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Association of mRNA expression levels of Cullin family members with prognosis in breast cancer

An online database analysis

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Abstract

Cullin proteins couple with RING-finger proteins, adaptor proteins and substrate recognition receptors to form E3 ubiquitin ligases for recognizing numerous substrates and participating in a variety of cellular processes, especially in genome stability and tumorigenesis. However, the prognostic values of Cullins in breast cancer remain elusive.

A "Kaplan–Meier plotter" (KM plotter) online survival analysis tool was used to evaluate the association of individual Cullin members' mRNA expression with overall survival (OS) in breast cancer patients.

Our results revealed that elevated mRNA expression of CUL4A and PARC were significantly associated with poor OS for breast cancer patients. While high mRNA expression of CUL2, CUL4B, and CUL5 were correlated with better survival for breast cancers.

The associated results suggested that some Cullin members could serve as new predictive prognostic indicators for breast cancer.

Abbreviations: CI = confidence interval, CRL = Cullin-Ring ubiquitin ligase, HR = hazard ratio, OR = odds ratio, OS = overall survival, PARC = p53-associated parkin-like cytoplasmic protein, SCF = SKP1/Cullin1/F-box.

Keywords: breast cancer, Cullins, KM plotter, prognostic value

1. Introduction

Breast cancer is a lethal disease that results in the second leading cause of cancer death in females worldwide.[1] The incidence of breast cancer continues to rise, while its mortality is decreasing with the advances achieved in screening and treatment modalities.[2] With earlier diagnoses of lower stage tumors, the treatment for breast cancer can entail moderate surgical procedure rather than the more aggressive one. Therefore, establishment of an early novel prognostic or therapeutic marker for breast cancer is the urgent requirement to improve clinical outcomes for breast cancer patients.

Ubiquitin-proteasome system is a major pathway controlling protein degradation.[3] There are 2 discrete steps involved in protein ubiquitination-mediated degradation: first, ubiquitins are catalyzed and transferred to the given substrates by the sequential actions of the activating (E1), conjugating (E2), and ligase (E3) enzymes. Then, the poly-ubiquitinated substrates are recognized and subsequently degraded by the 26S proteasome complex.[4] The Cullin-Ring ubiquitin ligase (CRL) is the largest family of E3 ubiquitin ligases, which executes the ubiquitination of about 20% of intracellular proteins.[5] Structurally, Cullin protein binds to an adaptor protein at the N-terminus serving as a scaffold, and interacts with a RING protein (RBX1 and RBX2) at the C-terminus, to form a CRL.[6] CRL activity is regulated by the interplay between several regulatory proteins, such as neural-precursor-cell-expressed developmentally down-regulated 8 (NEDD8), Cullin-associated NEDD8-dissociated protein 1 (CAND1), and COP9 (constitutive photomorphogenesis 9) signalosome complex (CSN). These proteins modulate the association/dissociation cycles of CRL subunits and thus altering the activity of CRLs.[7]

In mammals, there are 8 Cullin proteins that have been identified, including CUL1, CUL2, CUL3, CUL4A, CUL4B, CUL5, CUL7, and CUL9/the closely related p53-associated parkin-like cytoplasmic protein (PARC).[8–10] Several proto-oncoproteins and tumor suppressors are meditated by CRL-degradation pathway.[11] Abnormal expression of Cullins causes dysregulation of several cancer-related proteins, which may lead to tumorigenesis. Previous studies have reported that several Cullin members were overexpressed in breast cancers and their high expression were significantly associated with worse histology grade and poor survival.[12–14] However, some

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members of Cullins are rarely investigated in breast cancers, their prognostic values in breast cancer were still elusive.[15,16] Therefore, it is highly interesting to systemically investigate the prognostic roles of each individual Cullins for breast cancer patients.

An online KM plotter database, which is generated by using gene expression data and survival information downloaded from GEO (http://www.ncbi.nlm.nih.gov/geo/), has been widely used for prognostic analysis in various kinds of cancers.[17–19] This online survival analysis tool is used to assess the relevance of the expression levels of various genes on the clinical outcome both in untreated and treated breast cancer patients. The background database is established using gene expression data and survival information of 1809 patients downloaded from GEO (Affymetrix HGU133A and HGU133+2 microarrays).[17] Here, we used this online analysis tool and assessed the prognostic roles of each Cullin members in human breast cancer patients.

