ISSN: 1813-4890

Pan-cancer Analysis of the Role of RCE1

Huamin Sun[a], Qing Li[b]

School of Jinan, Jinan University, Guangzhou 510000, China

[a]sunhuamin9608@163.com, [b]scum0109@163.com

Abstract

Ras converting enzyme 1 (RCE1) is the glutamate intramembrane protease that is localized to the endoplasmic reticulum. RCE1 plays a crucial role in the cancerogenesis and development of various malignant tumors. However, a systematic assessment of the RCE1 across human cancers is lacking. We comprehensively analyzed the expression to assess the prognostic value and clinical significance of RCE1 in 33 cancer types based on TCGA databases. RCE1 was highly expressed in most cancers and correlates with pathological stage, and patient survival. RCE1 expression was negatively associated with CD8+ T-cell infiltration in several tumors, such as HNSC, LUSC, SKCM, and TGCT, while RCE1 expression was positively associated with cancer-associated fibroblast infiltration in ACC, HNSC, GBM, LIHC, THCA, UCEC and UVM. Furthermore, RCE1 expression was linked to several tumor biological processes such as inflammation, cell differentiation, cell repair, and tumor angiogenesis. KEGG and GO suggested that RCE1 may influence the occurrence and progression of certain tumors by interacting with other proteins such as the COG family and influencing biological processes such as cell proliferation, cell senescence, and apoptosis. Our study reveals that RCE1 may be a prognostic predictor, cancer biomarker and regulator in some tumors.

Keywords

Pan-cancer; RCE1; Prognosis.

1. Introduction

Worldwide, cancer has seriously jeopardized public health, and the incidence and mortality of cancer are rapidly increasing every year[1]. And it imposes a major health and economic burden on society[2]. Therefore, there is an urgent need to search for new methods to diagnose and treat cancer. Currently, the application of cancer biomarkers has promoting researchers to explore novel cancer biomarkers[3-5]. With the continuous development and improvement of public databases such as The Cancer Genome Atlas (TCGA)[6], it is possible to discover novel potential clinical biomarkers or new targets by performing Pan-cancer expression analysis of genes and evaluating their correlations with clinical prognosis and signaling pathways.

Ras converting enzyme 1 (*RCE1*) is an integral membrane endoprotease localized to the endoplasmic reticulum that mediates the cleavage of the carboxyl-terminal three amino acids from CAAX proteins[7-9]. Research works have shown that Rce1 plays a crucial role in the cancerogenesis and development of various malignant tumors, such as renal cell carcinoma[10], prostate cancer[11], and colorectal cancer[12]. RCE1 promoted tumorigenesis in a variety of ways, including maintaining proteostasis, reprogramming metabolism, facilitating cancer cell proliferation and migration, repairing the genome, preventing cell death, and altering the TME[11, 12]. However, the expression level and clinical significance of RCE1 in most types of cancer remain elusive. Therefore, it is crucial to deeply examine the expression and molecular mechanisms of RCE1 in a Pan-cancer dataset to provide new directions and strategies for the clinical treatment of cancer.

ISSN: 1813-4890

In this study, we represented a Pan-cancer analysis of RCE1 genes across 33 cancers using the Cancer Genome Atlas (TCGA) databases.

2. Organization of the Text

2.1. Expression Analysis of RCE1 Gene in Pan-cancer

The dysregulation of the *RCE1* expression between various types of cancer was investigated by combining the data from TCGA. RNA sequencing data and clinical follow-up information for patients with 33 cancer types including adrenocortical carcinoma(ACC), bladder urothelial carcinoma (BLCA), breast invasive carcinoma（BRCA）, cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), cholangiocarcinoma（CHOL）, colon adenocarcinoma (COAD), diffuse large B-cell lymphoma(DLBC), esophageal carcinoma(ESCA), glioblastoma multiforme (GBM), head and neck squamous cell carcinoma(HNSC), kidney chromophobe (KICH), kidney renal clear cell carcinoma(KIRC), kidney renal papillary cell carcinoma(KIRP), acute myeloid leukemia(LAML), lower grade glioma(LGG), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma(LUAD), lung squamous cell carcinoma(LUSC), mesothelioma (MESO), ovarian serous cystadenocarcinoma(OV), pancreatic adenocarcinoma(PADD), pheochromocytoma and paraganglioma(PCPG), prostate adenocarcinoma(PRAD), rectum adenocarcinoma（READ）, sarcoma（SARC）, skin cutaneous melanoma（SKCM）, stomach adenocarcinoma(STAD), testicular germ cell tumors(TGCT), thyroid carcinoma(THCA), thymoma (THYM), uterine corpus endometrial carcinoma(UCEC), uterine carcinosarcoma (UCS), uveal melanoma(UVM)

The whole data were filtered to remove missing and duplicated results, and transformed by $\log_2$(TPM+1) using R package of "rma" in an R environment (R version: 3.6.1). The differential expression of *RCE1* in tumor and paracancerous tissues was analyzed by Wilcoxon's test. The R package "ggpubr" was used to visualize pictures.

