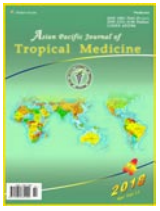


IF: 0.925

Asian Pacific Journal of Tropical Medicine

journal homepage: www.apjtm.org

doi: 10.4103/1995-7645.231471

EPOR mRNA level: A valuable prognostic indicator for patients with ER+ breast cancer

Jing Pan[1], Xing-Hua Han[1,2], Wei Wang[1,2], Yue-Yin Pan[1, 2]✉

[1]*Department of Medical Oncology, Anhui Provincial Hospital, Anhui Medical University, Hefei 230001, P.R. China*

[2]*Department of Medical Oncology, The First Affiliated Hospital of University of Science and Technology of China, Hefei 230001, P.R. China*

ARTICLE INFO

Article history:
Received 20 January 2018
Revision 23 February2018
Accepted 20 March 2018
Available online 2 April 2018

Keywords:
Breast cancer
EPOR
Prognosis
Bioinformatics

ABSTRACT

Objective: To explore the expression level and prognostic significance of erythropoietin receptor (EPOR) in patients with breast cancer (BRCA) based on estrogen receptor (ER) status and different molecular subtypes. **Methods:** The Cancer Genome Atlas (TCGA) and GTEx data were collected in GEPIA initially to identify the dysregulated genes. Further, bc-GenExMiner 4.1 online bioinformatics tool was used to evaluate EPOR mRNA differential expression level according to different classification of clinicopathologic parameters in patients with breast cancer. Additionally, the prognostic value between EPOR mRNA expression and free survival of metastatic relapse (MR) or any event (AE, namely any relapse or death) in patients with breast cancer was done. **Results:** EPOR mRNA was significantly downregulated in BRCA (1 085 cases) compared to normal tissues (291 cases) (P<0.05). Univariate Cox analysis revealed that high EPOR mRNA expression was remarkably correlated to a decreased risk of MR (*HR*: 0.79, P<0.000 1) and AE (*HR*: 0.87, P=0.000 7) especially in breast cancer patients with ER+. Besides, high EPOR mRNA level also associated with a favorable MR-free survival (*HR*: 0.81, P=0.007 2) and AE-free survival (*HR*: 0.88, P =0.029 9) in ER+ breast cancer patients. However, no similar above phenomenon was detected in ER- patients. Moreover, the subsequent prognostic adjusted analyses and univariate Cox analysis of AE based on SSP or SCM molecular subtypes validated the above results. **Conclusion:** EPOR mRNA level is a valuable prognostic indicator for patients with ER+ breast cancer.

1. Introduction

Breast cancer (BRCA) is one of the leading causes of cancer death among women worldwide and the number of newly diagnosed is gradually increasing in recent years[1,2]. To further acquire early detection and improve the treatment effect of advanced breast cancer, a great deal of efforts has been made. But unfortunately, the mortality of breast cancer patients is still maintaining higher and becoming a globally difficult question[3,4]. Recently, accumulating studies have demonstrated that exploration and validation of newly biomarkers or prognostic factors could improve clinical outcomes of breast cancer patients to some extent.

Erythropoietin receptor (EPOR) is a transmembrane protein of 484 amino acids and a calculated mass of 52.6 kDa[5], which increases to about 60 kDa due to glycosylation and phosphorylation. Recently, many studies focus their attention on this gene mainly due to the clinical use of recombinant human erythropoietin (rHuEPO) in cancer patients. In 2003, two large randomized controlled trials highlighting unexpected and deleterious effects of therapeutically-administered rHuEPO were published[6,7]. Starting from this, many controversies and conflicting conclusions have been gradually discovered. Some studies have found that rHuEPO can stimulate the

First author: Jing Pan, Department of Medical Oncology, Anhui Provincial Hospital, Anhui Medical University, Hefei 230001, P.R. China.

✉Corresponding author: Yue-Yin Pan, Department of Medical Oncology, The First Affiliated Hospital of University of Science and Technology of China, No.17 Lujiang Road, Luyang District, Hefei 230001, P.R.China.

E-mail: yueyinpan1965@163.com

Tel: +86-551-62283411

Fax: +86-551-62283411

Foundation project: This work was done in the Department of Medical Oncology, The First Affiliated Hospital of University of Science and Technology of China.

How to cite this article: Pan J, Han XH, Wang W, Pan YY. EPOR mRNA level: A valuable prognostic indicator for patients with ER+ breast cancer. Asian Pac J Trop Med 2018; 11(4): 297-304.

EPOR expression level through activating JAK2-STAT5 or PI3K-AKT signal pathway and high EPOR expression will induce tumor growth and metastasis in many kinds of malignances[8-11]. On the contrary, other studies have revealed that there is no influence of rHuEPO use on the EPOR expression, and even these corresponding authors have detected the low expression level or no expression of EPOR in malignancies[12-16]. They pointed out the potential reason may be partly associated with non-specificity of the antibodies used for detection of EPOR protein[17,18]. As for breast cancer, a similar paradoxical phenomenon exists as the above. Therefore, it is rather necessary to explore the expression of EPOR in breast cancer from the aspect of mRNA and whether it has a prognostic value.

Thus, in the present study, The Cancer Genome Atlas (TCGA) and GTEx data were collected in GEPIA initially to identify the dysregulated gene EPOR. Further, bc-GenExMiner 4.1 online bioinformatics tool was used to evaluate EPOR mRNA differential expression level according to different classification of clinicopathologic parameters in patients with breast cancer. Additionally, the prognostic value between EPOR mRNA level and free survival of metastatic relapse (MR) or any event (AE, namely any relapse or death) in patients with breast cancer was investigated.

2. Materials and methods

2.1. Mining of dysregulated EPOR gene in breast cancer

GEPIA (Website: http://gepia.cancer-pku.cn/) is a newly developed interactive web server for analyzing the RNA sequencing expression data of 9 736 tumors and 8 587 normal samples from the TCGA and the GTEx projects, using a standard processing pipeline[19]. Initially, to explore whether EPOR mRNA levels have the significance of differential expression between breast cancer and normal tissues, GEPIA was used to draw the "Expression on Box Plots" under the selection of "Breast Cancer (BRCA) Datasets". Besides, differential expression levels of EPOR mRNA in BRCA patients were analyzed by bc-GenExMiner v4.1 (breast cancer Gene-Expression Miner v4.1, website: http://bcgenex.centregauducheau.fr/BC-GEM/GEM-Accueil.php?js=1), a large free and open source database containing information about 5 861 cases of BRCA patients[20,21], based on different kinds of classified parameters: Receptor statuses (ER+ *vs*. ER-, PR+ *vs*. PR-, HER2+ *vs*. HER2-), Nodal status (N+ *vs*. N-), SBR (SBR1 *vs*. SBR2 *vs*. SBR3), NPI (NPI1 *vs*. NPI2 *vs*. NPI3), Age (≤40/40-70/≥70 and ≤51/>51), Molecular subtypes, Basal-like (PAM50) and/or TNBC.