2. Materials and methods

The online KM plotter database was established using gene microarray data and survival information of breast cancer patients downloaded from GEO, which including 1809 breast cancer patients, 1610 with distant metastasis free survival (DMFS) data and 1402 with overall survival (OS) data.[17] We put individual members of Cullins family into this online database (http://kmplot.com/analysis/index.php?p=service&cancer=breast) respectively and analyzed with the setting clinical parameters (ER, PR, HER2 status, lymph node status, differentiation grade, intrinsic subtype, and TP53 status). Then, Kaplan–Meier survival plots and hazard ratio (HR) with 95% confidence intervals (CI) were obtained on the webpage. P value of $<.05$ was represented as statistically significant. False discovery rate (FDR) approach was used to correct for multiple testing as previously described.[20] Suppose that there is a total of m P values. The original P value was arranged from small to large: $P(1), P(2), \ldots, P(m)$, FDP $P(i) = (P(i) \times m)/i$. In brief, the FDR P value was considered statistically significant only if it was less than .05 for FDR. UALCAN, an easy to use, interactive web-portal to perform the in-depth analyses of TCGA gene expression data, was used to validate the results. Given the results generated in this study were based on an online database analysis, ethical approval was not necessary, also no need to give patient consent.

3. Results

3.1. Prognostic roles of Cullin members in all breast cancer patients

Eight members of Cullins were polled in the online KM plotter database respectively, and the prognostic results of each individual member were showed in Figure 1A. Amongst 8 members, elevated expression of CUL4A (HR = 1.27 95%CI: 1.02–1.57, $P = .03$, Fig. 1B) and PARC (HR = 1.42 95%CI: 1.14–1.76, $P < .001$, Fig. 1C) were significantly associated with worse OS in all breast cancer patients. While high mRNA expression of CUL2 (HR = 0.83 95%CI: 0.73–0.94, $P < .01$, Fig. 1D), CUL4B (HR = 0.77 95%CI: 0.61–0.96, $P = .022$, Fig. 1E) and CUL5 (HR = 0.74 95%CI: 0.59–0.91, $P < .01$, Fig. 1F) were correlated with better survival. Whereas the mRNA expression levels of

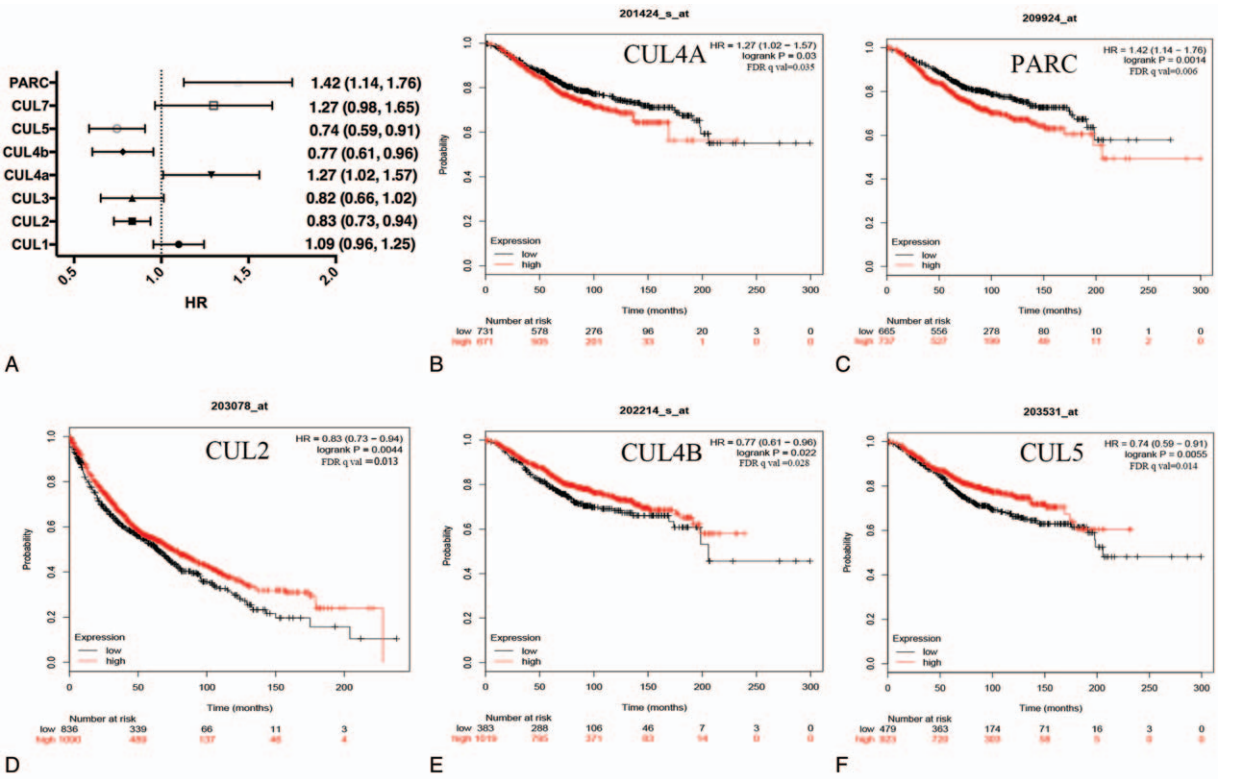

Figure 1. Prognostic values of Cullins for all breast cancers. The prognostic hazard ratios (HRs) value of individual Cullin members in all breast cancers (A). The survival curves of CUL4A (B), PARC (C), CULE (D), CUL4B (E) and CUL5 (F) were plotted for all breast cancers.

