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RORα and REV-ERBα are Associated With Clinicopathological Parameters and are Independent Biomarkers of Prognosis in Gastric Cancer

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Abstract

Retinoid-related orphan receptor alpha (RORα) and nuclear receptor subfamily 1 group D member 1 (REV-ERBα) play critical roles in many human cancers. Whether RORα and REV-ERBα expression levels are associated with clinical characteristics are poorly understood, and they may be independent predictors of overall survival (OS) and progression-free survival (PFS) in gastric cancer (GC). This study aimed to investigate the correlation of RORα and REV-ERBα expression levels with clinicopathological parameters, OS, and PFS in GC. Immunohistochemistry and quantitative reverse transcription-polymerase chain reaction (qRT-PCR) were employed to assess the expression levels of RORα and REV-ERBα, which were downregulated in GC tissues compared with normal gastric tissues ($P < .001$; $P < .001$) and were associated with several clinicopathological parameters, including histological grade ($P = .032$; $P < .001$), preoperative carcinoembryonic antigen (CEA) levels ($P = .004$; $P < .001$), and tumor-node-metastasis (TNM) stage ($P = .015$; $P < .001$). Additionally, low RORα and REV-ERBα expression levels were associated with poor OS and PFS in GC patients, respectively ($P < .001$; $P = .001$). Furthermore, univariate Cox regression model analysis showed that histological grade ($P < .001$; $P < .001$), preoperative CEA levels ($P < .001$; $P = .001$), TNM stage ($P < .001$; $P < .001$), lymph node metastasis ($P = .002$; $P = .002$), RORα expression levels ($P = .001$; $P < .001$), and REV-ERBα expression levels ($P < .001$; $P = .001$) were associated with OS and PFS in GC. Multivariate Cox regression model analysis indicated that RORα expression levels and REV-ERBα expression levels are independent factors of OS and PFS in GC. Besides, RORα and REV-ERBα expression may be positively correlated ($\chi^2 = 6.835$; $P = .009$), and GC patients with both high RORα and REV-ERBα expression levels had the best prognosis. In conclusion, RORα and REV-ERBα may coparticipate in tumor activities and show potential to estimate the prognosis of GC.

Keywords

RORα, rEV-ERBα, biomarkers, prognosis, gastric cancer

Abbreviations

CA99, carbohydrate antigen 199; CEA, carcinoembryonic antigen; 95% CI, 95% confidence interval; GC, gastric cancer; HR, hazard ratio; MOD, mean optical density; mRNA, messenger RNA; OS, overall survival; PFS, progression-free survival; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; RORα, retinoid-related orphan receptor alpha; REV-ERBα, nuclear receptor subfamily 1 group D member 1; TNM, tumor-node-metastasis.

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Introduction

Gastric cancer (GC) is the fifth most common malignancy, causing more than 770 000 deaths every year worldwide.[1] Advances in diagnosis and treatment have significantly improved in the past several decades. However, the incidence of advanced GC is high and the 5-year survival time is poor.[1]

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Additionally, the efficacy of chemotherapy is low, and drug resistance develops easily.[2,3] Thus, identifying discovery novel and practical biomarkers to promote diagnosis and improve prognosis is critical. Both retinoid-related orphan receptor alpha (RORα) and nuclear receptor subfamily 1 group D member 1 (REV-ERBα) belong to the nuclear receptor family and show apparent characteristics of circadian rhythm.[4,5] Furthermore, RORα and REV-ERBα are abundantly expressed in human organs and tissues such as skin, adipose tissues, muscle, and brain.[5] Accumulating studies suggest that RORα and REV-ERBα expression is downregulated and associated with poor prognosis in various tumors.[6–9] In GC, previous studies have reported RORα and REV-ERBα expression were associated with clinical and pathological features and induces cell apoptosis through certain molecular pathways.[10,11] However, the relationship of RORα and REV-ERBα expression with clinicopathology remains unclear in GC. Additionally, no integrated study has been performed to reveal the association of RORα and REV-ERBα expression with prognosis in GC. In the current study, we employed immunohistochemistry and quantitative reverse transcription-polymerase chain reaction (qRT-PCR) to further explore the clinicopathological features of RORα and REV-ERBα expression, prognosis, and correlation in GC.

Methods

Patients and Specimens (Ethics Approval Number: Quick-PJ2020-11-20)

All patients signed the informed consent before surgery. The study was approved by the Human Ethics Committee of Anhui Medical University, Hefei, Anhui, China and the justification of all methods was consistent with the institutional guideline. The calculation of differential expression was utilized through GraphPad Prism (GraphPad Software) and SPSS 17.0 software (SPSS). All formalin-fixed paraffin-embedded tissue specimens were collected from 208 patients who underwent radical GC surgical resection at The First Affiliated Hospital of Anhui Medical University (Hefei, Anhui) from August 2013 to August 2015. The average age of the research population was 61.8 years, range from 34 to 88 years, and the sex distribution was 116 males and 92 females. The eligible standards were as follows: (1) the pathological diagnosis of tumor tissues was gastric adenocarcinoma; (2) none of the patients had received radiotherapy or chemotherapy prior to surgery; (3) patients who were pregnant or breastfeeding were excluded and the function of lung, liver, renal, and blood, as well as bone marrow, were normal; (4) the Eastern Cooperative Oncology Group Performance Status scores were between 0 and 2.[12]