2.2. Correlation between EPOR mRNA expression levels and survival of breast cancer patients by bioinformatic analysis

To explore the correlation between EPOR mRNA level and the risk of MR or AE, MR-free and AE-free survival in BRCA patients, meta-analysis was done further through using bc-GenExMiner 4.1. Besides, subgroup analysis was done based on ER status, or molecular subtypes, or two types of molecular subtype predictors (SSP and SCM), respectively. The prognostic significance of EPOR mRNA level in BRCA patients was assessed by using univariate Cox regression model and drawing Kaplan-Meier curve and forest plot. Additionally, NPI-, AOL- and proliferation- adjusted analyses were further performed to verify the independent prognostic significance of EPOR mRNA in breast cancer.

3. Results

3.1. EPOR mRNA was downregulated in BRCA compared to normal tissues

Through searching the TCGA and GTEx data in GEPIA, we found that EPOR mRNA was dramatically downregulated in BRCA (1 085 cases) compared to normal tissues (291 cases) (P <0.05, Figure 1A). Then, in order to further know whether or not significantly differential expression levels of EPOR mRNA existed in breast cancer patients based on different kinds of classified parameters, bc-GenExMiner v4.1 was employed. The results showed that there were remarkably differential expression levels of EPOR mRNA in ER+ *vs*. ER- patients (ER+ > ER-, Figure 1B), PR+ *vs*. PR- patients (PR+ > PR-, Figure 1C), NPI1 *vs*. NPI2 *vs*. NPI3patients (NPI1 > NPI2 > NPI3), SBR1 *vs*. SBR2 *vs*. SBR3patients (SBR1 > SBR2 > SBR3), Basal-like *vs*. Not basal-like patients (Not basal-like >Basal-like, Figure1H), Basal-like and TNBC *vs*. Not basal-like and not TNBC patients (Not basal-like and not TNBC >Basal-like and TNBC, Figure 1I), TNBC *vs*. Not TNBC patients (Not TNBC >TNBC, Figure 1J), respectively. While, no significant expression difference of EPOR mRNA was found in HER2+ *vs*. HER2- patients (Figure 1D) or N+ *vs*. N- patients (Figure 1E).

3.2. High expression of EPOR mRNA was dramatically related to decreased risk of MR and AE in ERm or ER+ breast cancer patients

By exploring in bc-GenExMiner 4.1, totally 33 studies including 5 064 patients investigated the correlation between EPOR mRNA level and MR or AE (Table 1). Initially, a preliminary study was done to investigate the prognostic analysis for EPOR mRNA expression with any ER status, any nodal status and any event. As shown in Table 2, high EPOR mRNA expression was closely related to the decreased risk of MR and AE in ERm or ER+ patients. Nevertheless, the same relationship was not detected in ER- patients (Table 2). These results suggested that EPOR mRNA level might have diverse prognostic significance in different subtypes of breast cancer.

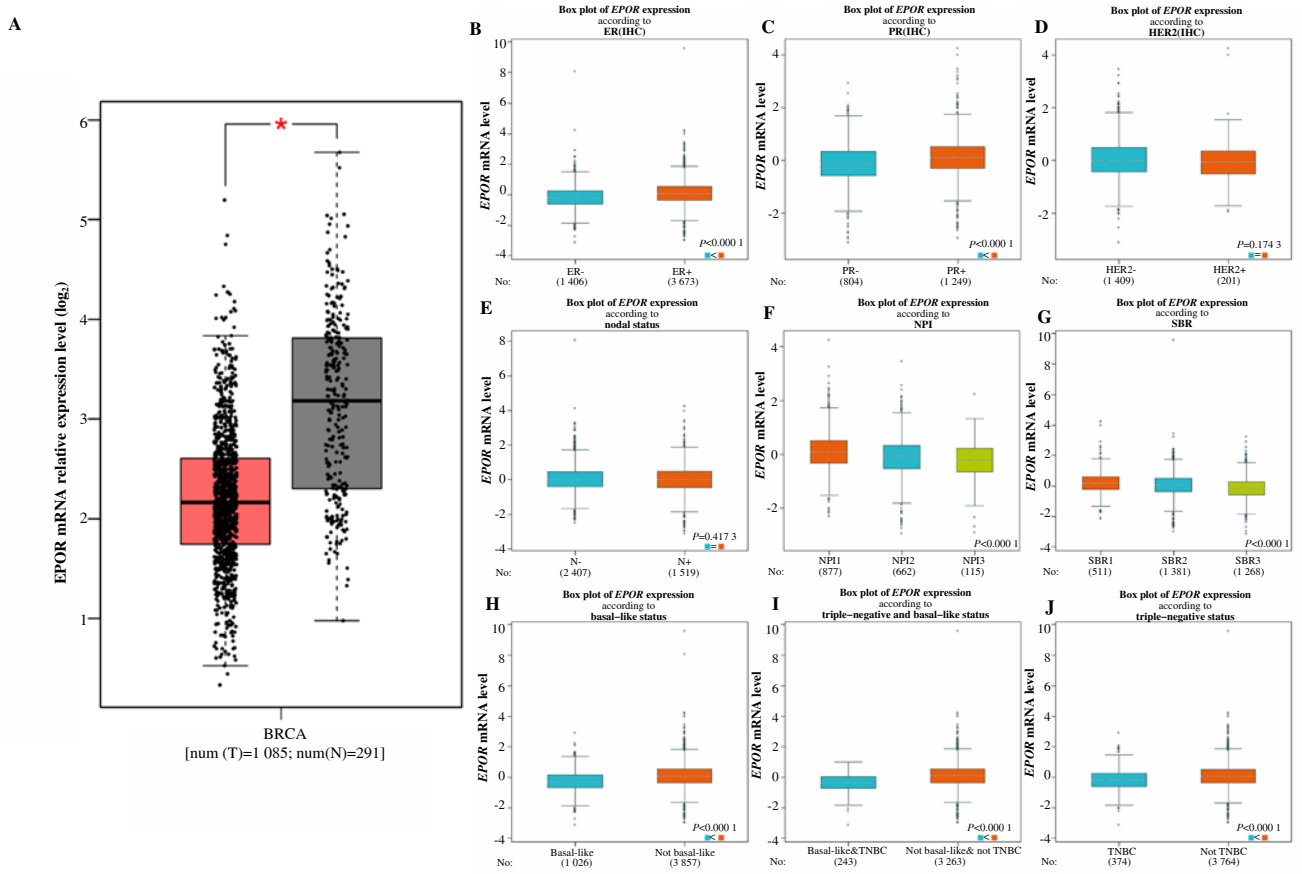


Figure 1. Differential expression levels of EPOR mRNA in breast cancer patients based on different kinds of classified parameters.
(A) TCGA data revealed that EPOR mRNA was significantly lower in breast cancer tissues (1 085 cases) than that in normal tissues (291 cases); (B-J) differential expression levels of EPOR mRNA in breast cancer patients were performed by bc-GenExMiner v4.1 based on different kinds of classified parameters: ER+ *vs*. ER- (B), PR+ *vs*. PR- (C), HER2+ *vs*. HER2- (D), N+ *vs*. N- (E), NPI1 *vs*. NPI2 *vs*. NPI3 (F), SBR1 *vs*. SBR2 *vs*. SBR3 (G), Basal-like *vs*. Not basal-like (H), Basal-like and TNBC *vs*. Not basal-like and not TNBC (I), TNBC *vs*. Not TNBC (J).