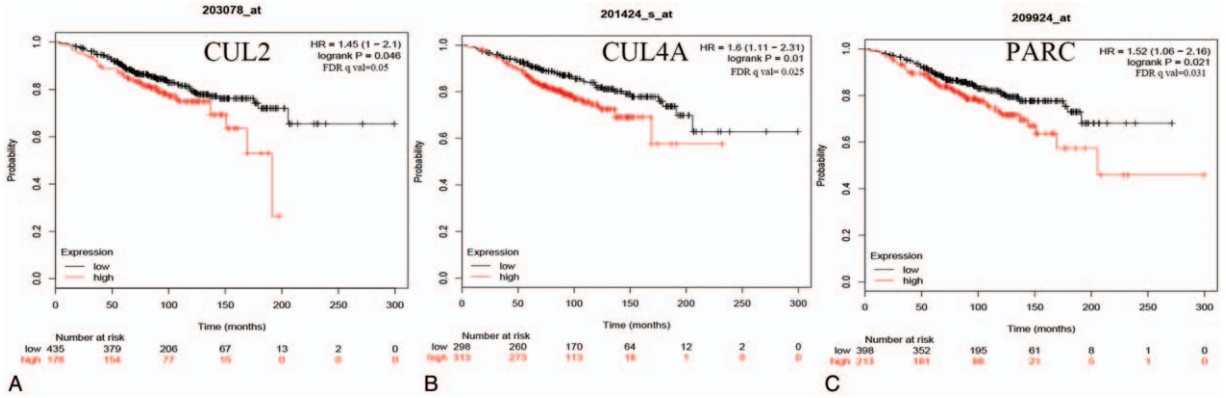

Figure 2. Prognostic roles of Cullins for luminal A type breast cancers. The overall survival (OS) curves of CUL2 (A), CUL4A (B), and PARC (C) were plotted in luminal A type breast cancer patients.

CUL1, CUL3, and CUL7 were not related to prognosis in all breast cancers (Fig. S1, http://links.lww.com/MD/D145). In order to exclude the possibility that the significant results were obtained circumstantially. UALCAN, an easy to use, interactive web-portal to perform the in-depth analyses of TCGA gene expression data, was used for validation. As expected, the results based on TCGA dataset confirmed that elevated CUL4A and PARC mRNA levels were associated with poor survival for all breast cancer patients. And high mRNA level of CUL5 were correlated with favorable prognosis, although high expression of CUL2 and CUL4B showed an moderate trend in associated with better survival for breast cancers (Fig. S2, http://links.lww.com/MD/D145).

3.2. The prognostic significance of mRNA expression of Cullins in different breast cancer subtypes

According to different molecular expression patterns, breast cancer is classified into 4 major molecular subtypes: luminal A and B, HER2-like and basal-like breast cancer. We then examined the prognostic effects of Cullins mRNA expression in these 4 different subtypes. In luminal A type breast cancers, high mRNA expression of CUL2 (HR = 1.45 95%CI: 1.00–2.10, $P = .046$), CUL4A (HR = 1.60 95%CI: 1.11–2.31, $P = .01$) and PRAC (HR = 1.52 95%CI: 1.06–2.16, $P = .021$) were associated with worse OS (Fig. 2A–C). While the other Cullins showed no correlation with OS (Fig. S3, http://links.lww.com/MD/D145).

In regarding to luminal B type breast cancers, only CUL7 (HR = 2.01 95%CI: 1.23–3.27, $P < .01$, Fig. 3A) was significantly associated with poor prognosis. The high mRNA levels of CUL4A (HR = 0.67 95%CI: 0.46–0.98, $P = .037$, Fig. 3B) and CUL5 (HR = 0.62 95%CI: 0.43–0.91, $P = .013$, Fig. 3C) were related to better OS. Whereas other Cullins did not relate to survival in luminal B type breast cancers (Fig. S4, http://links.lww.com/MD/D145).

In HER2-overexpressing type breast cancers, elevated mRNA expression of CUL2 was associated with poor survival, the HR was 2.07 (95%CI: 1.04–4.13 $P = .034$, Fig. 4A). While CUL1 (HR = 0.46 95%CI: 0.24–0.88, $P = .017$, Fig. 4B), CUL4B (HR = 0.31 95%CI: 0.11–0.89, $P = .021$, Fig. 4C) and PARC (HR = 0.32 95% CI: 0.11–0.90, $P = .023$, Fig. 4D) were significantly correlated with favorable OS. The rest of Cullins were not associated with survival in HER2-overexpressing type breast cancers according to the current analysis (Fig. S5, http://links.lww.com/MD/D145).