Immunohistochemistry

All specimens contained GC and normal gastric tissues (5 cm from the tumor region approximately) were performed on 5-μm-thick sections from wax blocks. The sections were deparaffinized in 100% xylene for 10 min and through a graded series of ethanol to wipe off xylene, and then were subjected to microwave with 10 mm citrate buffer (pH = 6.0) at 100 °C for 10 min. Subsequently, the sections were immersed in 3% hydrogen peroxide for 10 min at room temperature, and then incubated with primary RORα rabbit antibody (DF3161; 1:50 dilution; Affinity Biosciences) and REV-ERBα rabbit antibody (DF12430; 1:150 dilution; Affinity Biosciences) at 4 °C overnight, respectively. The sections were then incubated in a biotin-conjugated secondary antibody (PV6000;1:100 dilution; ZSGB-BIO; OriGene Technologies), after washing 3 times with phosphate-buffered saline, the sections were stained with 3,3′-diaminobenzidine (ZSGB-BIO; OriGene Technologies) for 5 min and 20% hematoxylin at room temperature. A fluorescent microscope was used to photograph in a single-blinded manner at a magnification of ×200 and ×400, respectively. The relative protein expression levels of RORα and REV-ERBα were calculated by the mean optical density (MOD) method according to the IPWIN Application software version 6.0.0260 (Media Cybernetics). The staining intensity were categorized by the proportion of positive cells: 0 (0%), 1 (1%–25%), 2 (26%–50%), 3 (51%–75%), and 4 (76%–100%). The score was counted: 0 (no staining); 1 to 2 (weakly stained); 3 (moderately stained); and 4 (strongly stained). The final score of low expression levels of RORα and REV-ERBα was defined from 0 to 2, and the high expression levels of RORα and REV-ERBα was defined as from 3 to 4.

Quantitative Reverse Transcription-Polymerase Chain Reaction

Total RNA was extracted from tissues using TRIzol® (Thermo Fisher Scientific) according to manual instructions. The complementary DNA was synthesized by PrimeScript RT Reagent kit (Takara Bio) and qRT-PCR was performed using GoTaq® Green Master Mix (Promega Corporation) on 7900 Thermal Cycler (Applied Biosystems; Thermo Fisher Scientific). The initial condition of denaturation at 95°C for 30 s, and then followed by 40 cycles at 95°C for 5 s and elongation at 60°C for 30 s. The primers of RORα, REV-ERBα, and β-actin as follows: RORα, 5′-ACTCCTGTCCTCGTCAGA AGA-3′ (forward) and 5′-CATCCCTACGGCAAGGCAT TT-3′ (reverse); REV-ERBα, 5′-ACAGAATCGAACTCTG CACTTCT-3′ (forward) and 5′-GGGGAGGGAGGCAGG TATT-3′ (reverse); and β-actin, 5′-CATGTACGTTGCTA TCCAGGC-3′ (forward) and 5′-CTCCTTAATGTCACGCA CGAT-3′ (reverse). The relative messenger RNA (mRNA) expression levels were calculated using the $2^{-\Delta\Delta Cq}$ method.[13] β-actin was used as an internal control.

Follow up

All patients (116 males and 92 females) were followed up every 3 months in the first year and every 6 months in the later time for a total of 5 years from November 2013 to August 2020. Abdominal and pelvic enhanced CT was recommended every 6 months at the first year and then in every year at a later time for a total of 5 years. Carcinoembryonic antigen

(CEA) and carbohydrate antigen 199 (CA199) were recommended every 6 months for a total of 5 years. A gastric endoscope also was suggested to perform every 2 years for a total of 5 years. A total of 204 (98.1%) patients survived in the first year and only 18 (8.7%) patients survived at the end of follow up. The definition of the median overall survival (OS) time was from the date of surgery to cancer-related death or last follow up. The median progression-free survival (PFS) time was complied with the criterion from the date of surgery to relapse or last follow up.

Statistical Analysis

The statistical analysis was performed using GraphPad Prism (GraphPad Software) and SPSS 17.0 software (SPSS). Multiple groups were compared by analysis of variance test. The chi-squared test was used to assess correlation and the relation of RORα and REV-ERBα expression levels with clinicopathological parameters. The Kaplan–Meier method and log-rank test were utilized to assess survival curves (the median OS and PFS time). The univariate and multivariate survival analyses were completed using the cox proportional hazards model. $P < .05$ was considered statistically significant.

Results

RORα and REV-ERBα Expression Levels are Downregulated in GC

Immunohistochemistry was used to detect the protein expression levels of RORα and REV-ERBα in normal gastric and GC tissues (Figures 1 and 2). The RORα and REV-ERBα protein expression levels were downregulated in GC tissues compared to normal gastric tissues (Figure 3a and b). Additionally, the RORα and REV-ERBα mRNA expression levels were also downregulated in GC tissues compared with normal gastric tissues, as demonstrated by qRT-PCR (Figure 4a and b).

RORα and REV-ERBα Expression Levels are Associated with Clinicopathological Parameters in GC

To illustrate the roles of RORα and REV-ERBα in GC, we analyzed the clinicopathological data and found that RORα and REV-ERBα expression levels were significantly associated with histological grade ($P = .032$; $P < .001$), preoperative CEA levels ($P = .004$; $P < .001$), and TNM stage ($P = .015$; $P < .001$). By contrast, age, gender, tumor size, primary tumor site, preoperative CA199 levels, nerve, and vascular invasion and lymph node metastasis were not related ($P > .05$) (Table 1).