3.3. EPOR univariate Cox regression analysis and adjusted analyses of MR based on ER status

To explore whether EPOR mRNA level had diverse prognostic significance in ER+ or ER- breast cancer patients, subgroup analysis was done further. As shown in Figure 2A, a total of 23 studies including 2 822 patients were searched to evaluate the correlation between EPOR mRNA level and MR risk in ER+ BRCA patients. The univariate Cox regression analysis demonstrated that high EPOR mRNA level was dramatically related to a lower risk of MR (*HR*: 0.79, *P*<0.000 1; Figure 2A) and also a better MR-free survival (*HR*: 0.81, 95%*CI*: 0.70-0.94, *P* =0.007 2; Figure 2B). Besides, NPI-, AOL- and proliferation- adjusted analyses validated that high EPOR mRNA level was significantly correlated to a lower risk of MR in ER+ patients (*HR*: 0.81, 0.66 and 0.76, respectively; *P*=0.017 4, 0.000 9 and <0.000 1, respectively; Figure 2C). As for ER- patients, a total of 21 studies including 1 073 patients were searched to evaluate the relationship between EPOR mRNA level and MR risk (Figure 2D). The results revealed no significant correlation no matter in univariate Cox regression analysis (Figure 2E) or in NPI-, AOL- and proliferation- adjusted analysis (Figure 2F).

3.4. EPOR univariate Cox regression analysis and adjusted analyses of AE based on ER status

Prognostic significance of EPOR mRNA on AE was examined among the breast cancer patients with different ER status. As shown in Figure 3A, a total of 31 studies including 3 631 patients were searched to evaluate the relationship between EPOR mRNA level and AR risk in ER+ BRCA patients. The univariate Cox regression analysis demonstrated that high EPOR mRNA level was dramatically related to a lower risk of AE (*HR*: 0.87, *P*=0.000 7; Figure 3A) and also a better AE-free survival (*HR*: 0.88, 95%*CI*: 0.78-0.99, *P*=0.029 9; Figure 3B). Besides, NPI-, AOL- and proliferation-adjusted analyses were further performed (Figure 3E-G), which showed that the significant impact of EPOR mRNA level on AE risk mainly in the ER+ combined N+ subgroup, but not in the ER+ combined N- subgroup. As for ER- patients, a total of 27 studies including 1 394 patients were searched to evaluate the significance of EPOR mRNA level on MR risk (Figure 3C).The results revealed no significant correlation both in univariate Cox regression analysis (Figure 3D) and NPI-, AOL- and proliferation- adjusted analyses (Figure 3H).

3.5. EPOR univariate Cox regression analysis of AE based on the molecular subtypes of SSP and SCM

Next, the prognostic significance of EPOR mRNA in breast cancer patients was evaluated under different molecular subtypes. As shown in Table 3, by using the SSP classification, high EPOR mRNA level was significantly related to decreased risk of AE in Luminal B breast cancer (*HR*: 0.82, 95%*CI*: 0.71-0.94, *P*=0.005 9),while no relationship was detected in basal-like, HER2+, Luminal A and normal basal-like subtypes. Moreover, to investigate the reliability of the results on the basis of SSP molecular subtype, EPOR univariate Cox analysis by SCM molecular subtype was done further. Similar to previous results, high EPOR mRNA level was significantly related to decreased risk in ER+/HER2-high proliferation group (*HR*: 0.86, 95%*CI*: 0.76-0.97, *P*=0.0152; Table 4), but not in ER-/HER2-, HER2+ or ER+/HER2- low proliferation group (Table 4).

Table 1

The basic characteristics of studies included.

Study code	Original data reference	Patients (*n*)	Filtered data (any N-ER and AE)	Final data [patients (*n*)]
Rosetta2002	[22]	295	295	295
PNAS1732912100	[23]	99	99	99
GSE1378	[24]	59	59	59
GSE2603	[25]	82	82	82
GSE1456	[26]	159	159	159
GSE2034	[27]	286	286	286
GSE2741	[28]	50	50	50
GSE3143	[29]	158	158	158
E_TABM_158	[30]	112	112	112
GSE4922	[31]	249	249	249
GSE8757	[32]	171	171	171
GSE7390	[33]	198	198	198
GSE6532	[34]	401	393	393
GSE5327	[35]	58	58	58
GSE7378	[36]	54	54	54
GSE7849	[37]	75	75	75
GSE9893	[38]	155	155	155
GSE9195	[39]	77	77	77
GSE11121	[40]	200	200	200
GSE10510	[41]	139	134	134
GSE16391	[42]	55	55	55
GSE12093	[43]	136	136	136
GSE19615	[44]	115	115	115
GSE17907	[45]	55	39	39
GSE22219	[46]	216	216	216
GSE20711	[47]	85	85	85
GSE26971	[48]	277	258	258
GSE25055	[49]	309	309	309
GSE20685	[50]	296	296	296
GSE21653	[51]	266	252	252
GSE16987	[52]	149	147	147
GSE33926	[53]	51	51	51
GSE45255	[54]	41	41	41
Total	33	5 861	5 790	5 064

Table 2

Exhaustive EPOR univariate Coxanalysis of MR and AE in breast cancer patients.

No.	Population and event criteria			*P*-value	*HR*	95% *CI*	Good prognosis' RNA level	Patients (*n*)	Events (*n*)
1	ERm	Nm	MR	< 0.000 1	0.78	0.72 - 0.85	High	3 925	1 023
2	ERm	Nm	AE	< 0.000 1	0.86	0.81 - 0.92	High	5 064	1 651
3	ERm	N+	MR	0.003 1	0.82	0.71 - 0.93	High	1 033	345
4	ERm	N+	AE	0.017 6	0.89	0.80 - 0.98	High	1 505	620
5	ERm	N-	MR	0.000 2	0.78	0.68 - 0.89	High	1 933	461
6	ERm	N-	AE	0.000 3	0.82	0.74 - 0.91	High	2 391	708
7	ER+	Nm	MR	< 0.000 1	0.79	0.71 - 0.88	High	2 822	673
8	ER+	Nm	AE	0.000 7	0.87	0.80 - 0.94	High	3 631	1 115
9	ER+	N+	MR	0.004 3	0.78	0.66 - 0.93	High	712	214
10	ER+	N+	AE	0.020 5	0.86	0.76 - 0.98	High	1 054	403
11	ER+	N-	MR	0.012 7	0.81	0.69 - 0.96	High	1 419	315
12	ER+	N-	AE	0.011 7	0.85	0.74 - 0.96	High	1 740	490
13	ER-	Nm	MR	0.089 0	0.88	0.76 - 1.02	High	1 073	345
14	ER-	Nm	AE	0.160 7	0.92	0.82 - 1.03	-	1 394	527
15	ER-	N+	MR	0.783 0	0.97	0.77 - 1.22	-	313	130
16	ER-	N+	AE	0.999 7	1.00	0.83 - 1.20	-	442	216
17	ER-	N-	MR	0.064 2	0.80	0.63 - 1.01	High	495	144
18	ER-	N-	AE	0.063 9	0.84	0.70 - 1.01	High	627	213

MR: metastatic relapse; AE: any event; ER (m, +, -): estrogen receptor status (mixed, positive, negative); N (m, +, -): Nodal status (mixed, positive, negative); *HR*: hazard ratio; *CI*: confidence interval.

