.org/10.1515/pteridines-2019-0020>

received March 20, 2019; accepted August 12, 2019.

Abstract: Objective: To further explore folate receptor 1 (*FOLR1*) gene expression in ovarian cancer and its association with patients' prognosis by deep mining the Oncomine and Kaplan-Meier plotter databases.

Methods: *FOLR1* mRNA expression data of ovarian cancer were retrieved from the Oncomine database and further analyzed by comparing tumor to healthy tissue. The prognostic value of *FOLR1* in ovarian cancer was analyzed by Kaplan-Meier Plotter, an online survival analysis database.

Results A total of 439 studies were included in the Oncomine database in multiple types of cancers. Of the 439 studies, there were 54 with statistical differences for the expression of *FOLR1*, 19 with increased expression of *FOLR1* and 35 with decreased expression comparing ovarian cancer to normal ovary tissue. After searching the Oncomine database, six datasets were discovered comparing the mRNA expression in ovarian tumor to healthy tissue. *FOLR1* mRNA expression in ovarian tumor was significantly higher than that of normal ovarian tissue (all $p < 0.05$). The Kaplan-Meier Plotter database analyzed the correlation between *FOLR1* expression and ovarian cancer patient's prognosis. A significant difference of progression-free survival between *FOLR1* high and low expressing groups was found in ovarian cancer patients (HR=1.14, 95%CI: 1.00-1.29, $p=0.043$). However, the overall survival was not statistically different between high and

low *FOLR1* expressing patients (HR=0.95, 95%CI: 0.84-1.09, $p=0.48$).

Conclusion *FOLR1* mRNA was found to be highly expressed in ovarian tumor compared to normal ovarian tissue. Elevated *FOLR1* mRNA expression was associated with the poor progression-free survival.

Keywords: Ovarian cancer; *FOLR1*; Oncomine, Data mining; Prognosis.

Introduction

Folate receptor 1 (*FOLR1*), also known as folate binding protein, is a glycosylphosphoinositol anchored membrane protein, which can mediate extracellular 5-methyltetrahydrofolate into cells and plays an important role in cell division, proliferation and tissue growth [1, 2]. In normal tissue the gene expression level of *FOLR1* is low, however, in many cancers such as lung [3, 4], breast [5] and colorectal [6] the *FOLR1* expression level is elevated. In ovarian cancer, the *FOLR1* gene is over expressed in tumor compared to normal ovarian tissues [7]. However, most previous studies used small sample sizes with limited statistical power and weak clinical evidence. In our study, microarray *FOLR1* mRNA expression data from the Oncomine database was used to determine prognosis.

The Oncomine database (<https://www.oncomine.org/>) is the largest oncogene chip databases with an integrated data-mining platform aimed at mining cancer gene information [8, 9]. At present, 715 gene expression datasets and 86,733 samples of cancerous and normal tissues have been made available. Oncomine can provide differentially expressed genes between tumor and normal tissues for many cancer types and subtypes which can be analyzed based on clinical and pathological data, differentially expressed sorting and co-expression analyses, location of differentially expressed genes, identification of target genes. These analyses can then be

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used to determine research directions, not only to save time but also reducing the cost of scientific research.

Material and methods

Data extraction from Oncomine database and analysis

The Oncomine database is a web-based gene database and an integrated data mining platform, in which data can be screened and mined in accordance with requirements. This platform aimed to stimulate discoveries from genome-wide expression analyses and compare the transcriptome data in most major cancer types. In our present work, folate receptor 1 (*FOLR1*) gene expression levels in ovarian cancer were compared to normal ovarian tissue and extracted and analyzed by the Oncomine database platform. The data search was as follows: (1) cancer type: ovarian cancer; (2) Gene: *FOLR1*; (3) Data type: mRNA; (4) Analysis type: Cancer vs normal; (5) Cut-off value: $p < 1E-4$, fold change > 2 ; gene rank = top 10%.

Survival analysis by Kaplan-Meier Plotter

The prognosis, in terms of overall survival (OS) and progression-free survival (PFS), was determined using tumor *FOLR1* mRNA expression levels for ovarian cancer patients and analyzed by the Kaplan-Meier plotter (www.kmplot.com). This database provided online survival analysis of lung, ovarian, gastric and breast cancer patients. In order to evaluate the prognostic significance of *FOLR1* expression on ovarian patient survival, the patients were divided into two cohorts according to median *FOLR1* mRNA expression (high versus low expression). The OS and PFS of the ovarian cancer patients were compared between *FOLR1* high and low expressing groups.