In addition, we searched *RCE1*, clicked SUBMIT, found Molecular subtype and immune subtype, selected cancer with a significant difference in online software TISIDB (http:// cis.hku. hk/ TISIDB/index.php).

2.2. Survival Analysis of RCE1 Gene in Pan-cancer

Cox regression analysis was used to evaluate the relationship between *RCE1* expression and overall survival and disease-specific survival of patients using the TCGA databases. The Kaplan-Meier method was used to assess the difference between "high" and "low" risk groups based on the best separation of *RCE1* expression, employing R packages of survminer and survival. The "surv-cutpoint" function in the survminer R package was performed to search for the best split by verifying all potential cut points. Subsequently, the patients were divided into high and low *RCE1* expression groups with the maximum-selected log-rank statistic. Log-rank P-value, hazard ratio (HR), and 95% confidence intervals were examined.

2.3. Immune Infiltration of RCE1 Gene in Pan-cancer

Tumor Immune Estimation Resource (TIMER2, http://timer.cistrome.org/), a web dedicates to comprehensively evaluating the abundances of tumor-infiltrating immune cells[13]. It provides 10 897 samples across 32 cancer types from the TCGA database.

The "Immune -Gene" module of the TIMER2.0 was applied to analyze and visualized the interaction between *RCE1*and $CD8^+$ T cells as well as cancer-related fibroblasts (CAFs) in TCGA datasets, using TIDE, XCELL, MCPCOUNTER, and EPIC algorithms. P-value and correlation were obtained by Spearman rank correlation test adjusted by ρ. The data is visualized as heatmap and scatter plot.

ISSN: 1813-4890

2.4. Expression Pattern of RCE1 in Single Cell

CancerSEA database was used to analyze expression pattern of *RCE1*. After searching for *RCE1* in CancerSEA, we selected Relevance of *RCE1* across 14 functional states in distinct cancers.

2.5. RCE1-related Co-expression Network and Gene Enrichment Analysis

STRING website was used to search a query of a single protein name (*RCE1*) and an organism (Homo sapiens). The following main parameters are set: Homo sapiens, Experiments, and update. Finally, the available experimental *RCE1* binding proteins are obtained. Using the GEPIA2-related gene detection module, the first 100 *RCE1*-binding genes were obtained according to the data set of all TCGA tumors and adjacent normal tissues.

Combined with *RCE1* binding protein and targeting gene, KEGG analyses were used to examine the biological and molecular functions of *RCE1*. KEGG was performed using the R package Cluster Profiler.

2.6. Statistical Analysis

All the data of gene expression were normalized by log2 transformation. The differential expression of *RCE1* in Pan-cancer was tested by Wilcox test. The Kaplan-Meier curve and Cox proportional hazards model were used for survival analysis. The Spearman method was used to study the correlation between two variables. value < 0.05 was considered as significant.

3. Results

3.1. RCE1 Expression was Significantly Differential in 16 Cancer Types

Among 21 cancer types with matched samples, *RCE1* expression was significantly differential in 16 cancer types, accounting for 76% of all cancer types (Figure 1A). Tumor tissues of BLCA, BRCA, CHOL, COAD, ESCA, GBM, HNSC, KIRC, LIHC, LUAD, LUSC, PRAD, STAD, UCEC (all $p<0.001$) and READ ($p<0.01$) had significantly higher *RCE1* expression when compared to corresponding paracancerous tissue (Figure 1A). Meanwhile, significantly decreased *RCE1* expression was observed in kidney chromophobe (KICH) tumor tissues ($p<0.001$) (Figure 1A). Compared with paired samples, the expression of *RCE1* in a variety of tumor tissues was significantly up-regulated, indicating that *RCE1* may be involved in the carcinogenesis of a variety of cancers and may be used as a marker of Pan-cancer.

3.2. RCE1 was Significantly Correlated with Molecular Subtype in 13 Cancer Types

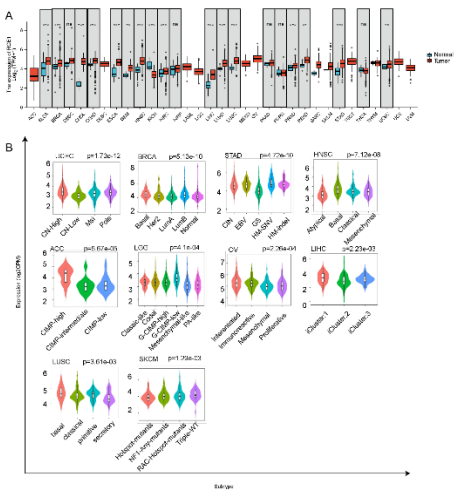

Figure 1. The mRNA expression profile of RCE1 in TCGA cohorts. A. Increased or decreased expression of RCE1 B. Correlations between the RCE1 expression and main molecular subtype

ISSN: 1813-4890

The high expression of *RCE1* was significantly correlated with the advanced stage of UCEC, BRCA, STAD, HNSC, ACC, LGG, OV, LIHC, LUSC and SKCM (Figure 1B). *RCE1* expression was up-regulated in various cancers with molecular subtypes. *RCE1* might be involved in the development of these cancers.