Table 3

EPOR univariate Cox analysis of AE by SSP molecular subtype.

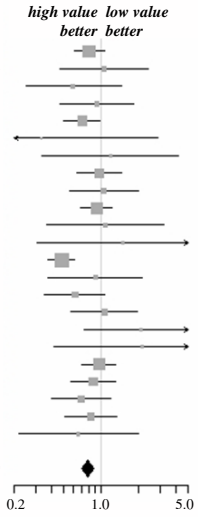
Molecular subtype	Analysis results	Single Sample Predictors (Hu's)
Basal-like	*P*-value	0.249 5
	HR (95% *CI*)	0.92 (0.79 - 1.06)
	Patients (*n*)	1 167
	Events (*n*)	410
HER2+	*P*-value	0.619 3
	HR (95% *CI*)	1.06 (0.84 - 1.35)
	Patients (*n*)	447
	Events (*n*)	189
Luminal A	*P*-value	0.917 2
	HR (95% *CI*)	1.01 (0.83 - 1.22)
	Patients (*n*)	121 8
	Events (*n*)	243
Luminal B	*P*-value	0.005 9
	HR (95% *CI*)	0.82 (0.71 - 0.94)
	Patients (*n*)	899
	Events (*n*)	386
Normal breast-like	*P*-value	0.174 3
	HR (95% *CI*)	0.86 (0.68 - 1.07)
	Patients (*n*)	732
	Events (*n*)	192

EPOR univariate Cox analysis of MR (ER+; Nm)

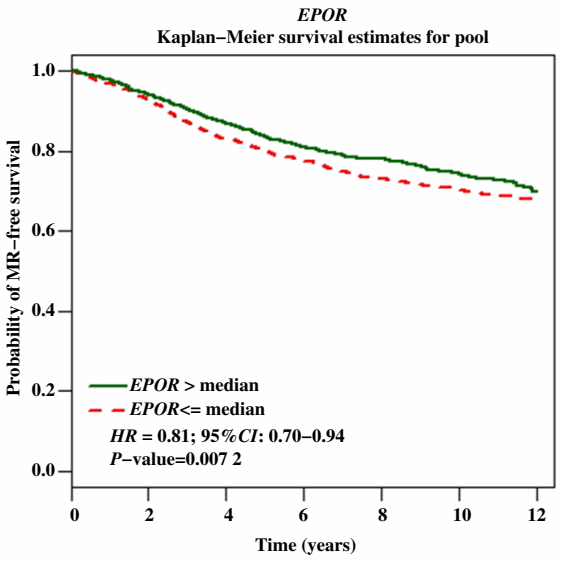
A

***EPOR*: Forest plot**

Study code	No. patients	No. MR	*P*-value	*HR*
Rosetta2002	226	70	0.137 8	0.81
PNAS1732912100	65	15	0.892 0	1.06
GSE2603	46	13	0.263 1	0.60
GSE1456	119	26	0.831 8	0.93
GSE2034	209	80	0.046 2	0.71
GSE2741	26	3	0.317 1	0.33
E_TABM_158	70	15	0.780 1	1.20
GSE8757	113	22	0.891 5	0.97
GSE7390	134	35	0.862 3	1.06
GSE6532	348	89	0.611 6	0.93
GSE7378	54	9	0.869 1	1.09
GSE7849	46	6	0.616 1	1.50
GSE9893	135	44	<0.000 1	0.49
GSE9195	77	10	0.807 4	0.90
GSE11121	154	34	0.084 1	0.62
GSE12093	136	20	0.841 2	1.07
GSE19615	70	4	0.169 7	2.10
GSE17907	14	4	0.360 2	2.14
GSE22219	134	49	0.827 0	0.97
GSE26971	258	58	0.498 3	0.87
GSE25055	173	23	0.195 2	0.70
GSE20685	188	34	0.438 1	0.83
GSE45255	27	10	0.455 3	0.66
Pool	**2 822**	**673**	**<0.000 1**	**0.79**



B



Patients at risk:

EPOR > median	1 411	1 274	1 092	819	557	340	131
EPOR<= median	1 411	1 256	1 041	761	530	325	153

C

Prognostic index–adjusted analyses of MR (ER+; Nm)

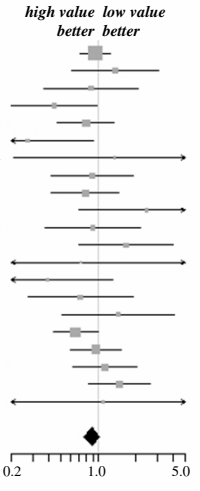
Results	*P*-value	*HR*	95%*CI*	No. Patients	No. MR
EPOR adjusted on NPI	0.017 4	0.81	0.67-0.96	927	222
EPOR adjusted on AOL	0.000 9	0.66	0.52-0.85	382	101
EPOR adjusted on proliferation	<0.000 1	0.76	0.67-0.87	1 791	460

EPOR univariate Cox analysis of MR (ER–; Nm)

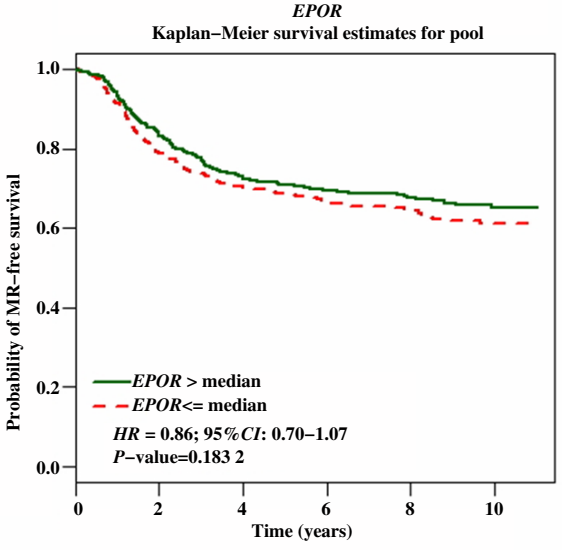
D

***EPOR*: Forest plot**

Study code	No. patients	No. MR	*P*-value	*HR*
Rosetta2002	69	31	0.677 2	0.94
PNAS1732912100	34	15	0.440 9	1.37
GSE2603	36	14	0.755 1	0.87
GSE1456	40	14	0.041 9	0.44
GSE2034	77	27	0.389 1	0.80
GSE2741	24	10	0.033 0	0.27
E_TABM_158	42	6	0.757 2	1.34
GSE8757	57	16	0.762 4	0.89
GSE7390	64	27	0.424 5	0.78
GSE6532	39	11	0.162 1	2.44
GSE5327	58	11	0.812 7	0.90
GSE7849	25	7	0.258 5	1.66
GSE9893	8	2	0.802 1	0.72
GSE11121	46	12	0.127 8	0.39
GSE19615	45	10	0.495 9	0.71
GSE17907	24	13	0.491 6	1.44
GSE22219	82	33	0.043 9	0.65
GSE25055	131	41	0.846 9	0.95
GSE20685	108	29	0.707 2	1.12
GSE33926	51	12	0.172 0	1.48
GSE45255	13	4	0.933 3	1.09
Pool	**1 073**	**345**	**0.089 0**	**0.88**