The Relationship of RORα and REV-ERBα Expression Levels with Survival Time (OS and PFS) in GC Patients

The prognosis of GC patients with different RORα and REV-ERBα expression levels was determined using the Kaplan–Meier method and log-rank test. The median OS time of patients with high RORα expression levels was significantly longer than that of patients with low RORα expression levels (Figure 5a), and patients with high REV-ERBα expression levels also had a longer OS time than those with low REV-ERBα expression levels (Figure 5b). Furthermore, the median PFS time of patients with high RORα expression levels was markedly longer than that of patients with low RORα expression levels (Figure 5c), and patients with high REV-ERBα expression levels also had a longer PFS time than those with low REV-ERBα expression levels (Figure 5d).

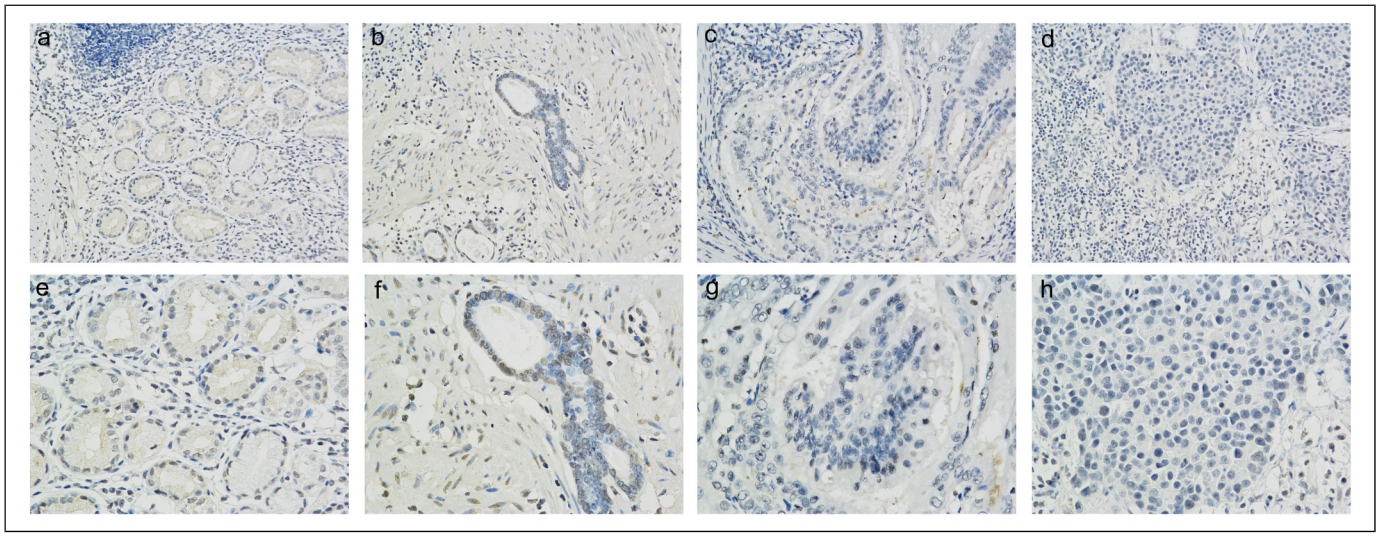

Figure 1. RORα was detected through immunohistochemistry stain in normal gastric and GC tissues. Original magnification, ×200: (a) normal gastric tissues. (b) High differentiation. (c) Moderate differentiation. (d) Low differentiation. Original magnification, ×400. (e) Normal gastric tissues. (f) High differentiation. (g) Moderate differentiation. (h) Low differentiation. Scale bar = 100 μm.
Abbreviations: RORα, retinoid-related orphan receptor alpha; GC, gastric cancer.