3.3. High RCE1 Expression was Associated with Poor Prognosis of Patients in 10 Cancer Types

Kaplan-Meier survival analysis demonstrated that higher *RCE1* expression was associated with shorter OS in cases of ACC(P<0.001), MESO(P<0.001), GBMLGG(P<0.001), LIHC(P=0.013), OV (P=0.018), LUAD(P=0.018), KIRC(P=0.022), SARC(P=0.027), OSCC(P=0.034), SKCM (P=0.043), THYM(P=0.05) (Figure 2A). Higher *RCE1* expression was associated with longer OS in cases of STAD(P=0.009) and READ(P=0.032).

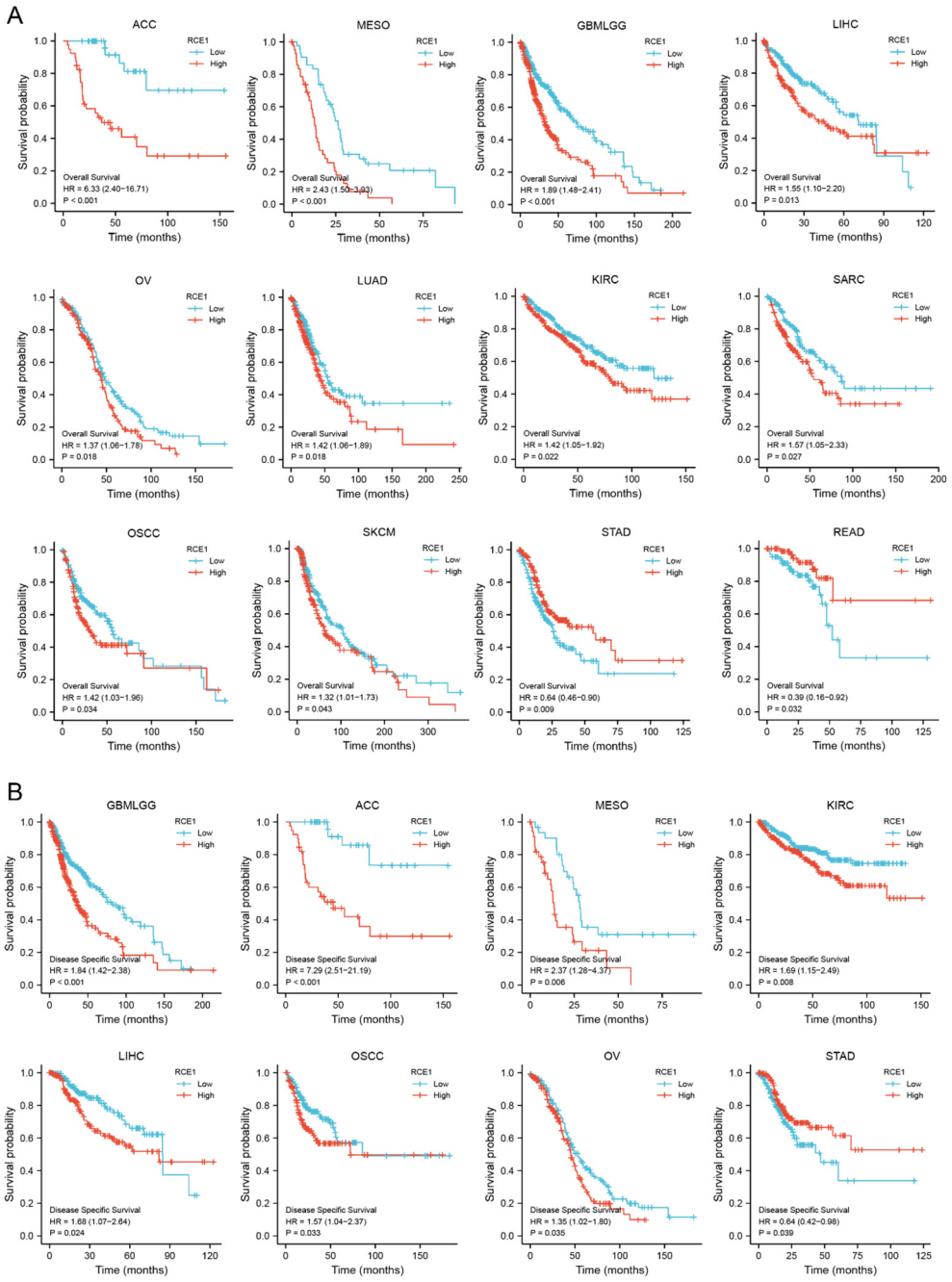

Figure 2. Overall survival (OS) and Disease-specific survival (DSS) of RCE1 in different cancer types. A.B. OS difference or DSS of RCE1 in different cancer types