The basal-like type breast cancer is characterized by worst pathological features with worst prognosis. We found that only increased CUL2 mRNA expression (HR = 1.93 95%CI: 1.14–3.30, $P = .013$, Fig. 5A) was highly associated with

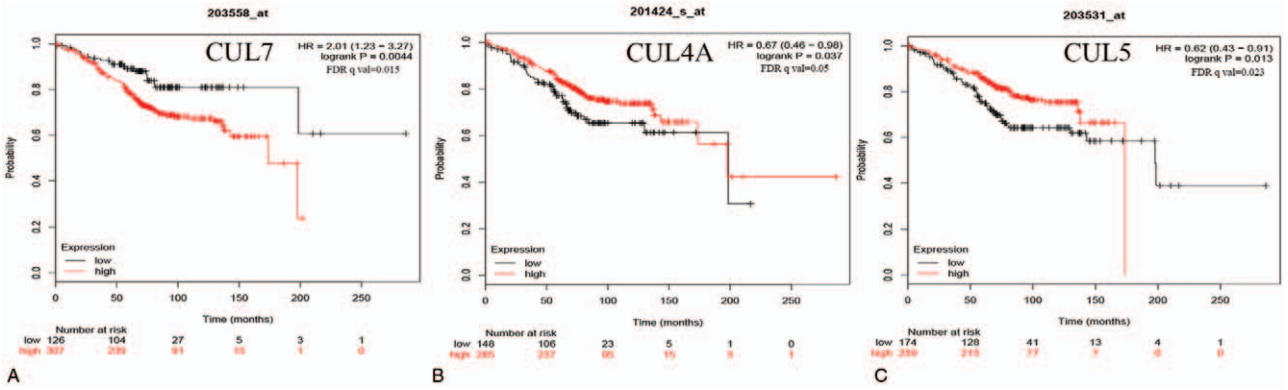

Figure 3. Prognostic significances of Cullins for luminal B type breast cancers. Survival curves of CUL7 (A), CUL4A (B), and CUL5 (C) were plotted for luminal B type breast cancer patients.

