Pan-cancer Analysis of a Cytoskeleton Mobility related ARHGAP44 gene with Potential Implications in Cancer Prognosis Risk Prediction and Immune Landscape Modulation

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Abstract

Background

Rho GTPases has been a well known family of small G proteins that regulate cellular cytoskeleton dynamics and involve in multiple critical steps of cancer progression. However, ARHGAP44 gene which is a member of GAP proteins that regulates the Rho GTPases cycling between their active GTP-bound and inactive GDP-bound state, its role in cancer development is still lack of understanding. The study is to analyze the function of ARHGAP44 gene in broad spectrum human cancers, thus aiding better understanding of the collaborative network of cytoskeleton related genes in cancers.

Methods

In the study, we started with the analysis of the genetic characteristics of ARHGAP44 gene, followed by its expression patterns, frequent alterations as well as survival prediction value in broad spectrum human cancers. Further, the probable reasons for the aberrant changed expression of ARHGAP44 in cancers comparing to corresponding normal control samples were investigated. Moreover, the correlation of ARHGAP44 with multiple critical clinical cancer parameters were in succession performed.

Results

Firstly, basic genetic physicochemical properties of ARHGAP44 were investigated including its aminoacid composition, estimated molecular weight and protein half life. Then, genetic alteration analysis revealed that ARHGAP44 expression various in human cancers, which was partly due to the modulation by DNA methylation and phosphorylation. Further, ARHGAP44 gene was indicated to be associated with multiple critical cancer traits including cancer stemness, cytoskeleton dynamics as well as immune infiltration in different human cancer types. Moreover, ARHGAP44 gene was also supported to be associated with the sensitivity of several chemotherapy related drugs.

Conclusions

Based on multiple bioinformatic analysis and TCGA pan-cancer data as well as certain local hospital samples, we revealed some valuable strategies to guide the therapeutic orientation concerning the role of ARHGAP44 gene in human cancers, although more detailed experiments and clinical trials are obligatory to support further clinical medical application of the gene, especially in each type of independent cancer.

Background

Rho GTPases are a family of small G proteins that have been well known for their ability to regulate cellular cytoskeletal dynamics, not just in morphology maintenance, cell growth and cell cycle...
progression, but also in cell migration process, vesicle trafficking as well as stem cell differentiation[1]. Considering cancer mobility especially cell migration and metastasis has been a major cause of cancer related death evolving intensive collaboration of cytoskeleton movement[2], it’s of clinical significance to investigate the detailed biological functions of Rho GTPases proteins in human cancers.

There are currently 20 mostly known Rho GTPases proteins that were classified into subfamilies as RHO (including RHOA, RHOB, RHOC), RAC (containing RAC1, RAC2, RAC3, RACG), CDC42 family (comprising of CDC42, RHOQ and RHOJ), as well as RhoD/RhoF cluster (namely RHOD and RHOF)[1]. And the best characterized of the 20 Rho GTPases were RHOA, RAC1 and CDC42, they have been reported to regulate cell cytoskeleton dynamics by activating multiple downstream effectors for instance protein kinases ROCK1 and ROCK2 which were involved in the regulation of multiple proteins that participate in cellular actin filaments stabilization and generation of actin-myosin contractile force[3, 4], as well as IQGAP family including IQGAP1, IQGAP2 and IQGAP3 which proteins have been reported to modulate cell shape and motility via regulating G-actin and F actin equilibrium[5–9]. Moreover, WASP, WAVE proteins and formins were also effective RHO GTPase effectors that modulate critical cellular cytoskeleton dynamics including actin polymerization, cell motility as well as cell mitosis process[10–12].

A similar function mode for above classic Rho GTPases proteins including RHOA, RAC1 and CDC42 is that they all cycle between an active GTP-bound and inactive GDP-bound state in cells[13]. And the cycling between the active and inactive states were tightly regulated by three clusters of proteins including guanine nucleotide exchange factors (GEFs), GTPase activating proteins (GAPs), and guanine nucleotide dissociation inhibitors (GDIs). GEFs destabilize the interaction between Rho GTPases proteins and their bounding GDP, resulting in interacting with GTP. Meanwhile, GAPs promote the inactivation of Rho GTPases proteins by activating the endogenous GTP hydrolyzing and exchange for interaction with inactive GDP[14–16]. Moreover, GDIs play roles in maintaining the inactive GDP-bound and cytoplasm locating state of RHO GTPases proteins. The activity of a specific Rho GTPase is actually the effect of a complex of related modulation factors[14].