ISSN: 1813-4890

Overall survival may be affected by non-tumor factors. Furthermore, DSS analysis showed that high *RCE1* expression was a marker for poor outcomes for patients with GBMLGG(P<0.001), ACC(P<0.001), MESO(P=0.006), KIRC(P=0.008), LIHC(P=0.024), OSCC(P=0.033), OV(P=0.0035) (Figure 2B). High *RCE1* expression was a marker for rich outcomes for patients with STAD (P=0.039) (Figure 2B). Those results indicated that increased *RCE1* expression was associated with poor prognosis and *RCE1* expression may be one of the prognostic factors in a variety of tumor types.

The above findings indicated that there was a differential correlation between *RCE1* expression and different tumors, and it was speculated that *RCE1* may play a role in different cancers via a common molecular mechanism. As a result, *RCE1* may be used as a tumor prognosis marker to help improve prognosis levels in cancer patients.

3.4. Pan-cancer Analysis of RCE1 Expression and Tumor Microenvironment

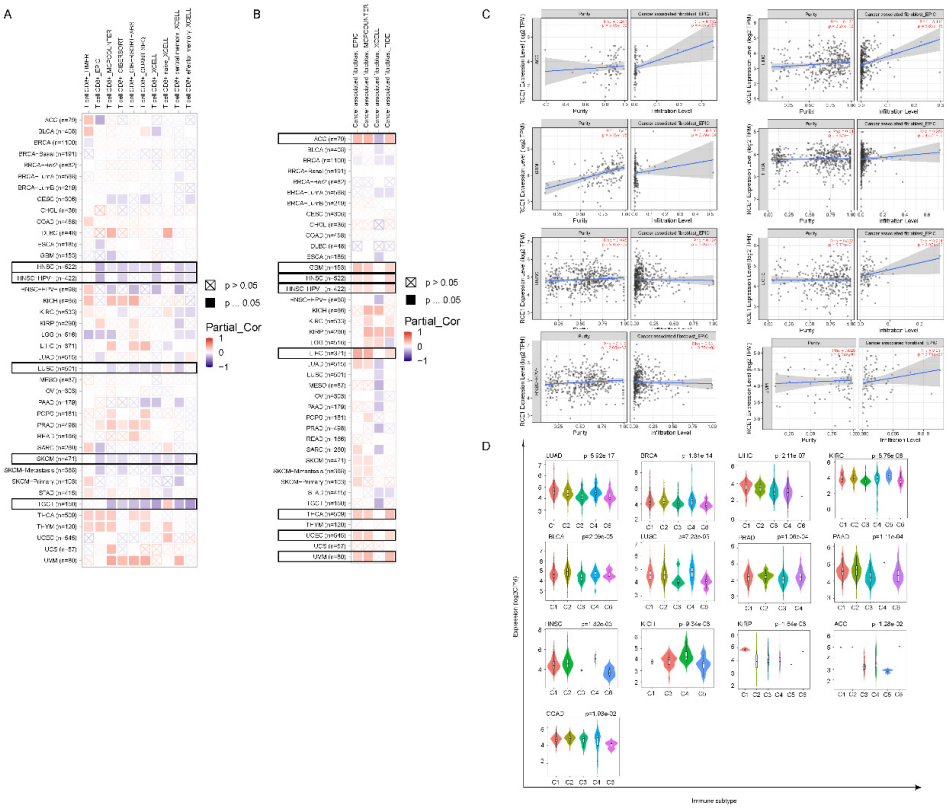

Figure 3. Correlations between RCE1 expression and immune infiltration. A.B.C.D. Correlations between RCE1 expression levels and immune cells or CAFs in some cancers or immune subtype

In most cancer types, the expression of *RCE1* is negatively correlated with the infiltration value of interstitial cells and immune cells in tumor tissues, such as HNSC, HNSC-HPV$^-$, LUSC, SKCM, TGCT (Figure 3A). These results suggest that *RCE1* may inhibit tumorigenesis and development by inhibiting the formation of the tumor immune microenvironment.