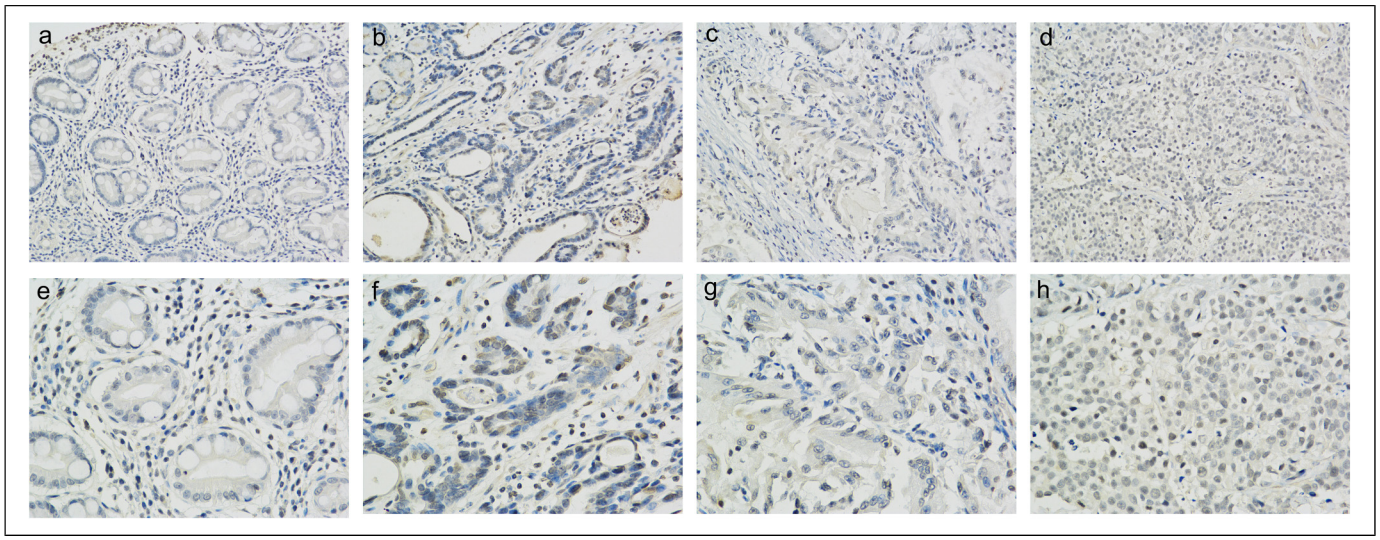

Figure 2. REV-ERBα was detected through immunohistochemistry stain in normal gastric and GC tissues. Original magnification ×200. (a) Normal gastric tissues. (b) High differentiation. (c) Moderate differentiation. (d) Low differentiation. Original magnification ×400. (e) Normal gastric tissues. (f) High differentiation. (g) Moderate differentiation. (h) Low differentiation. Scale bar = 100 μm.
Abbreviations: REV-ERBα, nuclear receptor subfamily 1 group D member 1; GC, gastric cancer.

Univariate and Multivariate Analyses of the Association of the Clinicopathological Parameters with OS and PFS in GC Patients

A univariate Cox regression model analysis was used to confirm that the median OS and PFS times among 208 GC patients were related to the histological grade ($P < .001$; $P < .001$), preoperative CEA levels ($P < .001$; $P = .001$), TNM stage ($P < .001$; $P < .001$), lymph node metastasis ($P = .002$; $P = .002$), RORα expression levels ($P = .001$; $P < .001$), and REV-ERBα expression levels ($P < .001$; $P = .001$). A multivariate Cox regression model analysis indicated that RORα and REV-ERBα expression levels are independent factors for OS and PFS in GC, respectively (Tables 2 and 3).

Correlation Analysis of RORα and REV-ERBα Expression Levels in GC

The correlation between RORα and REV-ERBα expression levels was calculated using the chi-squared test (Table 4), which showed a positive correlation in GC ($\chi^2 = 6.835$; $P = .009$).

The Expression Levels of Both RORα and REV-ERBα are Associated with OS and PFS in GC Patients

The Kaplan–Meier method and log-rank test were used to analyze the correlation between the median survival time (OS and PFS) and the expression levels of both RORα and

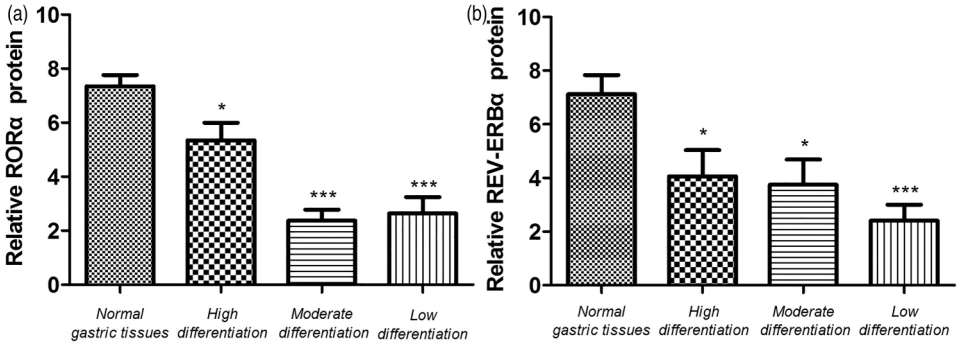

Figure 3. The relative protein expression levels of RORα and REV-ERBα were detected through immunohistochemistry stain in normal gastric and GC tissues. (a) MOD method illustrated the change of RORα relative protein expression levels. (b) MOD method illustrated the change of REV-ERBα relative protein expression levels. Data are represented as the mean ± standard deviation. $N = 10$. $^*P < .05$, $^{**}P < .01$, $^{***}P < .001$ versus normal gastric tissues.
Abbreviations: RORα, retinoid-related orphan receptor alpha; REV-ERBα, nuclear receptor subfamily 1 group D member 1; GC, gastric cancer; MOD, mean optical density.