Multiple modulation proteins including RHO GEFs, GAPs and GDIs are known to be altered through changed gene expression, genetic alteration or differed activity levels[17], and the altered modulation proteins may function as direct mechanism of changed Rho GTPase signaling and result in different cellular effects[18]. Nearly hundreds of RHO GEFs and GAPs have been identified over the years, and their expressions may vary in different types of cancers[1]. However, detailed investigations of these modulating RHO GEFs or GAPs are woefully lacking comparing to the extensive analysis towards Rho GTPases proteins RHOA, RAC1 or CDC42. Careful analysis of the modulating genes were also of potential clinical value and might shed promising insight for further understanding of the collaborative network of cellular mobility and cytoskeleton related genes in cancers.

In the study, we mainly focused on a GAP protein, namely ARHGAP44 which is short for Rho GTPase-activating protein 44 and has been known to work as a GAP at least for Rho GTPases CDC42 and RAC1[19, 20]. In neurons, it might involves in spine formation and synaptic plasticity[21]. Moreover,
ARHGAP44 has been supported to be associated with the survival of several cancers for instance osteosarcoma[22], hepatocellular carcinoma[23] and melanoma[24]. However, the full picture of ARHGAP44 gene in human cancers, especially the detailed function and working mechanism of gene has yet to be explained.

Based on TCGA pan-cancer data, we comprehensively studied the expression as well as clinical traits of the gene in human cancers. To be more specific, the study started with the investigation of basic genetic information and physicochemical property of ARHGAP44, and then the expression pattern, genetic alterations as well as prognostic value in pan-cancer, followed by potential post transcription modification including phosphorylation and methylation were included. Further more, the clinical traits including association with cancer stemness, cytoskeleton dynamics, immune infiltration and drug sensitivity were preliminary analyzed. The results shall provide promising insights for understanding the working mechanism of ARHGAP44 in human cancers and aid the unearthing of potential new prognostic indicators as well as cancer therapy targets.

**Materials and Methods**

**Data source: TCGA pan-cancer genes expression profiles**

TCGA pan-cancer data was accessed from UCSC Xena[25], and based on the data, not just the basic information regarding ARHGAP44 gene most importantly its expression levels, but also the human broad spectrum cancers data including the clinical pathological information, cancer subtypes, patients survival status were obtained. Most of the analysis in the study especially the association between ARHGAP44 gene expression and multiple clinical traits were conducted based on the TCGA pan-cancer data.

**Basic genetic information and physicochemical property analysis of ARHGAP44 gene**

A combine of three analysis platforms including ProtParam[26], ProtScale[27] and Human Protein Atlas[28] were accessed for understanding the basic information of ARHGAP44 gene including its genetic and physicochemical properties.

For starters, ProtParam and ProtScale were in succession applied to analyze the physicochemical properties of ARHGAP44 protein including its aminoacid composition, computed encoding protein molecular weight, theoretical isoelectric point, estimated protein half life, instability index and hydrophobicity as well as hydrophilicity.

Meanwhile, Human Protein Atlas which has also been effective for interpreting proteins information was accessed to predict the cellular location of ARHGAP44, and preliminary observe the gene expression in human normal tissues as well as certain cancers.
Expression patterns and association with clinical parameters

After understanding the basic genetic and encoding protein information of ARHGAP44, UALCAN[29] as well as GEPIA[30] were together used to analyze its expression patterns in human cancers. Firstly, ARHGAP44 gene expression in human broad spectrum cancers including but not limited to bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), cholangio carcinoma (CHOL), kidney clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), brain lower grade glioma (LGG), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), thymoma (THYM) comparing to corresponding normal control tissues were investigated. Besides, the association between ARHGAP expression and clinical parameters in these cancers were also observed.

Quantitative real-time PCR (QPCR) experiments

To preliminary validate the changed expression of ARHGAP44 gene in cancers, from us local hospital biobank, we selected tissues samples of four cancers including LUSC, LUAD, PAAD and KIRC, and extracted mRNA of 10 paired cancer tissues and adjacent paracancerous normal tissues of each cancer type for QPCR experiment of detecting ARHGAP44 gene expression.