E



Patients at risk:

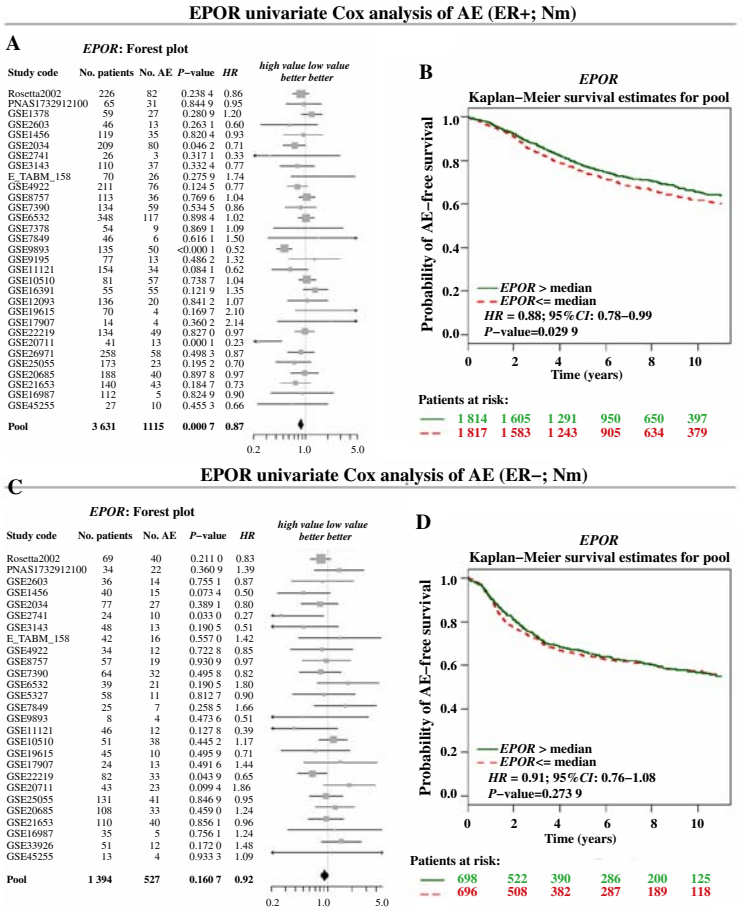
EPOR > median	538	407	320	241	170	110
EPOR<= median	535	405	330	255	169	105

F

Prognostic index–adjusted analyses of MR (ER–; Nm)

Results	*P*-value	*HR*	95%*CI*	No. Patients	No. MR
EPOR adjusted on NPI	0.227 7	0.84	0.63-1.12	346	111
EPOR adjusted on AOL	0.557 1	1.15	0.72-1.84	138	54
EPOR adjusted on proliferation	0.247 1	0.88	0.72-1.09	562	187

Figure 2. EPOR univariate Cox analysis and three types of prognostic index-adjusted analysis of MR based on ER status. Forest plots displaying univariate Cox's analysis of EPOR mRNA expression and the risk of MR in ER+ (A) and ER- (D) breast cancer patients; Kaplan-Meier curves of EPOR mRNA expression and MR-free survival in ER+ (B) and ER- (E) breast cancer patients; three types (NPI, AOL and proliferation) of prognostic index-adjusted analysis of the correlation between EPOR mRNA expression and the risk of MR in ER+ (C) and ER- (F) breast cancer patients. Data mining was done by bc-GenExMiner v4.1.



Study code	No. patients	No. AE	P–value	HR
Rosetta2002	226	82	0.238 4	0.86
PNAS1732912100	65	31	0.844 9	0.95
GSE1378	59	27	0.280 9	1.20
GSE2603	46	13	0.263 1	0.60
GSE1456	119	35	0.820 4	0.93
GSE2034	209	80	0.046 2	0.71
GSE2741	26	3	0.317 1	0.33
GSE3143	110	37	0.332 4	0.77
E_TABM_158	70	26	0.275 9	1.74
GSE4922	211	76	0.124 5	0.77
GSE8757	113	36	0.769 6	1.04
GSE7390	134	59	0.534 5	0.86
GSE6532	348	117	0.898 4	1.02
GSE7378	54	9	0.869 1	1.09
GSE7849	46	6	0.616 1	1.50
GSE9893	135	50	<0.000 1	0.52
GSE9195	77	13	0.486 2	1.32
GSE11121	154	34	0.084 1	0.62
GSE10510	81	57	0.738 7	1.04
GSE16391	55	55	0.121 9	1.35
GSE12093	136	20	0.841 2	1.07
GSE19615	70	4	0.169 7	2.10
GSE17907	14	4	0.360 2	2.14
GSE22219	134	49	0.827 0	0.97
GSE20711	41	13	0.000 1	0.23
GSE26971	258	58	0.498 3	0.87
GSE25055	173	23	0.195 2	0.70
GSE20685	188	40	0.897 8	0.97
GSE21653	140	43	0.184 7	0.73
GSE16987	112	5	0.824 9	0.90
GSE45255	27	10	0.455 3	0.66
Pool	**3 631**	**1115**	**0.000 7**	**0.87**

Study code	No. patients	No. AE	P–value	HR
Rosetta2002	69	40	0.211 0	0.83
PNAS1732912100	34	22	0.360 9	1.39
GSE2603	36	14	0.755 1	0.87
GSE1456	40	15	0.073 4	0.50
GSE2034	77	27	0.389 1	0.80
GSE2741	24	10	0.033 0	0.27
GSE3143	48	13	0.190 5	0.51
E_TABM_158	42	16	0.557 0	1.42
GSE4922	34	12	0.722 8	0.85
GSE8757	57	19	0.930 9	0.97
GSE7390	64	32	0.495 8	0.82
GSE6532	39	21	0.190 5	1.80
GSE5327	58	11	0.812 7	0.90
GSE7849	25	7	0.258 5	1.66
GSE9893	8	4	0.473 6	0.51
GSE11121	46	12	0.127 8	0.39
GSE10510	51	38	0.445 2	1.17
GSE19615	45	10	0.495 9	0.71
GSE17907	24	13	0.491 6	1.44
GSE22219	82	33	0.043 9	0.65
GSE20711	43	23	0.099 4	1.86
GSE25055	131	41	0.846 9	0.95
GSE20685	108	33	0.459 0	1.24
GSE21653	110	40	0.856 1	0.96
GSE16987	35	5	0.756 1	1.24
GSE33926	51	12	0.172 0	1.48
GSE45255	13	4	0.933 3	1.09
Pool	**1 394**	**527**	**0.160 7**	**0.92**

E

Prognostic index–adjusted analyses of AE (ER+; Nm)

Results	P–value	HR	95%CI	No. Patients	No. AE
EPOR adjusted on NPI	0.137 5	0.90	0.78-1.03	1 227	355
EPOR adjusted on AOL	0.010 4	0.77	0.64-0.94	434	167
EPOR adjusted on proliferation	0.008 2	0.88	0.80-0.97	2 252	766

F

Prognostic index–adjusted analyses of AE (ER+; N+)