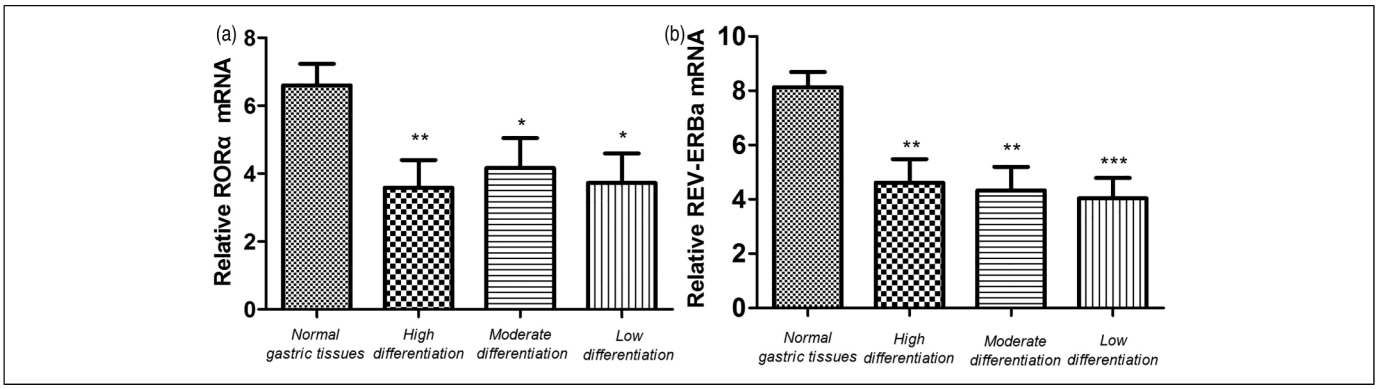

Figure 4. The relative mRNA expression levels of RORα and REV-ERBα were detected through qRT-PCR in normal gastric and GC tissues. (a) The 2-ΔΔCq method calculated the change of RORα relative mRNA expression levels. (b) The 2-ΔΔCq method calculated the change of REV-ERBα relative mRNA expression levels. Data are represented as the mean ± standard deviation. $N = 20$. $^{**}P < .01$, $^{***}P < .001$ versus normal gastric tissues.

Abbreviations: mRNA, messenger RNA; RORα, retinoid-related orphan receptor alpha; REV-ERBα, nuclear receptor subfamily 1 group D member 1; qRT-PCR, quantitative reverse transcription-polymerase chain reaction.

Table 1. The relationship of the expression levels of RORα and REV-ERBα with clinicopathological parameters in GC tissues.

Clinicopathological parameters	Total case (n) $n = 208$	RORα expression levels (n) Low ($n = 121$)	High ($n = 87$)	χ^2	P-value	REV-ERBα expression levels (n) Low ($n = 113$)	High ($n = 95$)	χ^2	P-value
Age (years)									
<65	101	55	46	1.115	.291	53	48	0.271	.602
≥65	107	66	41			60	47		
Gender									
Male	116	71	45	0.992	.319	67	49	1.245	.265
Female	92	50	42			46	46		
Tumor size (cm)									
<5	133	76	57	0.161	.688	74	59	0.161	.688
≥5	75	45	30			39	36		
Primary tumor site									
Gastric cardia or fundus	84	44	40	1.493	.163	42	42	1.063	.302
Gastric antrum or body	124	77	47			71	53		
Histological grade									
High and moderate differentiation	78	38	40	4.585	.032	26	52	22.167	<.001
Low differentiation and undifferentiation	130	93	47			87	43		
Preoperative CEA levels (ng/ml)									
<5	88	41	47	8.409	.004	35	53	13.022	<.001
≥5	120	80	40			78	42		
Preoperative CA199 levels (U/ml)									
<40	100	59	41	0.054	.816	55	45	0.035	.851
≥40	108	62	46			58	50		
TNM stage									
I-II	78	37	41	5.913	.015	23	55	31.034	<.001
III-IV	130	84	46			90	40		
Nerve and vascular invasion									
No	93	56	37	0.288	.591	53	40	0.127	.721
Yes	115	65	50			60	55		
Lymph node metastasis									
No	72	41	31	0.068	.794	34	38	2.240	.134
Yes	136	80	56			79	57		

Abbreviations: TNM, tumor-node-metastasis; CEA, carcinoembryonic antigen; CA199, carbohydrate antigen 199; RORα, retinoid-related orphan receptor alpha; REV-ERBα, nuclear receptor subfamily 1 group D member 1; GC, gastric cancer.