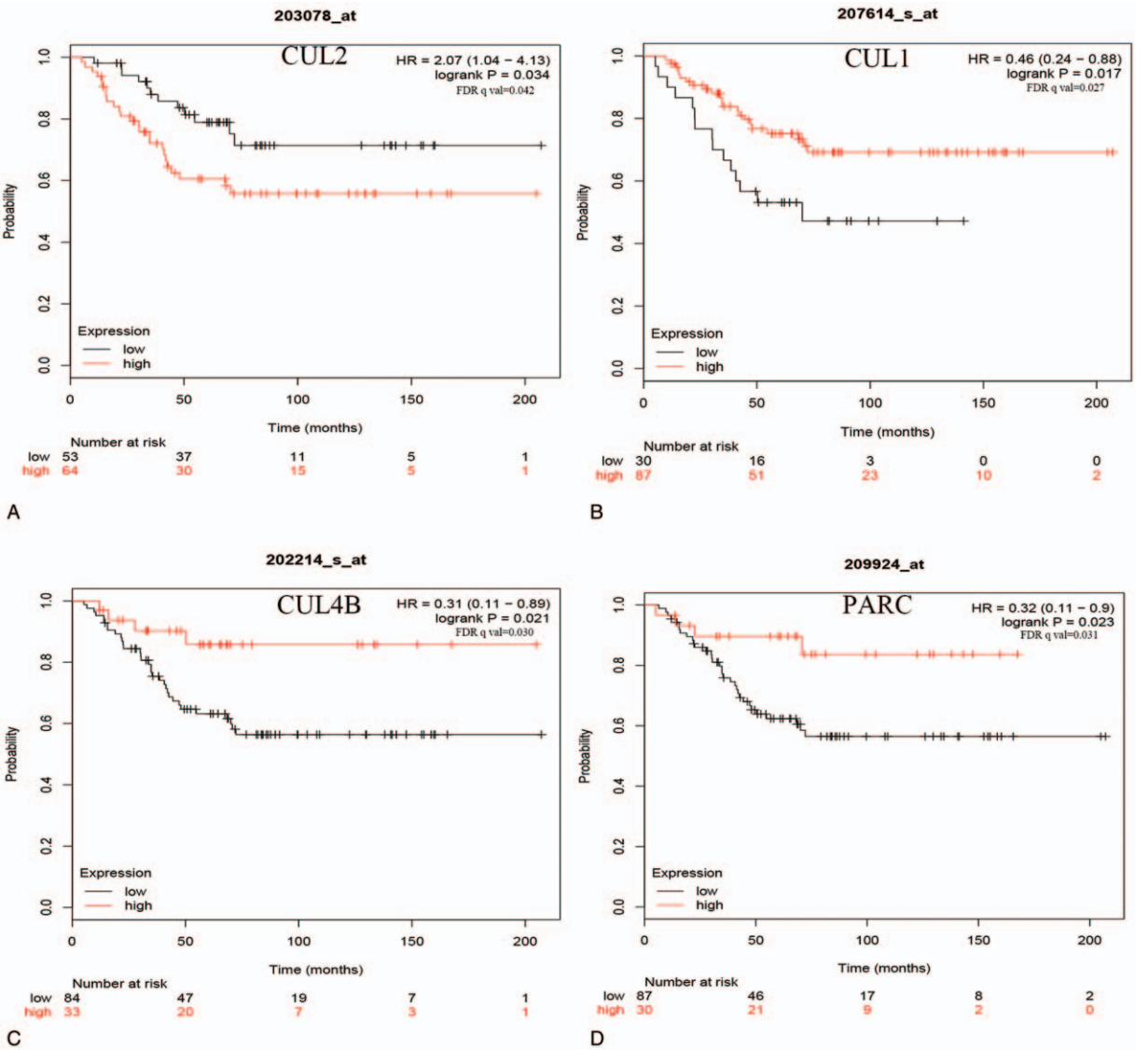

Figure 4. Prognostic values of Cullins for HER2-overexpressing type breast cancers. Survival curves of the overall survival (OS) curves of CUL2 (A), CUL1 (B), CUL4B (C), and PARC (D) were plotted for HER2-overexpressing type breast cancer patients.

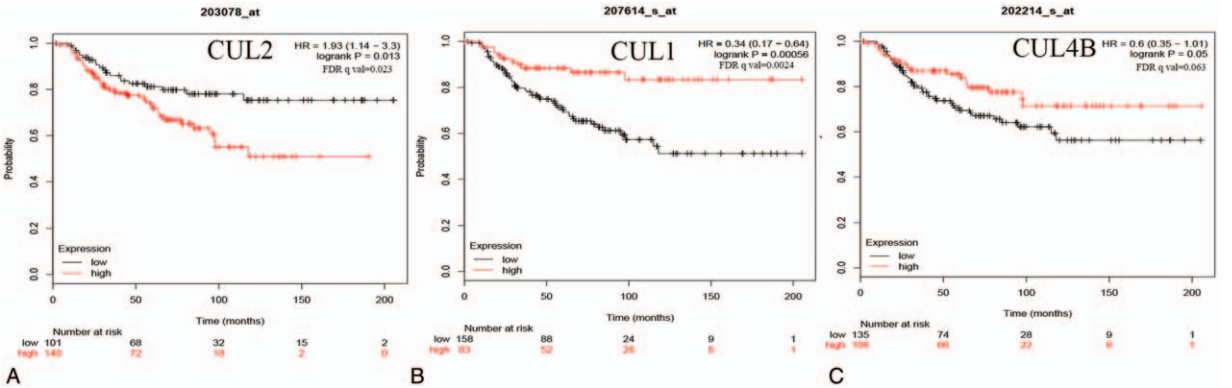

Figure 5. Prognostic values of Cullins for basal-like type breast cancers. Survival curves of the overall survival (OS) curves of CUL2 (A), CUL2 (1), and CUL4B (C) were plotted for basal-like type breast cancer patients.

unfavorable prognosis in basal-like breast cancers. The higher expression of CUL1 (HR = 0.34 95%CI: 0.17–0.64, $P < .001$, Figure 5B) and CUL4B (HR = 0.60 95%CI: 0.35–0.01, $P < .05$, Fig. 5C) mRNA level was associated with better OS. The other Cullins members did not show any correlation with OS in basal-like type breast cancers (Fig. S6, http://links.lww.com/MD/D145).

3.3. The relationship between Cullins and prognosis in breast cancers with different clinicopathological parameters

Breast cancer is a heterogeneous disease presented by different phenotypes. The prognostic values of Cullins were distinct in breast cancer with different clinicopathological features. We observed that only higher CUL2 mRNA level was correlated to prognosis in grade I breast cancer patients, the HR was 0.28 (95%CI: 0.11–0.74, $P < .001$). In grade II breast cancer patients, elevated mRNA expression of CUL2 did not relate to prognosis, but higher PARC mRNA level was associated with poor prognosis (HR = 1.91 95%CI: 1.24–2.94, $P = .013$), and CUL5 was associated with better prognosis (HR = 0.48 95%CI: 0.31–0.75, $P < .001$). While in grade III patients, higher mRNA expression of CUL2 (HR = 1.54 95%CI: 1.11–2.15, $P = .010$), CUL4A (HR = 1.57 95%CI: 1.12–2.18, $P < .001$), CUL5 (HR = 1.51 95%CI: 1.01–2.24, $P = .041$) and CUL7 (HR = 1.82 95% CI: 1.30–2.56, $P < .001$) were associated with worse OS. Increased CUL1 (HR = 0.61 95%CI: 0.44–0.86, $P < .001$) and PARC (HR = 0.53 95%CI: 0.35–0.80, $P < .001$) mRNA were apparently associated with better OS in grade III patients (Table 1).