Results	P–value	HR	95%CI	No. Patients	No. AE
EPOR adjusted on NPI	0.018 3	0.76	0.60-0.95	245	93
EPOR adjusted on AOL	0.007 3	0.70	0.54-0.91	130	66
EPOR adjusted on proliferation	0.015 7	0.84	0.72-0.97	629	276

G

Prognostic index–adjusted analyses of AE (ER+; N–)

Results	P–value	HR	95%CI	No. Patients	No. AE
EPOR adjusted on NPI	0.966 8	1.00	0.84-1.20	982	262
EPOR adjusted on AOL	0.698 4	0.94	0.68-1.29	304	101
EPOR adjusted on proliferation	0.237 5	0.92	0.79-1.06	1 346	403

H

Prognostic index–adjusted analyses of AE (ER–; Nm)

Results	P–value	HR	95%CI	No. Patients	No. AE
EPOR adjusted on NPI	0.218 2	0.85	0.66-1.10	401	149
EPOR adjusted on AOL	0.523 3	1.15	0.75-1.76	138	68
EPOR adjusted on proliferation	0.170 5	0.90	0.77-1.05	695	294

Figure 3. EPOR univariate Cox analysis and three types of prognostic index-adjusted analysis of AE based on ER status.
Forest plots displaying univariate Cox's analysis of EPOR mRNA expression and the risk of AE in ER+ (A) and ER- (C) breast cancer patients; Kaplan-Meier curves of EPOR mRNA expression and AE-free survival in ER+ (B) and ER- (D) breast cancer patients; three types (NPI, AOL and proliferation) of prognostic index-adjusted analysis of the correlation between EPOR mRNA expression and the risk of AE in ER+ (E) and ER- (H) breast cancer patients; in ER+ patients, subgroup analysis was further made on the basis of nodal- positive (F) or negative (G) status. Data mining was done by bc-GenExMiner v4.1.

Table 4

EPOR univariate Cox analysis of AE by SCM molecular subtype.

Molecular subtype	Analysis results	Subtype clustering models (SCMOD1)
ER-/HER2-	*P*-value	0.993 3
	HR (95% *CI*)	1.00 (0.86 - 1.16)
	Patients (*n*)	942
	Events (*n*)	352
HER2+	*P*-value	0.935 5
	HR (95% *CI*)	0.99 (0.86 - 1.15)
	Patients (*n*)	833
	Events (*n*)	286
ER+/HER2- low proliferation	*P*-value	0.251 3
	HR (95% *CI*)	0.92 (0.80 - 1.06)
	Patients (*n*)	1 561
	Events (*n*)	377
ER+/HER2- high proliferation	*P*-value	0.015 2
	HR (95% *CI*)	0.86 (0.76 - 0.97)
	Patients (*n*)	1 396
	Events (*n*)	519

4. Discussion

Treatment of cancer patients with recombinant human erythropoiesis stimulating agents (rhESA) reduces transfusion requirements and improves quality of life[55-57]. Anemia prevention is pivotal with a view to hypoxia-driven tumor progression. Nevertheless, the negative outcomes of high-dose rhESA therapy trials on patients with breast[6] or head and neck cancers[7] have raised concern that EPO may boost tumor growth. A prerequisite for effects of EPO is the existence of functional EPOR. Previous studies have provided conflicting results[56,58], which may be partly due to nonspecificity of the antibodies used for detection of EPOR protein[17,18].Therefore, to avoid the bias induced by the protein level detection of EPOR, bioinformatic mining method was performed to explore the expression level of EPOR mRNA in breast cancer and its potential prognostic significance.

First, TCGA and GTEx data in GEPIA was used to found surprisingly that EPOR mRNA was dramatically downregulated in BRCA (1 085 cases) compared to normal tissues (291 cases). Then, in order to further know whether or not significantly differential expression levels of EPOR mRNA existed in breast cancer patients based on different kinds of classified parameters, subgroup analysis was employed by bc-GenExMiner v4.1. The findings revealed that there were remarkably differential expression levels of EPOR mRNA between the favorable prognostic parameter group and unfavorable prognostic parameter group. The differential expression levels of

EPOR mRNA in each subgroup were listed as follows: ER+ > ER-, PR+ > PR-, NPI1 > NPI2 > NPI3, SBR1 > SBR2 > SBR3, Not basal-like >Basal-like, Not basal-like and not TNBC >Basal-like and TNBC and Not TNBC >TNBC, respectively. As we known, breast cancer patients with the above favorable prognostic parameters were to be considered commonly had a better survival time[59-62]. Thus, these results suggested that high EPOR mRNA level might be served as a protective role in breast cancer patients for longer survival.Second, the subsequent validation analyses were performed to investigate whether or not differential expression levels of EPOR mRNA were associated with the risk of MR and AE or MR-free and AE-free survival of breast cancer patients. Consistent with previous hypothesis, we found that high EPOR mRNA expression in ERm or ER+ patients (any type of N status) was remarkably related to decreased the risk of MR and AE. Simultaneously, ER+ patients with high EPOR mRNA level also had much more better MR-free and AE-free survival than those with low EPOR mRNA level. However, no similar phenomenon was detected in patients with ER-.Besides, NPI-, AOL- and proliferation- adjusted analyses were further performed to verify the above findings and the results were consistent. Additionally, univariate Cox analysis of AE was done to evaluate the prognostic significance of EPOR in BRCA patients based on SSP or SCM molecular subtypes. The results showed that high EPOR mRNA level was significantly associated with decreased risk of AE in BRCA patients with Luminal B or ER+/HER2- high proliferation group under SSP and SCM classification, respectively. Taken together, the above findings suggested that high EPOR mRNA level might be a significantly favorable indicator to predict low risk of MR and AE or longer survival in ER+ breast cancer patients.

In conclusion, the present bioinformatic mining findings suggested that EPOR mRNA level might be a significant indicator to predict the risk of MR and AE in ER+ breast cancer patients. In future, this interesting observation is worthy of deeper exploration and validation from the aspect of real fundamental experiments and clinical trials.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Acknowledgement

This study was supported by the Science and Technology Research Project of Anhui Province (No. 1704a0802148).

References

[1] Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2017. *CA Cancer J Clin* 2017; **67**(1): 7-30.

[2] Xu H, Yu S, Liu Q, Yuan X, Mani S, Pestell RG, et al. Recent advances of highly selective CDK4/6 inhibitors in breast cancer. *J Hematol Oncol* 2017; **10**(1): 97.

[3] Shan M, Yin H, Li J, Li X, Wang D, Su Y, et al. Detection of aberrant methylation of a six-gene panel in serum DNA for diagnosis of breast cancer. *Oncotarget* 2016; **7**(14): 18485-18494.

[4] Baldwin RM, Haghandish N, Daneshmand M, Amin S, Paris G, Falls TJ, et al. Protein arginine methyltransferase 7 promotes breast cancer cell invasion through the induction of MMP9 expression. *Oncotarget* 2015; **6**(5): 3013-3032.

[5] Sinclair AM, Coxon A, McCaffery I, Kaufman S, Paweletz K, Liu L, et al. Functional erythropoietin receptor is undetectable in endothelial, cardiac, neuronal, and renal cells. *Blood* 2010; **115**(21): 4264-4272.