Statistical analysis

Data were analyzed by SPSS17.0 (<http://www-01.ibm.com/software/analytics/spss/>) and expressed by $\bar{x} \pm s$. The between group comparison of the *FOLR1* expression was conducted using a student t-test, and the counted data was expressed by rate. The between group comparison was made by χ^2 -test or Fisher's exact test. A proportional hazard ratio was used for survival analysis through

Disease Summary for FOLR1

Analysis Type by Cancer	Cancer vs. Normal	Cancer vs. Cancer	
		Cancer Histology	Multi-Cancer
Bladder Cancer		1	1
Brain and CNS Cancer	2	1	1
Breast Cancer	12		
Cervical Cancer			
Colorectal Cancer	4	1	2
Esophageal Cancer			
Gastric Cancer	3		
Head and Neck Cancer	2		
Kidney Cancer	3	2	2
Leukemia			1
Liver Cancer			1
Lung Cancer	12	10	10
Lymphoma	2	1	2
Melanoma	2		2
Myeloma			1
Other Cancer	3	1	2
Ovarian Cancer	6	3	1
Pancreatic Cancer	1		
Prostate Cancer			2
Sarcoma	1	1	1
Significant Unique Analyses	19	35	18
Total Unique Analyses	439	739	264

Figure 1: *FOLR1* expression in multiple cancers from comparing tumor with healthy control tissue in the Oncomine database.

a log-rank test and survival curve. $P < 0.05$ was taken as a statistically significant difference.

Results

FOLR1 expression in multiple cancers

A total of 439 studies of multiple cancers types were included from the Oncomine database (**Figure 1**). Of the 439 studies, there were 54 with statistical differences for the expression of *FOLR1*, 19 with increased expression of *FOLR1* and 35 with decreased expression when comparing ovarian tumors to normal ovarian tissues.

Table 1: Characteristics of the included six studies.

Study	Sample size (n)		Pathology type	Microarray	Year
	Cancer	Control			
Bonome Ovarian	185	10	Serous Adenocarcinoma	Human Genome U133A Array	2008
Hendrix Ovarian	8	4	Clear cell Adenocarcinoma	Human Genome U133A Array	2006
Hendrix Ovarian	37	4	Endometrioid Adenocarcinoma	Human Genome U133A Array	2006
Hendrix Ovarian	41	4	Serous Adenocarcinoma	Human Genome U133A Array	2006
Welsh Ovarian	28	4	Ovarian Serous Surface Papillary Carcinoma	HumanGeneFL Array	2001
Yoshihara Ovarian	43	10	Ovarian Serous Adenocarcinoma	Agilent Human 1A Oligo Microarray v2	2009

Figure 2: Expression of *FOLR1* in ovarian cancer in the studies identified by the Oncomine database. 1-6 represent the six datasets expressing *FOLR1* in ovarian tumors. The darker the red, the higher the *FOLR1* gene expression from the microarray.

FOLR1 expression in ovarian cancer

After searching the Oncomine database, six datasets [10-15] were discovered after comparing the mRNA expression in tumor with normal tissue (**Table 1**). *FOLR1* was highly expressed in ovarian tumors when compared to normal ovarian tissue in all of the six series, **Figure 2**.

FOLR1 expression by microarray analysis

FOLR1 mRNA expression in ovarian tumor versus normal ovarian tissue by microarray analysis of the six data sets were shown in **Figure 3**. *FOLR1* mRNA expression in ovarian tumor was significantly higher than that from normal ovary (all $p < 0.05$).

FOLR1 expression and prognosis

The correlation between *FOLR1* expression and ovarian cancer patients prognosis was analyzed by the Kaplan-Meier Plotter database[16]. A significant difference between PFS and *FOLR1* high and low expressing groups was found in ovarian cancer patients (HR=1.14, 95%CI: 1.00-1.29, $p=0.043$). However, the OS was not statistically significantly different between high and low *FOLR1* expressing patients (HR=0.95, 95%CI: 0.84-1.09, $p=0.48$), **Figure 4**. We further performed subgroup survival analysis of *FOLR1* expression according to clinical stage and histology, **Table 2**. The prognostic significance of *FOLR1* expression for subgroup analysis was demonstrated in **Figure 5**. The PFS was different between *FOLR1* high and low expressing groups in stage 3 and 4 subgroups and

Figure 3: Expression of *FOLR1* in ovarian cancer in different studies identified from the Oncomine database. (A: data form Hendrix ovarian statistics for normal tissue versus ovarian serous adenocarcinoma; B: data from Hendrix ovarian statistics for normal tissue versus ovarian endometrioid adenocarcinoma; C: data from Hendrix ovarian statistics for normal tissue versus ovarian clear cell adenocarcinoma; D: data from Welsh ovarian statistics for normal tissue versus ovarian serous surface papillary adenocarcinoma; E: data from Yoshihara ovarian statistics for normal tissue versus ovarian serous adenocarcinoma; F: data from Adib ovarian statistics for normal tissue versus ovarian serous adenocarcinoma; G: data from Lu ovarian statistics for normal tissue versus several kinds of adenocarcinoma; H: data from TCGA databases for normal tissue versus ovarian serous cystadenocarcinoma).

Figure 4: Overall survival (OS) and progression-free survival (PFS) curve of ovarian cancer (A: OS of ovarian cancer; B: PFS of ovarian cancer)

Table 2: Subgroup analysis of *FOLR1* gene expression and patient prognosis according to stage and history. (NA = not applicable, HR = Hazard ratio, OS = overall survival, CI = confidence interval).