Table 1

The association between the Cullin members and the prognosis of breast cancer with different grade status.

Culling family	Affymetrix IDs	Grade	HR 95%CI	*P* value
CUL1	207614_s_at	Grade1	1.76 (0.67, 4.63)	.250
		Grade2	0.70 (0.45, 1.08)	.110
		Grade3	0.61 (0.44, 0.86)	.004*
CUL2	203078_at	Grade1	0.28 (0.11, 0.74)	.005*
		Grade2	0.75 (0.48, 1.17)	.200
		Grade3	1.54 (1.11, 2.15)	.010*
CUL3	201372_s_at	Grade1	0.42 (0.15, 1.16)	.083
		Grade2	0.67 (0.42, 1.06)	.084
		Grade3	1.29 (0.93, 1.79)	.120
CUL4A	201424_s_at	Grade1	1.74 (0.70, 4.31)	.230
		Grade2	0.70 (0.45, 1.10)	.120
		Grade3	1.57 (1.12, 2.18)	.007*
CUL4B	202214_s_at	Grade1	0.46 (0.18, 1.14)	.086
		Grade2	0.65 (0.41, 1.02)	.057
		Grade3	0.75 (0.54, 1.04)	.084
CUL5	203531_at	Grade1	0.51 (0.20, 1.29)	.150
		Grade2	0.48 (0.31, 0.75)	.001*
		Grade3	1.51 (1.01, 2.24)	.041*
CUL7	203558_at	Grade1	2.18 (0.85, 5.54)	.095
		Grade2	1.38 (0.79, 2.42)	.250
		Grade3	1.82 (1.30, 2.56)	.000*
PARC	209924_at	Grade1	1.56 (0.61, 3.97)	.350
		Grade2	1.91 (1.24, 2.94)	.0026*
		Grade3	0.53 (0.35, 0.80)	.0025*

CI = confidence interval, HR = hazard ratio.
* $P < .05$.

Table 2

The association between the Cullin members and the prognosis of breast cancer with different lymph node status.

Culling family	Affymetrix IDs	Lymph node	HR 95%CI	*P* value
CUL1	207614_s_at	Positive	0.58 (0.36, 0.92)	.020*
		Negative	0.74 (0.51, 1.07)	.110
CUL2	203078_at	Positive	0.64 (0.42, 0.98)	.037*
		Negative	1.31 (0.91, 1.90)	.150
CUL3	201372_s_at	Positive	0.81 (0.55, 1.21)	.310
		Negative	1.07 (0.74, 1.55)	.730
CUL4A	201424_s_at	Positive	0.76 (0.51, 1.12)	.160
		Negative	0.86 (0.59, 1.25)	.430
CUL4B	202214_s_at	Positive	0.61 (0.41, 0.90)	.013*
		Negative	0.70 (0.48, 1.02)	.065
CUL5	203531_at	Positive	0.54 (0.36, 0.81)	.003*
		Negative	0.91 (0.63, 1.33)	.630
CUL7	203558_at	Positive	1.66 (1.10, 2.50)	.015*
		Negative	1.18 (0.81, 1.71)	.380
PARC	209924_at	Positive	0.79 (0.53, 1.18)	.240
		Negative	1.21 (0.84, 1.75)	.310

CI = confidence interval, HR = hazard ratio.
* $P < .05$.

As shown in Table 2, none of the Cullin members were associated with OS in lymph node negative breast cancer patients. Whereas in lymph node positive breast cancer patients, increased mRNA expression of CUL7 was correlated with worse survival (HR = 1.66 95%CI: 1.10–2.50, $P < .015$), and increased expression of CUL1, CUL2, CUL4B, CUL5, and PARC were significantly correlated with favorable OS.

P53 is a tumor suppressor protein which is widely muted in human cancers.[21] High mRNA expression of CUL1 (HR = 0.27 95%CI: 0.10–0.72, $P < .001$) and CUL4A (HR = 0.25 95%CI: 0.06–1.06, $P = .042$) were associated with better prognosis in mutant-p53-type breast cancer. While in wild-p53-type breast cancer patients, CUL4A (HR = 0.34 95%CI: 0.12–0.96, $P = .032$) and CUL5 (HR = 0.40 95%CI: 0.21–0.78, $P < .001$) were found to be correlated to better survival. Whereas PARC (HR = 2.5 95%CI: 1.30–4.78, $P < .001$) was significantly associated with poor survival in wild-p53-type breast cancers (Table 3).