The total mRNA of above four cancer types tissues and adjacent paracancerous normal tissues were extracted using RNAiso-Plus (TAKARA, DaLian, China). And then 1 µg extracted mRNA was used for cDNA synthesis with commercial cDNA synthesis kit (TAKARA, DaLian, China) based on the kit operating instruction. Further, qPCR was performed on Roche z 480 and the primers used were listed as below:

**ARHGAP44:**

Former: ACAACAACATCCGATACTTGA

Reverse: TTGGCATTATGGTTCACG

**GAPDH:**

Former: AGAAGGCTGGGGCTCATTTG

Reverse: AGGGGCCATCCACAGTCTTC

The PCR cycling condition was set as: 95°C 5 min for 1 cycle; 95°C 5 s, 60°C 30 s, and 72°C 34 s for 35 cycles followed by the melting curve stage. And the relative gene expression in each sample was recorded as the average $2^{-\Delta\Delta CT}$ calculation result of three replicates. Further, T-test was used for detailed statistical analysis. $P < 0.05$ was considered statistically significant.

Survival analysis of ARHGAP44 gene in human cancers

Survival analysis is both an important and necessary aspect for evaluating the clinical effect of altered genes. Kaplan-Meier plotter[31] has been a widely used open access online service containing over
10,000 cases samples of human pan-cancer types for analyzing patients overall survival (OS) as well as recurrence free survival (RFS). In the study, the survival correlation of ARHGAP44 gene in broad spectrum human cancers were evaluated based on Kaplan-Meier plotter platform, both OS and RFS.

**Post transcription modulation of ARHGAP44 gene in cancers**

For understanding the reasons for altered expression of ARHGAP44 in different types of human cancers, we preliminary explored the common post transcription modulation including methylation and phosphorylation levels of ARHGAP44 gene in cancers which were all based on UALCAN platform[29].

**Genetic alterations analysis**

Besides altered mRNA expression, other types of genetic variations for instance gene mutation, copy number variation, amplification and deletion may change certain gene's working mode and affect cellular biological functions. cBioPortal[32] has been one of the largest open access cancer genomics data website containing over 126 large-scale tumor research projects and it has been working effectively as a rich data resource for world wide researches investigation of certain genes variations.

In the study, the “cancer types summary” module of “quick search” section in cBioPortal database was applied to uncover the genetic alteration characteristics of ARHGAP44 in human cancers, meanwhile, the “mutation” module of same database was accessed to display the mutated site information of the gene in 3D protein structures.

**Protein-protein interaction (PPI) network construction and related genes analysis**

After recognizing the expression as well as alteration patterns of ARHGAP44 in cancers, to explore the detailed cellular function and potential mechanism behind the regulation, STRING[33], which is short for Search Tool for the Retrieval of Interacting Genes was used to construct the PPI network which was centered on ARHGAP44 gene for investigating its surrounding interacting genes.

Further, genes enrichment analysis were applied to annotate basic biological attributes of ARHGAP44 and its connecting genes including their main cellular location, involved biological processes, molecular functions and the signaling pathways the genes mainly enriched in.

**Cytoskeleton mobility related genes expression association analysis**

ARHGAP44 was known as a GAP protein that was supposed to modulate RHO GTPases proteins activity thus affecting cell mobility as well as other cytoskeleton related cellular biological processes. To validate the association between ARHGAP44 gene and cytoskeleton related genes, 29 genes that were previously supported to be related with cytoskeleton dynamics for instance actin filaments stabilization, F-actin
polymerization, G-actin and F actin equilibrium and generation of actin-myosin contractile force were selected (listed in Supplementary Table 1).

Based on TCGA pan-cancer expression data, we analyzed the correlation between ARHGAP44 gene and the 29 genes signature for preliminary validating the association between ARHGAP44 and cytoakeleton dynamics in cancers.

**Correlation with main Rho GTPase proteins RHOA, RAC1 and CDC42 in human cancers**

Among currently identified RHO GTPases proteins, the best characterized were RHOA, RAC1 and CDC42, which proteins have been known to regulate different aspects of cellular cytoskeleton movements. Even though previous reports have indicated that ARHGAP44 works as a GAP modulation protein for RAC1 and CDC42 in some cancers, the correlation between ARHGAP44 and all three proteins RHOA, RAC1 and CDC42 were yet to be comprehensively explained in pan-cancer.

In the study, based on TCGA pan-cancer data, the correlation between ARHGAP44 gene and each of RHOA, RAC1 as well as CDC42 RHO GTPases proteins was orderly investigated in each type of human cancers, thus aiding more better understanding of the mechanism behind ARHGAP44 regulation of RHO GTPases related cell mobility.