Group	Median PFS		HR(95%CI)	p-value	Median OS		HR(95%CI)	p-value
	High	Low			High	Low		
Stage								
1	NA	NA	1.40(0.48-4.06)	0.54	63.90	81.23	1.06(0.34-3.28)	0.92
2	35.19	39.00	1.52(0.75-3.09)	0.25	71.61	44.30	0.38(0.13-1.14)	0.073
3	18.83	15.00	0.81(0.70-0.95)	0.0082	48.37	40.13	0.83(0.71-0.99)	0.033
4	13.00	20.07	1.86(1.23-2.81)	0.0029	27.30	38.40	1.48(1.02-2.14)	0.037
Histology								
Endometrioid	9.00	34.00	4.83(1.84-12.66)	0.00046	NA	NA	0.44(0.07-2.64)	0.36
Serous	17.43	18.40	1.12(0.95-1.33)	0.17	48.37	40.10	0.81(0.70-0.95)	0.0097

Figure 5: Subgroup survival analysis for *FOLR1* gene expression survival curves according to clinical stage and histology (A: OS of stage 1 ovarian cancer; B: PFS of stage 1 ovarian cancer; C: PFS of stage 2 ovarian cancer; D: OS of stage 2 ovarian cancer; E: PFS of stage 3 ovarian cancer; F: OS of stage 3 ovarian cancer; G: PFS of stage 4 ovarian cancer; H: OS of stage 4 ovarian cancer; I: DFS of endometrioid ovarian cancer; J: OS of endometrioid ovarian cancer; K: DFS of serous ovarian cancer; L: OS of serous ovarian cancer).

endometrioid histology subgroup ($p < 0.05$). For OS, there was a statistical difference in stage 3 and 4 subgroups and serous histology subgroup ($p < 0.05$).

Discussion

Ovarian tumors are one of the most common carcinomas in female genital organs [17]. The incidence of ovarian cancer ranks third, after cervical and uterine body cancers [18, 19]. However, the mortality rate of epithelial ovarian cancer is the highest among all gynecological carcinomas [20]. The cancer epidemiological study from the United States in 2019, showed that there were 22,530 new cases of ovarian cancer and 19,380 deaths, and only ~40% of ovarian cancer patients were suitable for curative resection [17]. Epithelial cancer is the most common malignant ovarian tumors, followed by malignant germ cell tumors [21]. In patients with ovarian cancer, only 30% of the tumors were discovered to be confined to the ovary [22, 23], the majority had spread to the uterus, bilateral appendages, omentum and pelvic organs [23, 24].

The prognosis of patients with ovarian cancer is poor when diagnosed in advanced stages, and much more is needed to be done to improve early diagnostic methods. There are many factors which can affect the prognosis of ovarian cancer patients such as clinical stage, pathological classification, post-operative treatment, and surgical methods [25, 26]. Studies have also shown that the expression of a variety of genes negatively correlates to patient prognoses, such as CA-125 [27, 28] and *FOLR1* [29].

FOLR1 is a member of the human folate-binding protein family (three isomers of folate-binding protein have been identified: *FOLR1*, *FOLR2* and *FOLR3*). The gene is located on chromosome 11q13.3-13.5. Its protein is exposed to the outside of the cell and anchored by GPI on the cell membrane. *FOLR1* transports extracellular 5-methyltetrahydrofolate into the cell and participates in DNA replication and replication as a coenzyme component. The expression level of *FOLR1* is closely related to tumor progression and cell proliferation. Several studies have evaluated *FOLR1* expression and ovarian cancer patient prognosis, but the results were inconclusive [29-31]. Kimberly et al. [32] examined *FOLR1* protein expression in 186 primary ovarian cancer patients and 27 cases of recurrence disease by immunohistochemical assay. The authors found that the expression of *FOLR1* did not correlate with the OS of the ovarian cancer patients. However, Fu et al. [33] evaluated the correlation between

FOLR1 mRNA expression and prognosis in patients with serous ovarian carcinoma by RT-PCR. They found high expressing *FOLR1* was correlated with poor prognosis, which was in accordance with our results.

In our present work, six comparisons of tumor versus normal tissue from ovarian cancer patients were included here. *FOLR1* gene expression in ovarian cancer was analyzed and compared with normal ovarian tissue. Of the six data sets, three were from three studies which compared three different ovarian cancer subtypes. All six data sets confirmed *FOLR1* gene expression levels from tumor were higher when compared to healthy control tissue. This may indicate that *FOLR1* plays an important role in ovarian cancer development. We also found that elevated *FOLR1* mRNA expression was associated with poor PFS. The correlation between elevated *FOLR1* mRNA expression and poor prognosis indicated that *FOLR1* mRNA could be used as a molecular biomarker for the prediction of ovarian cancer patient prognosis.

Conclusion

The deep mining of *FOLR1* gene expression confirmed that the gene is highly expressed in ovarian tumors, and negatively correlates with ovarian cancer patient prognosis.

Conflict of interest: Authors state no conflict of interest

Founding: None

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