[6] Leyland-Jones B, BEST Investigators and Study Group. Breast cancer trial with erythropoietin terminated unexpectedly. *Lancet Oncol* 2003; **4**(8): 459-460.

[7] Henke M, Laszig R, Rübe C, Schäfer U, Haase KD, Schilcher B, et al. Erythropoietin to treat head and neck cancer patients with anaemia undergoing radiotherapy: randomised, double-blind, placebo-controlled trial. *Lancet* 2003; **362**(9392): 1255-1260.

[8] Julius A, Desai A, Yung RL. Recombinant human erythropoietin stimulates melanoma tumor growth through activation of initiation factor eIF4E. *Oncotarget* 2017; **8**(18): 30317-30327.

[9] Chan KK, Matchett KB, Coulter JA, Yuen HF, McCrudden CM, Zhang SD, et al. Erythropoietin drives breast cancer progression by activation of its receptor EPOR. *Oncotarget* 2017; **8**(24): 38251-38263.

[10]Ilkovi ová L, Trošt N, Szentpéteriová E, Solár P, Komel R, Debeljak N. Overexpression of the erythropoietin receptor in RAMA 37 breast cancer cells alters cell growth and sensitivity to tamoxifen. *Int J Oncol* 2017; **51**(2): 737-746.

[11]Miao S, Wang SM, Cheng X, Li YF, Zhang QS, Li G, et al. Erythropoietin promoted the proliferation of hepatocellular carcinoma through hypoxia induced translocation of its specific receptor. *Cancer Cell Int* 2017; **17**: 119.

[12]Sinclair AM, Rogers N, Busse L, Archibeque I, Brown W, Kassner PD, et al. Erythropoietin receptor transcription is neither elevated nor predictive of surface expression in human tumour cells. *Br J Cancer* 2008; **98**(6): 1059-1067.

[13]Swift S, Ellison AR, Kassner P, McCaffery I, Rossi J, Sinclair AM, et al. Absence of functional EpoR expression in human tumor cell lines. *Blood* 2010; **115**(21): 4254-4263.

[14]Küster O, Simon P, Mittelbronn M, Tabatabai G, Hermann C, Strik H, et al. Erythropoietin receptor is expressed in meningiomas and lower levels are associated with tumour recurrence. *Neuropathol Appl Neurobiol* 2009; **35**(6): 555-565.

[15]Frille A, Leithner K, Olschewski A, Olschewski H, Wohlkönig C, Hrzenjak A. No erythropoietin-induced growth is observed in non-small cell lung cancer cells. *Int J Oncol* 2018; **52**(2): 518-526.

[16]Elliott S, Swift S, Busse L, Scully S, Van G, Rossi J, et al. Epo receptors are not detectable in primary human tumor tissue samples. *PLoS One* 2013; **8**(7): e68083.

[17]Laugsch M, Metzen E, Svensson T, Depping R, Jelkmann W. Lack of functional erythropoietin receptors of cancer cell lines. *Int J Cancer* 2008; **122**(5): 1005-1011.

[18]Fandrey J. Erythropoietin receptors on tumor cells: what do they mean? *Oncologist* 2008; **13**(Suppl 3): 16-20.

[19]Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. *Nucleic Acids Res* 2017; **45**(W1): W98-W102.

[20]Jézéquel P, Campone M, Gouraud W, Guérin-Charbonnel C, Leux C, Ricolleau G, et al. bc-GenExMiner: an easy-to-use online platform for gene prognostic analyses in breast cancer. *Breast Cancer Res Treat* 2012; **131**(3): 765-775.

[21]Jézéquel P, Frénel JS, Campion L, Guérin-Charbonnel C, Gouraud W, Ricolleau G, et al. bc-GenExMiner 3.0: new mining module computes breast cancer gene expression correlation analyses. *Database (Oxford)* 2013; **2013**: bas060.

[22]van de Vijver MJ, He YD, van't Veer LJ, Dai H, Hart AA, Voskuil DW, et al. A gene-expression signature as a predictor of survival in breast cancer. *N Engl J Med* 2002; **347**(25): 1999-2009.

[23]Collins KAL, Stuhlmiller TJ, Zawistowski JS, East MP, Pham TT, Hall CR, et al. Proteomic analysis defines kinase taxonomies specific for subtypes of breast cancer. *Oncotarget* 2018; **9**(21): 15480-15497.

[24]Ma XJ, Wang Z, Ryan PD, Isakoff SJ, Barmettler A, Fuller A, et al. A two-gene expression ratio predicts clinical outcome in breast cancer

patients treated with tamoxifen. *Cancer Cell* 2004; **5**(6): 607-616.

[25]Minn AJ, Gupta GP, Siegel PM, Bos PD, Shu W, Giri DD, et al. Genes that mediate breast cancer metastasis to lung. *Nature* 2005; **436**(7050): 518-524.

[26]Pawitan Y, Bjöhle J, Amler L, Borg AL, Egyhazi S, Hall P, et al. Gene expression profiling spares early breast cancer patients from adjuvant therapy: derived and validated in two population-based cohorts. *Breast Cancer Res* 2005; **7**(6): R953-64.

[27]Wang Y, Klijn JG, Zhang Y, Sieuwerts AM, Look MP, Yang F, et al. Gene-expression profiles to predict distant metastasis of lymph-node-negative primary breast cancer. *Lancet* 2005; **365**(9460): 671-679.

[28]Weigelt B, Hu Z, He X, Livasy C, Carey LA, Ewend MG, et al. Molecular portraits and 70-gene prognosis signature are preserved throughout the metastatic process of breast cancer. *Cancer Res* 2005; **65**(20): 9155-9158.

[29]Bild AH, Yao G, Chang JT, Wang Q, Potti A, Chasse D, et al. Oncogenic pathway signatures in human cancers as a guide to targeted therapies. *Nature* 2006; **439**(7074): 353-357.

[30]Chin K, DeVries S, Fridlyand J, Spellman PT, Roydasgupta R, Kuo WL, et al. Genomic and transcriptional aberrations linked to breast cancer pathophysiologies. *Cancer Cell* 2006; **10**(6): 529-41.

[31]Ivshina AV, George J, Senko O, Mow B, Putti TC, Smeds J, et al. Genetic reclassification of histologic grade delineates new clinical subtypes of breast cancer. *Cancer Res* 2006; **66**(21): 10292-10301.

[32]Chin SF, Teschendorff AE, Marioni JC, Wang Y, Barbosa-Morais NL, Thorne NP, et al. High-resolution aCGH and expression profiling identifies a novel genomic subtype of ER negative breast cancer. *Genome Biol* 2007; **8**(10): R215.

[33]Desmedt C, Piette F, Loi S, Wang Y, Lallemand F, Haibe-Kains B, et al. Strong time dependence of the 76-gene prognostic signature for node-negative breast cancer patients in the TRANSBIG multicenter independent validation series. *Clin Cancer Res* 2007; **13**(11): 3207-3214.

[34]Loi S, Haibe-Kains B, Desmedt C, Lallemand F, Tutt AM, Gillet C, et al. Definition of clinically distinct molecular subtypes in estrogen receptor-positive breast carcinomas through genomic grade. *J Clin Oncol* 2007; **25**(10): 1239-1246.