Previous studies have found that cancer-associated fibroblasts in the stroma are involved in the regulation of different tumor-infiltrating immune cells. To validate this hypothesis, we investigate the correlation between cancer-associated fibroblast infiltration and *RCE1* expression in different malignancies. *RCE1* expression was positively correlated with cancer-associated fibroblast infiltration in ACC(Rho=0.332, *P*=4.07e-3), GBM(Rho=0.306, *P*=2.79e-4), HNSC(Rho=0.126, *P*=5.05e-3), HNSC-HPV$^-$(Rho=0.131, *P*=8.77e-3), LIHC(Rho=0.416, *P*=6.65e-

ISSN: 1813-4890

16), THCA(Rho=0.262, *P*=4.47e-9), UCEC(Rho=0.221, *P*=3.87e-2) and UVM(Rho=0.237, *P*=3.79e-2) (Figure 3B-C). In order to further explore the internal mechanism of the correlation between PDCD2L expression and CAF, we analyzed the expression markers of CAF in pan-carcinoma. *RCE1* expression was found to be significantly associated with immune subtypes, including C1(wound healing), C2(IFN-gamma dominant), C3(inflammatory), C4(lymphocyte depleted), C5(immunologically), C6(TGF-b dominant). We found that *RCE1* expression was significantly associated with immune subtype of 13 cases including LUAD, BRCA and LIHC (Figure3D).

Above all, in most cancer types, the scatterplots were positively correlated based on most algorithms, which indicated the immune suppression role of *RCE1* in cancer patients.

3.5. Expression Pattern of RCE1 in Single Cell and its Relationship with Cancer Functional Status

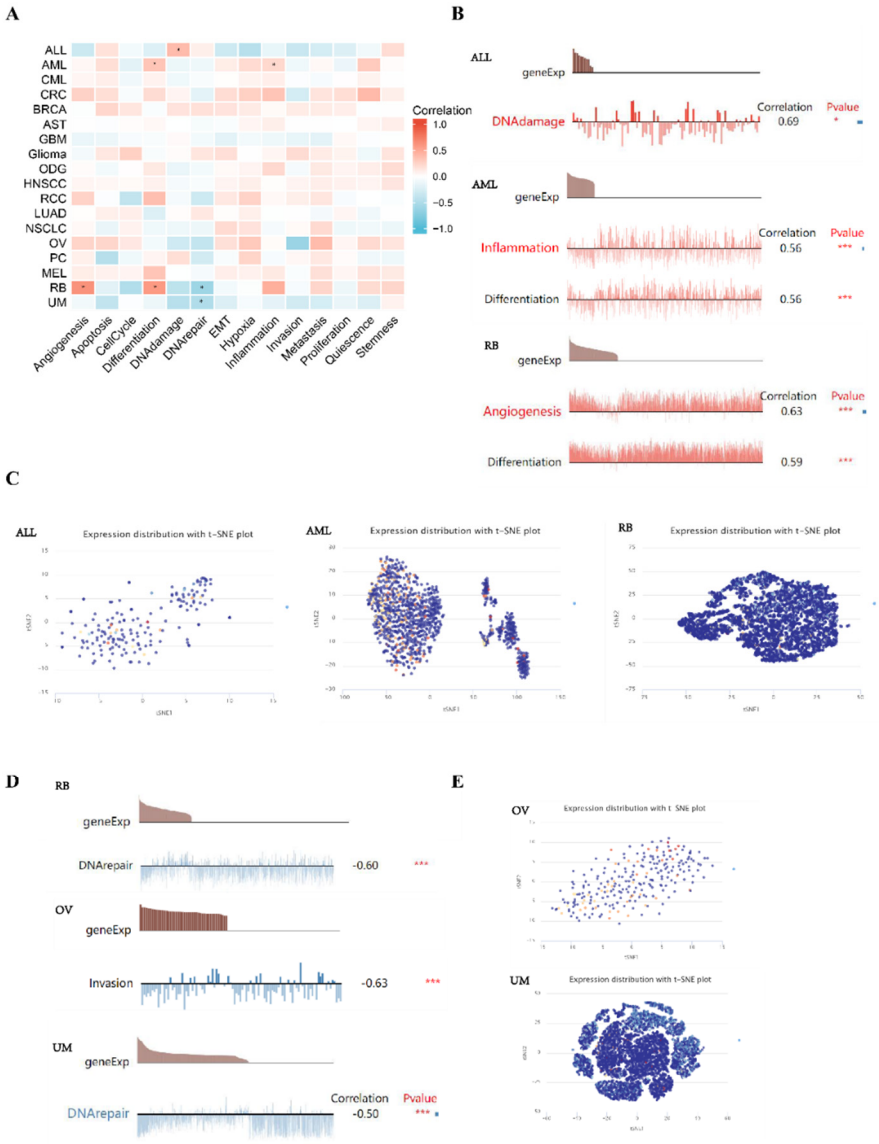

Figure 4. Expression pattern of RCE1 in single cell sequencing and its correlation with tumor functional status. A. Heatmap between RCE1 expression and different tumor functional statuses B.D. Correlation between RCE1 and positively or negatively functional states C. E. RCE1 expression profiles of some samples

ISSN: 1813-4890

RCE1 expression is linked to the biological process among several different cancers (Figure 4A). For example, *RCE1* expression in AML was found to be significantly positively associated with inflammation and differentiation (Figure 4B-C), indicating that *RCE1* expression in AML promoted inflammatory response and cell differentiation. While there was a significant negative correlation between *RCE1* expression and DNA repair in UM (Figure 4D-E), indicating that *RCE1* expression in UM was devastating to the cell repair process. The findings suggest that *RCE1* may play an important role in the progression of cancer.