**Extra cellular matrix (ECM) degradation association analysis**

Metastasis has been a major cause of cancer related death evolving not just intensive collaboration of cytoskeleton movements, but also changes of the structure of cellular microenvironment for instance extra cellular matrix (ECM). ECM degradation has been commonly known as a major step of cancer metastasis. After analyzing the association between ARHGAP44 gene and cytoskeleton dynamics, for better understanding the potential effect ARHGAP44 gene has on cancer metastasis, we explored the association between the gene and ECM degradation in cancers.

In the study, 23 genes that have been previously supported to be related with ECM degradation were selected (listed in Supplementary Table 2), and based on TCGA pan-cancer expression data, we analyzed the correlation between ARHGAP44 and the 23 genes signature for preliminary exploring the potential effect ARHGAP44 gene has on ECM structure.

**Cancers stemness and HRR score correlation analysis**

Homologous Recombination Deficiency (HRD) has been increasingly known as an important clinical trait which correlates not only with disease procession but also PARPi related drugs therapies efficiency[34, 35]. To further analyze the HRD signature in pan-cancer, 27 usually adopted Homologous Recombination Repair (HRR) signaling participated genes (listed in Supplementary Table 3) were collected and input into GEPIA2.0 to calculate their correlations with ARHGAP44. Meanwhile, RNA based cancer stemness index (RNAss) of each cancer type was calculated based on TCGA pan-cancer data using “gelnet” R package, followed by investigating its correlation with ARHGAP44 gene expression.
Cancer proliferation and cell cycle G2M proteins association analysis

Uncontrolled cell proliferation has always been one of the most major hallmarks of cancers[36], for comprehensively understanding ARHGAP44 gene's role in cancer development besides cytoskeleton modulation, the potential connection between ARHGAP44 expression and cancer proliferation as well as cell cycles were explored.

Based on TCGA pan-cancer data, 14 genes that were commonly known to be able to represent cell proliferation ratio and 199 G2M gene checkpoints relating with cell cycle were selected (listed in Supplementary Table 4), further, the correlation between ARHGAP44 expression and the gene clusters were explored respectively.

Correlation with cancer EMT signature analysis

To next step seek the relevance with cancer Epithelial Mesenchymal Transition (EMT) which has been another critical and important step for cancer metastasis, 14 characteristic EMT genes (listed in Supplementary Table 5) were selected and input into GEPIA2.0 for calculating their correlations with ARHGAP44.

Immune infiltration cells and immune checkpoints correlation

For characterizing the microenvironment immune landscape between ARHGAP44-high and low expression cancer samples, CIBERSORT algorithm was performed to calculate the relative contents of 22 tumor infiltrating immune cells (TICs) based on TCGA profiles data, followed by analyzing the relationship between ARHGAP gene and the 22 TICs in different types of cancers.

Moreover, considering immune checkpoints especially CTLA4, PD-L1, TIGIT, TIM-3 and LAG-3 have been showing promising drug targeting effects in multiple cancers and they have been increasing accepted to be clinical biomarkers for selecting potential cancer patients that were most likely to benefit from immunotherapy, we preliminary investigated the association between ARHGAP44 and these immune checkpoints expression.

Further, the association between ARHGAP44 expression and cytotoxic T lymphocytes (CTLs) and patients survival rates were subsequently presented based on TIDE analysis[37], above all for the purpose of preliminary investigating ARHGAP44 gene role in pan-cancer immune microenvironment modulation.

Tumor mutation burden (TMB) and microsatellite instability (MSI) analysis

Immune checkpoints especially PD-L1 expression, MSI and TMB have been three most widely used clinical biomarkers for predicting the potential benefit of cancer patients from immune targeting
therapies. Therefore, after analyzing the association with immune checkpoints expression, ARHGAP44 gene expression correlation with pan-cancer TMB and MSI scores were in succession evaluated based on ACLBI online database[38] which has been an effective open access and users friendly data analysis platform.

**Drug sensitivity analysis**

To preliminary investigate the effects of ARHGAP44 on the routine therapeutic response for human cancers, based on ROC plotter analysis platform[39] which has been a well accepted online tool which links genes expression to clinical therapy responses using cancers transcriptome level data, the ARHGAP44 gene expression differences between responders and non-responders to certain drugs were displayed as well as the receiver operating characteristic curves (ROC) of therapy-related survival.

**Statistical Analysis**

Most of the bioinformatics analyses were conducted on relative online platforms using tools that were supplied on the platform. And as for the processing of downloaded TCGA pan-cancer data and local hospital samples QPCR experiment data, all statistical analysis were performed using SPSS 26.0. For the enumeration data for instance the analysis of genes expression difference in cancers vs normal control samples, the data were analyzed using t test. As for the measurement data for instance the association between gene expression and cancer clinical parameters, the data were analyzed by χ² test. And for correlation analysis, for instance the correlation between gene expression and immune checkpoints expression, the data were analyzed by Spearman analysis. p < 0.05 was considered statistically significant. (For all analysis results, * represents p < 0.05, ** represents p < 0.01, *** represents p < 0.001).