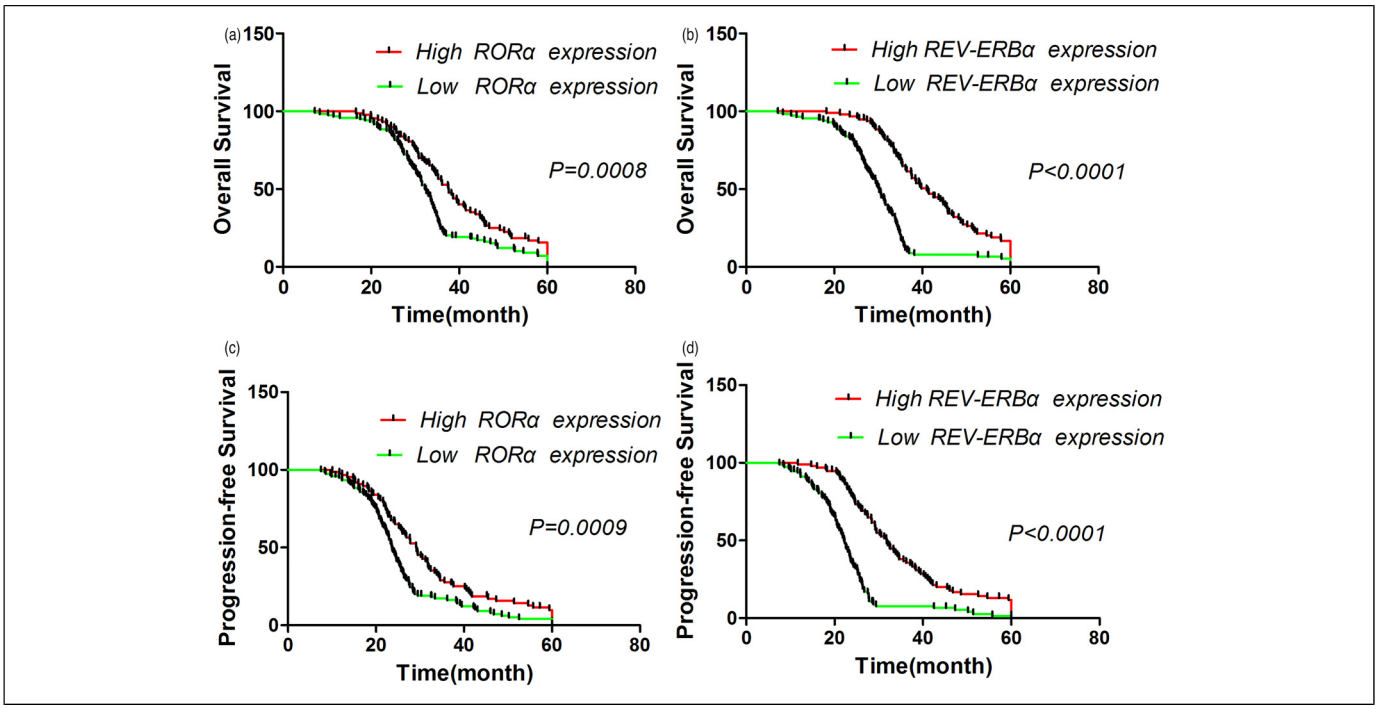

Figure 5. The median OS and PFS times were generated through the Kaplan–Meier method and log-rank test according to RORα and REV-ERBα expression levels in GC patients. (a) The median OS time of high and low RORα expression levels in GC patients. (b) The median PFS time of high and low RORα expression levels in GC patients. (c) The median OS time of high and low REV-ERBα expression levels in GC patients. (d) The median PFS time of high and low REV-ERBα expression levels in GC patients.
Abbreviations: OS, overall survival; PFS, progression-free survival ; RORα, retinoid-related orphan receptor alpha; REV-ERBα, nuclear receptor subfamily 1 group D member 1; GC, gastric cancer.

Table 2. Univariate and multivariate Cox regression analyses of prognostic parameters in GC patients for OS.

Parameters	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Age (years)						
<65 versus ≥65	0.838	0.630-1.114	.223			
Gender						
Male versus female	0.855	0.642-1.139	.284			
Tumor size (cm)						
<5 versus ≥5	0.885	0.653-1.199	.430			
Primary tumor site						
Gastric cardia or fundus versus gastric antrum or body	1.319	0.982-1.773	.066			
Histological grade						
High and moderate differentiation versus low differentiation and undifferentiation	2.656	1.951-3.616	<.001	2.434	1.732-3.422	<.001
Preoperative CEA levels (ng/ml)						
<5 versus ≥5	1.762	1.317-2.357	<.001	1.083	0.786-1.491	.625
Preoperative CA199 levels, u/ml						
<40 versus ≥40	1.103	0.830-1.467	.500			
TNM stage						
I-II versus III-IV	2.846	2.084-3.885	<.001	2.371	1.659-3.389	<.001
Nerve and vascular invasion						
No versus yes	0.970	0.729-1.290	.832			
Lymph node metastasis						
No versus yes	1.602	1.185-2.166	.002	1.673	1.225-2.284	.001
RORα expression levels						
Low versus high	1.604	1.198-2.147	.001	1.511	1.105-2.068	.010
REV-ERBα expression levels						
Low versus high	2.679	1.988-3.609	<.001	1.621	1.177-2.232	.003

Abbreviations: GC, gastric cancer; OS, overall survival; HR, hazard ratio; 95% CI, 95% confidence interval; CEA, carcinoembryonic antigen; TNM, tumor-node-metastasis; CA199, carbohydrate antigen 199; RORα, retinoid-related orphan receptor alpha; REV-ERBα, nuclear receptor subfamily 1 group D member 1.

Table 3. Univariate and multivariate Cox regression analyses of prognostic parameters in GC patients for PFS.

Parameters	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Age (years)						
<65 versus ≥65	0.816	0.613-1.086	.164			
Gender						
Male versus female	0.833	0.625-1.111	.214			
Tumor size (cm)						
<5 versus ≥5	0.901	0.666-1.220	.502			
Primary tumor site						
Gastric cardia or fundus versus gastric antrum or body	1.272	0.947-1.709	.109			
Histological grade						
High or moderate differentiation versus low or undifferentiation	2.909	2.126-3.981	<.001	2.728	1.921-3.874	<.001
Preoperative CEA levels (ng/ml)						
<5 versus ≥5	1.821	1.358-2.440	.001	1.091	0.791-1.504	.596
Preoperative CA199 levels (U/ml)						
<40 versus ≥40	1.086	0.817-1.444	.570			
TNM stage						
I-II versus III-IV	2.824	2.069-3.853	<.001	2.285	1.599-3.266	<.001
Nerve and vascular invasion						
No versus yes	0.930	0.697-1.239	.618			
Lymph node metastasis						
No versus yes	1.629	1.204-2.205	.002	1.760	1.286-2.410	<.001
RORα expression levels						
Low versus high	1.617	1.207-2.164	.001	1.522	1.112-2.082	.009
REV-ERBα expression levels						
Low versus high	2.839	2.100-3.837	<.001	1.693	1.225-2.339	.001

Abbreviations: GC, gastric cancer; PFS, progression-free survival; HR, hazard ratio; 95% CI, 95% confidence interval; TNM, tumor-node-metastasis; CEA, carcinoembryonic antigen; CA199, carbohydrate antigen 199; RORα, retinoid-related orphan receptor alpha; REV-ERBα, nuclear receptor subfamily 1 group D member 1.