3.6. RCE1-related Co-expression Network and Gene Enrichment Analysis

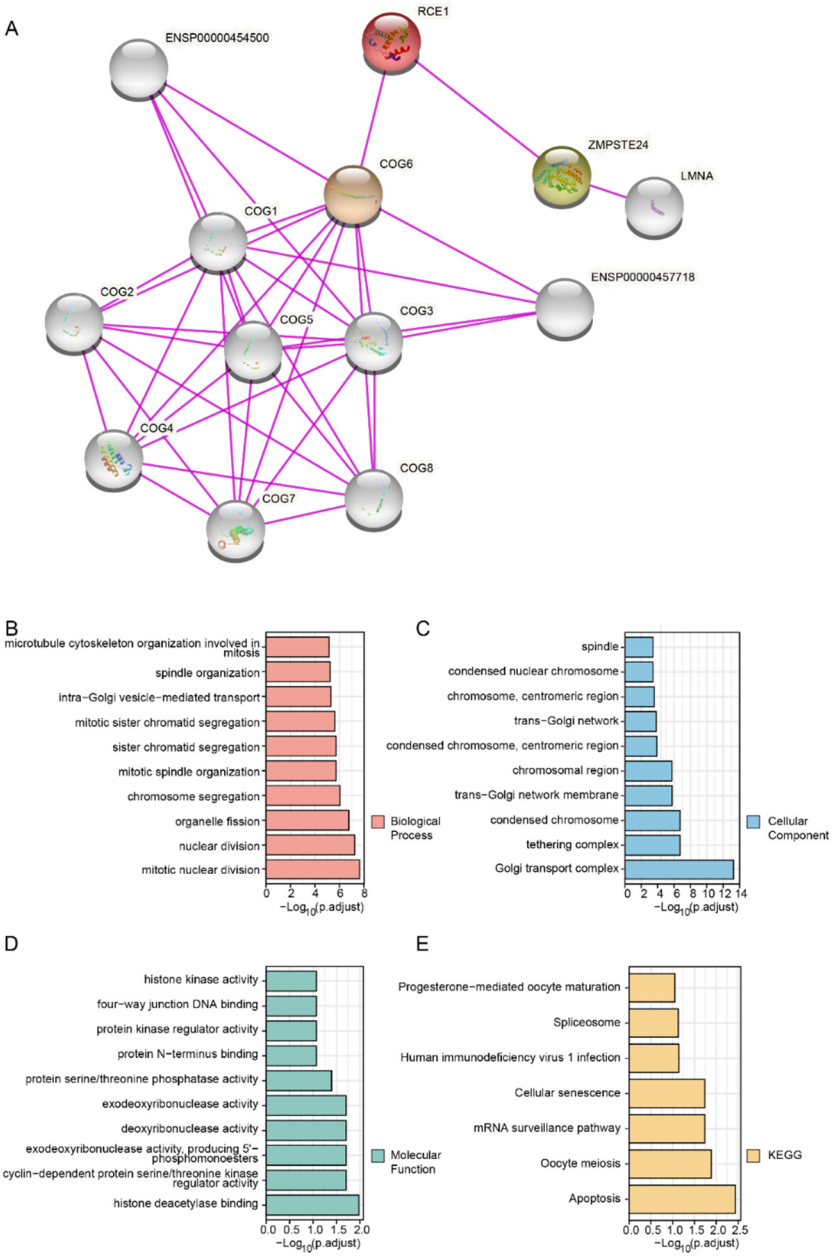

Figure 5. Co-expression network of *RCE1* and enrichment pathway analysis. A. The binding proteins of *RCE1* B. Biological process C. Cellular component D. Molecular function E. KEGG pathways

ISSN: 1813-4890

RCE1 primarily interacts with the conserved oligomeric Golgi apparatus (COG) family (Figure 5A). COG complex is a COG1-8 peripheral Golgi matrix protein that regulates membrane transport and protein glycosylation and is involved in a variety of human diseases, including cancer. COG complex subunits, particularly COG3, COG6 and COG8 are potential prognostic biomarkers in KIRC patients. By regulating protein glycosylation, abnormal COG complex expression can influence tumor invasion and metastasis. It implied that *RCE1* may influence tumor progression via its interaction with the COG family.

Among the 12 interacting proteins, COG6 and ZMPSTE24 interact directly with *RCE1*. ZMPSTE24 (zinc metallopeptidase STE24) is a transmembrane metalloproteinase. ZMPSTE24 has recently emerged as a key protease involved in human progeria. This implied that *RCE1* affects tumor progression through its interaction with ZMPSTE24.

Then to investigate the functional mechanism of *RCE1* in carcinogenesis, we used GEPIA2 to extract the top 100 genes which expression patterns resemble *RCE1* from all tumor types in the TCGA datasets. Following that, we combined *RCE1* interacting protein and top 100 genes to validate the result of KEGG and Gene Ontology enrichment analysis.