4. Discussion

Cullin proteins are implicated in tumorigenesis via degradation of numerous tumor suppressor proteins or oncoproteins.[22] In the current study, by using an online survival analysis tool, we comprehensively analyzed the prognostic significances of individual Cullins mRNA expression in breast cancer and suggested that high mRNA expression of CUL4A and PARC were significantly associated with poor OS in all breast cancer patients. Whereas high levels of CUL2, CUL4B, and CUL5 were correlated with better survival of breast cancers.

CUL1 was the first identified scaffold protein that constitutes a heterotrimeric complex of SKP1/Cullin1/F-box (SCF).[23] Knockout of CUL1 in mice resulted in embryonic lethality because of the failure of cyclin E turnover.[24] CUL1 protein overexpression has been proved to be correlated with poor survival in breast cancer.[13,25] However, we found that CUL1 mRNA expression did not relate to prognosis for all breast cancer patients. Previous results showed that CUL1 staining was significantly increased in

Table 3

The association between the Cullin members and the prognosis of breast cancer with different p53 status.

Culling family	Affymetrix IDs	p53	HR 95%CI	*P* value
CUL1	207614_s_at	Mutant	0.27 (0.10, 0.72)	.005*
		Wild	0.48 (0.20, 1.16)	.095
CUL2	203078_at	Mutant	1.56 (0.73, 3.36)	.250
		Wild	1.36 (0.70, 2.64)	.360
CUL3	201372_s_at	Mutant	2.57 (0.77, 8.54)	.110
		Wild	0.62 (0.32, 1.21)	.160
CUL4A	201424_s_at	Mutant	0.25 (0.06, 1.06)	.042*
		Wild	0.34 (0.12, 0.96)	.032*
CUL4B	202214_s_at	Mutant	0.57 (0.26, 1.28)	.170
		Wild	0.64 (0.32, 1.28)	.200
CUL5	203531_at	Mutant	0.6 (0.28, 1.28)	.180
		Wild	0.4 (0.21, 0.78)	.005*
CUL7	203558_at	Mutant	0.55 (0.25, 1.17)	.110
		Wild	1.83 (0.96, 3.49)	.064
PARC	209924_at	Mutant	0.33 (0.10, 1.09)	.055
		Wild	2.5 (1.30, 4.78)	.004*

CI = confidence interval, HR = hazard ratio.
* $P < .05$.

breast cancers with histology grade III, negative ER, negative PR, and positive HER2.[13] We here observed that higher mRNA expression of CUL1 was significantly associated with better OS in grade III, HER2-overexpressing, lymph node positive and basal-like breast cancer patients. Interestingly, CUL1 protein expression was associated with p53 expression in breast cancers.[25,26] Our study showed that elevated CUL1 mRNA expression was related to favorable OS in mutant-p53-type breast cancer.

CUL2 interacts with elongins B and C and a RING finger protein Rbx1 to form a SCF-like E3 ubiquitin ligase VHL.[27] Previous studies indicated that higher CUL2 mRNA expression was associated with therapy response and prolonged survival in esophageal carcinoma.[28] Whereas CUL2 overexpression in cervical cells was likely to accelerate HPV16-induced cervical carcinogenesis.[29] The role of CUL2 implicated in breast cancer development is still elusive. Here, we showed that higher CUL2 mRNA expression level was correlated with better survival of breast cancer.

The constitutive deletion of CUL3 caused embryonic lethality by the dysregulation of cyclin E degradation.[30] Furthermore, conditional CUL3 deletion in various organs lead to cyclin E accumulation and induced renal fibrosis and functional maintenance of hepatic progenitors.[24,31] The expression of CUL3 in breast cancer was significantly associated with tumor stage.[14] However, our results showed that there was no relation between the mRNA level of CUL3 and the prognosis of breast cancer.

CUL4 family has 2 members, including CUL4A and CUL4B, they share 83% sequence identity.[32] CUL4A-deleted male mice exhibited severe deficiencies in spermatogenesis and caused infertility, but CUL4A was dispensable for embryonic development.[33] CUL4A is regarded as an oncogene, as several well-defined tumor suppressor genes, such as p53, p21, and p27, are regulated by CUL4A-mediate ubiquitination and degradation.[34–36] Accumulating of evidences have suggested that CUL4A was highly expressed in breast cancers comparing to normal tissues, overexpression of CUL4A in breast cancer cells induced EMT process in vitro and promoted metastasis in vivo.[37] Furthermore, CUL4A overexpression was significantly related to tumor aggressiveness and poor prognosis in breast cancers.[38,39] Consistently, our results indicated that higher mRNA level of CUL4A was associated with worse survival in breast cancers. Unlike the counterpart CUL4A, CUL4B was critical for early embryogenesis. Constitutive deletion of CUL4B resulted in embryonic lethality.[40] The critical roles of CUL4B in cell proliferation, DNA damage and repair, cell invasion and metastasis and signaling pathways have been widely investigated.[41] CUL4B was supposed to extensively expressed in a variety of human cancers.[42–44] However, the prognostic significance of CUL4A in breast cancer is never know. In the current study, we suggested that elevated CUL4B mRNA level was indicated a favorable OS in breast cancer, especially in HER2-overexpresing and lymph node positive breast cancers.