REV-ERBα in GC patients. The median OS time of GC patients with both high RORα and REV-ERBα expression levels was significantly longer than that of GC patients with high RORα and low REV-ERBα expression levels (Figure 6a), low RORα and high REV-ERBα expression levels (Figure 6b), and both low RORα and REV-ERBα expression levels (Figure 6c). Furthermore, the median PFS time of GC patients with both high RORα and REV-ERBα expression was longer than that of GC patients with high RORα and low REV-ERBα expression levels (Figure 6d), low RORα and high REV-ERBα expression levels (Figure 6e), and both low RORα and REV-ERBα expression levels (Figure 6f).

Table 4. The correlation analysis of RORα and REV-ERBα expression levels in GC.

	REV-ERBα expression levels, n		χ^2	*P*-value
	High	Low	6.835	.009
RORα expression levels (*n*)				
High	49	38		
Low	46	75		

Abbreviations: RORα, retinoid-related orphan receptor alpha; REV-ERBα, nuclear receptor subfamily 1 group D member 1; GC, gastric cancer.

Discussion

Accumulating evidence suggests that abnormalities in the circadian rhythm lead to gene dysfunction related to metabolic disorders and tumors.[5,14] Additionally, clinicopathological staging is a common method to predict prognosis in recent years. However, patients with identical stages manifest tremendous discrepancies in tumor recurrence and metastasis. Thus, it is meaningful to probe innovative biomarkers to predict prognosis and assist in the choice of optimized chemotherapy.

The ROR nuclear receptor family comprises RORα, RORβ, and RORγ members, which participate in many molecular pathways to regulate physiological activities.[4] Previous studies have shown that RORα and RORγ are the most important participants in the immune system and are associated with the pathogenesis of ovarian cancer.[15] They can regulate the expression of T-helper cell 17, which is a T-cell subgroup that secretes indispensable inflammatory factors, such as interleukin 17 and interleukin 22, during bacteria and virus infection.[15] Additionally, the RORα overexpression inhibited tumor cell invasion by inducing SEMA3F transcription in breast cancer. SEMA3F is a tumor microenvironmental suppressive factor and is regarded as a RORα target gene.[8] By contrast, silencing the SEMA3F gene cannot impede tumor growth and suggesting multiple target genes are involved in the downstream of RORα.[8]

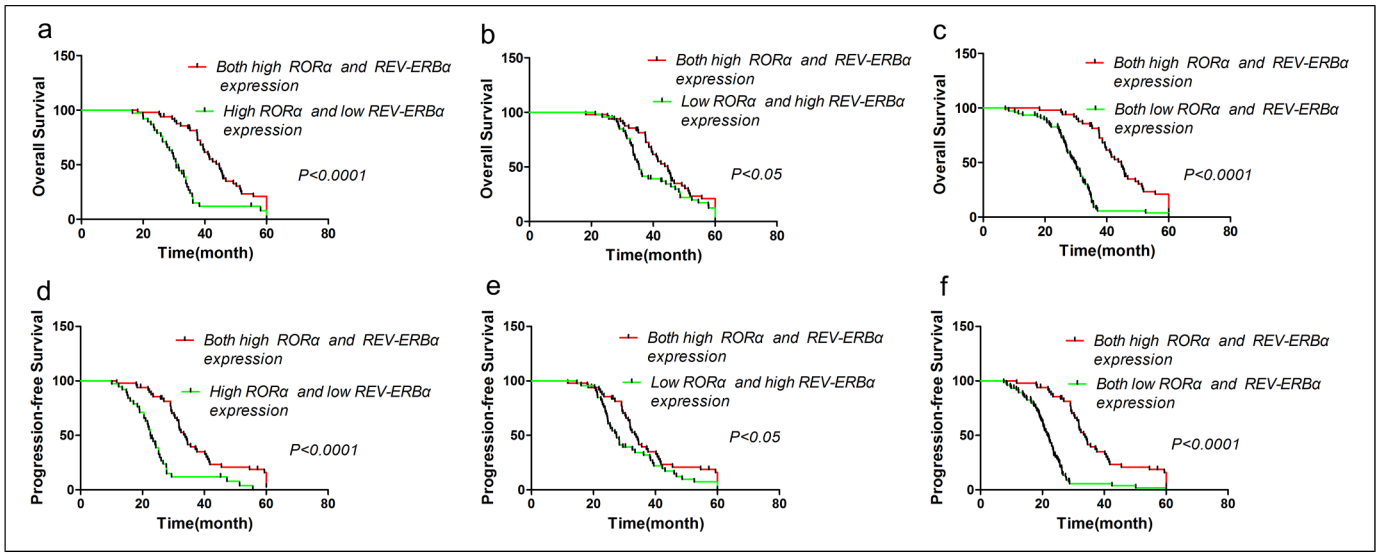

Figure 6. The median OS and PFS times were generated through the Kaplan–Meier method and log-rank test according to the expression levels of both RORα and REV-ERBα in GC patients. The median OS time in GC patients: (a) Both high RORα and REV-ERBα versus high RORα and low REV-ERBα. (b) Both high RORα and REV-ERBα versus low RORα and high REV-ERBα. (c) Both high RORα and REV-ERBα versus both low RORα and REV-ERBα. The median PFS time in GC patients: (d) both high RORα and REV-ERBα versus high RORα and low REV-ERBα. (e) Both high RORα and REV-ERBα versus low RORα and high REV-ERBα. (f) Both high RORα and REV-ERBα versus both low RORα and REV-ERBα.