According to KEGG data, the analysis results found that apoptosis and cell aging pathways were positively correlated with *RCE1* expression levels, which suggested that *RCE1* may play a role in tumors by influencing cellular senescence and apoptosis.

Gene Ontology enrichment analysis indicated that these genes were closely linked to Multiple cellular biological processes (Figure 5A). Using BP enrichment results as an example, *RCE1* is involved in related biological processes such as cell division and proliferation. *RCE1* may be involved in a variety of kinase activity alteration processes, the majority of which are related to cell biology, according to the results of MF enrichment.

The data presented above suggest that *RCE1* may influence the occurrence and progression of certain tumors by interacting with other proteins such as the COG family and influencing biological processes such as cell proliferation, cell senescence, and apoptosis.

4. Discussion

Cancer is a serious threat to human health due to its high morbidity and mortality[14]. Pan-cancer analysis could reveal the similarities and differences between different cancers and provide deep insights into the design of cancer prevention and personalized treatment strategies[15]. A growing number of recent studies focused on genome-wide Pan-cancer analysis to reveal cancer-driving genes related to the initiation and development of cancer, which is of great significance for the early diagnosis of cancer and the identification of sensitive biomarkers[16-18].

Here we have identified that *RCE1* plays a key role in various human cancers. The present study first comprehensively examined the expression of *RCE1* in a Pan-cancer dataset. The results from the analysis of 33 cancer data sets from the TCGA were demonstrated that *RCE1* was significantly upregulated in BLCA, BRCA, CHOL, COAD, ESCA, GBM, HNSC, KICH, KIRC, LIHC, LUAD, LUSC, PRAD, READ, STAD, THCA and UCEC compared to paracancerous tissues, whereas the expression of *RCE1* was down-regulated in KICH. Therefore, *RCE1* may serve different functions in different types of cancers. In addition, the expression of *RCE1* was significantly and positively correlated with the increase in molecular subtype in multiple cancer types. And our results demonstrated that the expression of *RCE1* can affect cancer patient prognosis. Among 33 cancer types in the TCGA dataset, the high expression of *RCE1* was correlated with poor prognosis in 13 cancer types, which further supports its oncogenic role in tumor progression.

Next, we sought to elucidate the oncogenic role by analyzing its association with immune infiltration. The *RCE1* expression was significantly associated with immune infiltration and immune checkpoint markers in various types of cancer. As for immune cell infiltrations, the

ISSN: 1813-4890

most relevant immune cells of *RCE1* included CD8+ T cells and CD8 + T cells memory. HNSC, LUSC, SKCM and TGCT all demonstrated significant correlation with immune cell infiltration. CAFs in the stroma of the tumor microenvironment were extendedly researched recently and were believed to regulate the function of tumor-infiltrating immune cells including CD8+ T cells and monocytes[19, 20]. We found that the *RCE1* expression was positively correlated with the infiltration of CAFs, such as ACC, GBM, HNSC, LIHC, THCA, UCEC and UVM. Among 33 cancer types in the TCGA dataset, the high expression of *RCE1* was correlated with immune subtype in 13 cancer types, which further supports its oncogenic role in immune system. The close relation between *RCE1* and the immune system might offer a new idea for future studies on immune therapy against cancer.

To understand its functional activity, we explored the expression pattern of *RCE1* in single cell and its relationship with cancer functional status, which revealed *RCE1* expression is linked to the biological process among several different cancers. Furthermore, we also found that *RCE1* might work together in carcinogenesis as significant correlations were detected such as *COG6*, and *ZMPSTE24*, which have well-characterized functions in invasion and metastasis, DNA repair and cell cycle regulation. This Pan-cancer study revealed the prognostic value and oncogenic role of *RCE1* across multiple tumor types. Consistent with previous studies, our KEGG analysis suggested that *RCE1* was significantly associated with many signaling pathways. *RCE1* significantly contributed to the activation or suppression of various oncogenic pathways including apoptosis and cell aging. Gene Ontology analysis showed that *RCE1* was co-expressed with genes involved in biological processes such as cell cycle and mitotic regulation. All these findings indicated that targeting *RCE1* could be a promising strategy and warrants further investigation. Although our study provides useful evidence for tumor patient prognosis and tumor microenvironment, it has some limitations that need to be addressed and confirmed by additional laboratory studies and large-scale clinical trials.

In summary, our study systematically demonstrated the expression, prognostic value, tumor microenvironment and its relationship with tumor function, PPI and functional pathways of *RCE1* across a series of cancers. The expressions of *RCE1* suggested a significant association with oncogenic pathways including protein secretion, mitotic spindle, cell cycle and showed a correlation with immune regulations of CD8+ T cells and CAFs. The evaluation of *RCE1* distributions could predict the prognosis of cancer patients. These findings provide novel evidence for the investigation of *RCE1* in the development and therapy of cancer in the future.