[35]Minn AJ, Gupta GP, Padua D, Bos P, Nguyen DX, Nuyten D, et al. Lung metastasis genes couple breast tumor size and metastatic spread. *Proc Natl Acad Sci U S A* 2007; 104(16): 6740-6745.

[36]Zhou Y, Yau C, Gray JW, Chew K, Dairkee SH, Moore DH, et al. Enhanced NF kappa B and AP-1 transcriptional activity associated with antiestrogen resistant breast cancer. *BMC Cancer* 2007; **7**: 59.

[37]Anders CK, Acharya CR, Hsu DS, Broadwater G, Garman K, Foekens JA, et al. Age-specific differences in oncogenic pathway deregulation seen in human breast tumors. *PLoS One* 2008; **3**(1): e1373.

[38]Chanrion M, Negre V, Fontaine H, Salvetat N, Bibeau F, Mac Grogan G, et al. A gene expression signature that can predict the recurrence of tamoxifen-treated primary breast cancer. *Clin Cancer Res* 2008; **14**(6): 1744-1752.

[39]Loi S, Haibe-Kains B, Desmedt C, Wirapati P, Lallemand F, Tutt AM, et al. Predicting prognosis using molecular profiling in estrogen receptor-positive breast cancer treated with tamoxifen. *BMC Genomics* 2008; **9**: 239.

[40]Schmidt M, Böhm D, von Törne C, Steiner E, Puhl A, Pilch H, et al. The humoral immune system has a key prognostic impact in node-negative breast cancer. *Cancer Res* 2008; **68**(13): 5405-5413.

[41]Calabrò A, Beissbarth T, Kuner R, Stojanov M, Benner A, Asslaber M, et al. Effects of infiltrating lymphocytes and estrogen receptor on gene expression and prognosis in breast cancer. *Breast Cancer Res Treat* 2009; **116**(1): 69-77.

[42]Desmedt C, Giobbie-Hurder A, Neven P, Paridaens R, Christiaens MR, Smeets A, et al. The Gene expression Grade Index: a potential predictor of relapse for endocrine-treated breast cancer patients in the BIG 1-98 trial. *BMC Med Genomics* 2009; **2**: 40.

[43]Zhang Y, Sieuwerts AM, McGreevy M, Casey G, Cufer T, Paradiso A, et al. The 76-gene signature defines high-risk patients that benefit from adjuvant tamoxifen therapy. *Breast Cancer Res Treat* 2009; **116**(2): 303-309.

[44]Li Y, Zou L, Li Q, Haibe-Kains B, Tian R, Li Y, et al. Amplification of LAPTM4B and YWHAZ contributes to chemotherapy resistance and recurrence of breast cancer. *Nat Med* 2010; **16**(2): 214-218.

[45]Sircoulomb F, Bekhouche I, Finetti P, Adélaïde J, Ben Hamida A, Bonansea J, et al. Genome profiling of ERBB2-amplified breast cancers. *BMC Cancer* 2010; **10**: 539.

[46]Buffa FM, Camps C, Winchester L, Snell CE, Gee HE, Sheldon H, et al. microRNA-associated progression pathways and potential therapeutic targets identified by integrated mRNA and microRNA expression profiling in breast cancer. *Cancer Res* 2011; **71**(17): 5635-45.

[47]Dedeurwaerder S, Desmedt C, Calonne E, Singhal SK, Haibe-Kains B, Defrance M, et al. DNA methylation profiling reveals a predominant immune component in breast cancers. *EMBO Mol Med* 2011; **3**(12): 726-741.

[48]Filipits M, Rudas M, Jakesz R, Dubsky P, Fitzal F, Singer CF, et al. A new molecular predictor of distant recurrence in ER-positive, HER2-negative breast cancer adds independent information to conventional clinical risk factors. *Clin Cancer Res* 2011; **17**(18): 6012-20.

[49]Hatzis C, Pusztai L, Valero V, Booser DJ, Esserman L, Lluch A, et al. A genomic predictor of response and survival following taxane-anthracycline chemotherapy for invasive breast cancer. *JAMA* 2011; **305**(18): 1873-1881.

[50]Kao KJ, Chang KM, Hsu HC, Huang AT. Correlation of microarray-based breast cancer molecular subtypes and clinical outcomes: implications for treatment optimization. *BMC Cancer* 2011; **11**: 143.

[51]Sabatier R, Finetti P, Cervera N, Lambaudie E, Esterni B, Mamessier E, et al. A gene expression signature identifies two prognostic subgroups of basal breast cancer. *Breast Cancer Res Treat* 2011; **126**(2): 407-420.

[52]Wang DY, Done SJ, McCready DR, Boerner S, Kulkarni S, Leong WL. A new gene expression signature, the ClinicoMolecular Triad Classification, may improve prediction and prognostication of breast cancer at the time of diagnosis. *Breast Cancer Res* 2011; **13**(5): R92.

[53]Kuo WH, Chang YY, Lai LC, Tsai MH, Hsiao CK, Chang KJ, et al. Molecular characteristics and metastasis predictor genes of triple-negative breast cancer: a clinical study of triple-negative breast carcinomas. *PLoS One* 2012; **7**(9): e45831.

[54]Nagalla S, Chou JW, Willingham MC, Ruiz J, Vaughn JP, Dubey P, et al. Interactions between immunity, proliferation and molecular subtype in breast cancer prognosis. *Genome Biol* 2013; **14**(4): R34.

[55]Rizzo JD, Lichtin AE, Woolf SH, Seidenfeld J, Bennett CL, Cella D, et al. Use of epoetin in patients with cancer: evidence-based clinical practice guidelines of the American Society of Clinical Oncology and the American Society of Hematology. *Blood* 2002; **100**(7): 2303-2320.

[56]Glaspy JA. Cancer patient survival and erythropoietin. *J Natl Compr Canc Netw* 2005; **3**(6): 796-804.

[57]Varlotto J, Stevenson MA. Anemia, tumor hypoxemia, and the cancer patient. *Int J Radiat Oncol Biol Phys* 2005; **63**(1): 25-36.

[58]Hardee ME, Arcasoy MO, Blackwell KL, Kirkpatrick JP, Dewhirst MW. Erythropoietin biology in cancer. *Clin Cancer Res* 2006; **12**(2): 332-339.

[59]Alobaedi OH, Talib WH, Basheti IA. Antitumor effect of thymoquinone combined with resveratrol on mice transplanted with breast cancer. *Asian Pac J Trop Med* 2017; **10**(4): 378-386.

[60]Zhou YF, Sun Q, Zhang YJ, Wang GM, He B, Qi T, et al. Targeted inhibition of Notch1 gene enhances the killing effects of paclitaxel on triple negative breast cancer cells. *Asian Pac J Trop Med* 2017; **10**(2): 172-176.

[61]Nicolini A, Ferrari P, Duffy MJ. Prognostic and predictive biomarkers in breast cancer: Past, present and future. *Semin Cancer Biol* 2017; doi: 10.1016/j.semcancer.2017.08.010

[62]Fragomeni SM, Sciallis A, Jeruss JS. Molecular subtypes and local-regional control of breast cancer. *Surg Oncol Clin N Am* 2018; **27**(1): 95-120.