CUL5 is the least conserved member of Cullin family, it was recently discovered as a Cullin protein that could bind to TRAF6 and promote TRAF6 polyubiquitination to repress inflammatory response following LPS (lipopolysaccharide) stimulation.[45] In breast cancer cells, CUL5 overexpression lead to cell growth inhibition.[46] A significantly ~2.2 fold decreased expression of CUL5 mRNA was observed in breast tumor tissues comparing to normal tissues.[15] However, the prognostic value of CUL5 in breast cancer is still unclear. According to the results analyzed by KM plotter, we found that higher mRNA expression level of CUL5 was significantly associated with favorable survival in breast cancer. While its elevated level in grade III breast cancer was indicated a poor survival, which suggested that CUL5 might implicated in the process of tumor pathological differentiation.

CUL7 binds to SKP1 and F-box to form an SCF-like complex. Knockout of CUL7 caused early embryonic death.[47] In breast cancer cells, silencing of CUL7 significantly inhibited cell growth and invasion. Moreover, high CUL7 protein expression in breast cancer was associated with poor clinical characteristics and worse outcomes.[12,48] In the current study, we found that higher mRNA expression of CUL7 showed an trend in association with poor prognosis in all breast cancer patients, although it did not reach a significant statistic difference (HR = 1.65 95%CI: 0.98–1.27, $P = .072$). Furthermore, elevated level of CUL7 mRNA predicted worse OS in poor differentiation and lymph node positive breast cancers, which suggested that CUL7 might contribute to tumor pathogenesis and metastasis.[49]

PARC has significant sequence similarity with CUL7 and both contain a CPH domain and a DOC domain.[50] The structures and functions of PARC are rarely reported.[10] PARC is indispensable for embryonic development, as its knockout mice exhibited no apparent phenotype.[51] To our knowledge, there was no study evaluated the prognostic role of PARC either at mRNA or protein levels in breast cancer. Our results based on online database revealed that overexpression of PARC mRNA was significantly correlated to poor OS in breast cancer.

TP53, as an important tumor suppressor gene, is mutated in over 50% of human malignancies.[52] It plays an important role in regulation of DNA repair, cell cycle and apoptosis, thereby playing an essential role in maintaining genetic stability.[53] Mutations of TP53, always resulting in bear ubiquitination, are suggested to be implicated in the pathogenesis of human cancers.[54,55] CUL4A was significantly correlated with TP53 expression in colorectal cancers.[56] Here, we showed that CUL4A was associated with better prognosis independent of mutant-p53-type breast cancer. PARC was identified as a p53 activator, it regulated cell proliferation and maintaining genomic integrity through p53.[26] On the other hand, PARC was capable

of inactivate of p53 in the cytoplasm via interaction with the C terminus of p53.[57] Our results showed that PARC mRNA expression was significantly associated with poor OS in p53-wild-type, but not in p53-mutute-type breast cancer.

However, we must note the study has a number of limitations. First of all, the results presented here were based on the analysis using of the median (or upper/lower quartile) sample to classify the samples into higher and lower expression groups. If we could determinate an exact cutoff value for each transcript, that should improve the quality of results. In some cases, there exists contradictory results regarding the significances of Cullins between at mRNA levels and at protein levels, which might be caused by post-translational modification of Cullins, such as ubiquitination. As such, this might be a confounder of the results we obtained. Therefore, it is highly needed to confirm the results by independent methods like RT–PCR or immunohistochemistry. Besides, the information of patients involved in this online database is partial, such as the clinical stage of the patients or whether a patient had received adjuvant therapy or not is unclear. Thus, further studies are needed for the sake of getting more detail understanding of functional characterization of each Cullin members and determining whether they can be potential prognostic targets of breast cancer.

Taken together, our study revealed that 5 members of Cullins were significantly associated with survival and could be served as potential prognostic biomarkers for breast cancers. The prognostic values of some Cullins are completely opposite in different types of breast cancers, suggesting that Cullins may interact with diverse signaling pathways and exert distinct functions in the process of different types of breast cancer development. Overall, our study provides a novel insight regarding the characteristics of Cullins in contributing to breast cancer progression. Further studies are required in order to get more specific understanding of functional characterization of each Cullin members and determining whether some of them can be used as potential treatment targets for breast cancer.

Author contributions

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