References

[1] C. de Martel, D. Georges, F. Bray, J. Ferlay, G.M. Clifford, Global burden of cancer attributable to infections in 2018: a worldwide incidence analysis, The Lancet. Global health, 8 (2020) e180-e190.

[2] G. Lippi, F. Sanchis-Gomar, G. Cervellin, Global epidemiology of atrial fibrillation: An increasing epidemic and public health challenge, International journal of stroke : official journal of the International Stroke Society, 16 (2021) 217-221.

[3] O. Kucuk, Cancer biomarkers, Molecular aspects of medicine, 45 (2015) 1-2.

[4] N.L. Henry, D.F. Hayes, Cancer biomarkers, Molecular oncology, 6 (2012) 140-146.

[5] T. Stachowicz-Stencel, A. Synakiewicz, Biomarkers for pediatric cancer detection: latest advances and future perspectives, Biomarkers in medicine, 14 (2020) 391-400.

[6] A. Blum, P. Wang, J.C. Zenklusen, SnapShot: TCGA-Analyzed Tumors, Cell, 173 (2018) 530.

[7] S.E. Hampton, T.M. Dore, W.K. Schmidt, Rce1: mechanism and inhibition, Critical reviews in biochemistry and molecular biology, 53 (2018) 157-174.

[8] J. Jaworski, U. Govender, C. McFarlane, M. de la Vega, M.K. Greene, N.D. Rawlings, J.A. Johnston, C.J. Scott, J.F. Burrows, A novel RCE1 isoform is required for H-Ras plasma membrane localization and is regulated by USP17, The Biochemical journal, 457 (2014) 289-300.

[9] I. Manolaridis, K. Kulkarni, R.B. Dodd, S. Ogasawara, Z. Zhang, G. Bineva, N.O. Reilly, S.J. Hanrahan, A.J. Thompson, N. Cronin, S. Iwata, D. Barford, Mechanism of farnesylated CAAX protein processing by the intramembrane protease Rce1, Nature, 504 (2013) 301-305.

[10] J. Li, D. Wang, J. Liu, Y. Qin, L. Huang, Q. Zeng, M. Xiao, J. Hu, Q. Yang, J. He, L. Mai, Y. Li, W. Liu, Rce1 expression in renal cell carcinoma and its regulatory effect on 786-O cell apoptosis through endoplasmic reticulum stress, Acta biochimica et biophysica Sinica, 49 (2017) 254-261.

[11] L. Huang, M. Li, D. Wang, J. He, W. Wu, Q. Zeng, J. Li, M. Xiao, J. Hu, Y. He, Y. Li, L. Mai, W. Liu, Overexpressed Rce1 is positively correlated with tumor progression and predicts poor prognosis in prostate cancer, Human pathology, 47 (2016) 109-114.

[12] B. Shi, X. Zhou, L. He, M. Liang, Y. Luo, P. Jiang, Reduced RCE1 expression predicts poor prognosis of colorectal carcinoma, BMC Cancer, 17 (2017) 414.

[13] T. Li, J. Fu, Z. Zeng, D. Cohen, J. Li, Q. Chen, B. Li, X.S. Liu, TIMER2.0 for analysis of tumor-infiltrating immune cells, Nucleic Acids Res, 48 (2020) W509-w514.

[14] J.J. Wang, K.F. Lei, F. Han, Tumor microenvironment: recent advances in various cancer treatments, Eur Rev Med Pharmacol Sci, 22 (2018) 3855-3864.

[15] Á. Nagy, G. Munkácsy, B. Győrffy, Pancancer survival analysis of cancer hallmark genes, Sci Rep, 11 (2021) 6047.

[16] W.X. Xu, J. Zhang, Y.T. Hua, S.J. Yang, D.D. Wang, J.H. Tang, An Integrative Pan-cancer Analysis Revealing LCN2 as an Oncogenic Immune Protein in Tumor Microenvironment, Frontiers in oncology, 10 (2020) 605097.

[17] K. Wang, C. Guan, X. Shang, X. Ying, S. Mei, H. Zhu, L. Xia, Z. Chai, A bioinformatic analysis: the overexpression and clinical significance of FCGBP in ovarian cancer, Aging (Albany NY), 13 (2021) 7416-7429.

[18] D. Sahu, Y.L. Chang, Y.C. Lin, C.C. Lin, Characterization of the Survival Influential Genes in Carcinogenesis, Int J Mol Sci, 22 (2021).

[19] X. Chen, E. Song, Turning foes to friends: targeting cancer-associated fibroblasts, Nat Rev Drug Discov, 18 (2019) 99-115.

[20] M. Nurmik, P. Ullmann, F. Rodriguez, S. Haan, E. Letellier, In search of definitions: Cancer-associated fibroblasts and their markers, Int J Cancer, 146 (2020) 895-905.