Abbreviations: OS, overall survival; PFS, progression-free survival ; RORα, retinoid-related orphan receptor alpha; REV-ERBα, nuclear receptor subfamily 1 group D member 1; GC, gastric cancer.

Besides, RORα expression reduction could attenuate the Wnt/β-catenin signaling pathway, an important reason for the poor prognosis in liver cancer.[16] In the present study, immunohistochemistry and qRT-PCR are employed to illustrate that RORα expression levels were downregulated in GC tissues compared with that in normal gastric tissues. These results were the same as those reported by researchers who revealed that reduced RORα expression could inhibit cell apoptosis and tumor suppressor genes overexpression in GC.[10] The patients with low RORα expression levels were significantly associated with histological grade, preoperative CEA levels, and TNM stage and showed an increased risk of death compared with those with high RORα expression levels at the median OS and PFS times. The univariate and multivariate Cox regression model indicated that RORα expression levels, histological grade, preoperative CEA levels, TNM stage, and lymph node metastasis are associated with the prognosis of GC. Furthermore, the RORα expression levels can be considered an independent prognostic factor in GC.

REV-ERBα is also a nuclear receptor that belongs to one of the crucial clock genes.[5] In a previous study, REV-ERBα was mainly regulated in the metabolism of lipids and inflammation, a common event in humans.[5,17] However, the relationship between REV-ERBα and the mechanism of tumor generation and progression is not clear. Some scholars illustrated that breast cancer cells exhibit suppressed cell cycle progression and proliferation when REV-ERBα is overexpressed by adding a synthetic REV-ERB agonist.[18] Moreover, several other scholars demonstrated that REV-ERBα inhibits proliferation through glycolysis and the pentose phosphate pathway in GC cells.[19] In the present study, we found that REV-ERBα expression levels are also downregulated in GC tissues compared with those in normal gastric tissues, similar to that reported in our former study.[10] Therefore, we expanded the number of samples and increased the depth of analysis, and found that the GC patients with low REV-ERBα expression levels are markedly related with histological grade, preoperative CEA levels, and TNM stage. Additionally, GC patients with low REV-ERBα expression levels show an increased risk of death compared with those with high REV-ERBα expression levels at the median OS and PFS times. Besides, the univariate and multivariate Cox regression models were employed to determine whether REV-ERBα expression levels, histological grade, preoperative CEA levels, TNM stage, and lymph node metastasis were related to the prognosis of GC. Furthermore, REV-ERBα expression levels could be an independent prognostic factor in GC.

RORα and REV-ERBα have similar mechanisms of regulation in organs and tissues.[20] On the one hand, they are bound in a specific form to the response element of the promoter and then recruit particular target genes to participate in physiological activities.[21] On the other hand, coactivators and cosuppressors are integrated with RORα and REV-ERBα to regulate the inscription of target genes in the progression of histone acetylation and deacetylation.[22] Further research showed that RORα and REV-ERBα compete for interactions and reveal opposite functions through transcription.[4,5,23] However, no study has reported RORα and REV-ERBα coexpression in GC. Thus,

we hypothesized that RORα and REV-ERBα are coexpressed to participate in physiological activities, and the expression levels of both RORα and REV-ERBα are also associated with prognosis in GC. We found that RORα expression levels were associated with REV-ERBα expression levels, indicating a possible coexpression of RORα and REV-ERBα to participate in GC regulation. Additionally, GC patients with both high RORα and REV-ERBα expression levels had the best prognosis. However, this study had several limitations. Firstly, the deep molecular mechanism of RORα and REV-ERBα expression was not clear. Secondly. The study of survival time was a retrospective research. So, if the samples belonged to a abundant and multicentric database, the results manifested more representative. In addition, the hypothesis of RORα and REV-ERBα coexpression was based on this study and got no further verification and exploration.

Conclusion

RORα and REV-ERBα expression levels are downregulated in GC, and are associated with histological grade, preoperative CEA levels, and TNM stage. Additionally, GC patients with low RORα expression levels or low REV-ERBα expression levels show a poor prognosis, and the univariate and multivariate Cox regression models implicate RORα and REV-ERBα as potential biomarkers to predict the prognosis of GC, respectively. Furthermore, in GC, the expression levels of both RORα and REV-ERBα were first investigated and found to be positively correlated, and patients with both high RORα and REV-ERBα expression levels had the best prognosis.

Authors Contributions

XSW and RJ designed the study. XSW performed immunohistochemistry to determine the expression levels of RORα and REV-ERBα. RJ performed qRT-PCR to detect the expression levels of RORα and REV-ERBα. KC collected and calculated a clinical database of prognosis. XSW drafted the manuscript.

Ethical Approval

This research was conducted acccording to the Helsinki Declaration and was approved by Anhui Medical University, Hehei, China (Ethics Approval Number: Quick-PJ2020-11-20). Informed consent was obtained from each patient participating in this study.

Declaration of Conflicting Interests

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