**Results**

**ARHGAP44 genetic and physicochemical property analysis**

Based on multiple analysis platforms, the physiochemical property of ARHGAP44 gene was preliminary interpreted before further deeper investigating the gene biological functions. And the results revealed that ARHGAP44, which is short for Rho GTPase-activating protein 44 and also known as RICH2, NPC-A-10 locates in 17p12 containing 22 exons. Meanwhile, the gene encodes a 818 amino acids containing protein, and the protein formula was computed as C_{3917}H_{6283}N_{1087}O_{1216}S_{37} with estimated weighing as 89.2KD and theoretical isoelectric point to be 6.13. Moreover, the instability index of the protein is computed to be 71.22 and the grand average of hydrophobic value is -0.461 indicating ARHGAP44 works as a cellular unstable and hydrophilic protein.

As for the cellular location, ARHGAP44 is predicted by Human Protein Atlas to locates in convoluted dendritic plasma membrane sections enriched in polymerized actin and myosin patches, and the gene has been reported to involve in the regulation of plasma membrane bounded cell projection organization.
ARHGAP44 expression various in human cancers and its association with clinical parameters

ARHGAP44 mRNA expression in broad spectrum human cancers and corresponding normal control samples were investigated, and the results revealed that its expression seemed to be down regulated in multiple cancers including BLCA, CESC, GBM, KIRP, LGG, LUAD, LUSC, OV, SKCM and UCEC. Meanwhile, as for other cancers for instance CHOL, DLBC, KICH, PCPG, STAD and THYM, the expression was higher in cancers comparing to control samples (Fig. 1A), and an inspiring fact was that ARHGAP44 gene expression various in human cancers including in different subtypes of a specific cancer indicating its diversified functions in cancers (Fig. 1B-1D). For four types of the cancers including LUAD, LUSC, PAAD and KIRC, local hospital samples were also applied and validated the changed expression of the gene in cancers comparing to corresponding normal tissues (data not shown), although the gene expression in other cancers hasn’t been validated in the study yet because of the limitation of cancer types that were currently available in us hospital, the results shall provide meaning support for next step research of the gene.

As for the clinical pathological parameters association, analysis result indicated that although ARHGAP44 gene expression seems to be keeping decreasing as the stages of some cancers advancing, for instance in BLCA, LUAD and READ, especially in the early stage I comparing to more advanced stages (Fig. 1E-1G), most of the difference were not statistical significant, for example in BRCA, LUSC and KIRC indicating ARHGAP44 doesn’t directly affecting cancer stages related advancement (Fig. 1H-1J). And no specific correlation has been observed between ARHGAP44 gene and patients age, gender, race, smoking, drinking or other specific cancer occurrence relating life habits (data not shown).

ARHGAP44 affects patients survival in multi human cancers

To evaluate the clinical significance of the altered expression of ARHGAP44 in cancers, prognostic association analysis was conducted based on KM plotter platform, and the results revealed that high expression of ARHGAP44 was associated with better overall survival (OS) and longer recurrence free survival (RFS) in LUAD, READ, PAAD and KIRP (Fig. 2A-2D). Meanwhile, in contrast with these cancers, high expression of the gene correlates with worse OS and shortening RFS in BRCA, LUSC, CESC and ESCA (Fig. 2E-2H). Different expression patterns as well as the diverse prognosis evaluation value of ARHGAP44 in various cancers indicating its diversified functions in cancers originated from different tissues, which shall also be a phenomenon reflecting tumor heterogeneity as well as the complex regulation network inside tumor individual cells.

Post transcription modulation of ARHGAP44 gene in cancers

For understanding the reasons for altered expression of ARHGAP44 in different types of human cancers, common post transcription modulation including methylation and phosphorylation levels of ARHGAP44
gene in cancers were explored. And the results revealed that ARHGAP44 methylation level was higher in BRCA, KIRC, COAD, GBM, CESC and HNSC which was in consistent with the lower gene expression in the cancers comparing to corresponding normal control samples (Fig. 3A-3F). Meanwhile, the gene methylation level was lower in PCPG and THYM which was in keeping with the higher expression in cancers vs normal control (Fig. 3G, 3H). However, ARHGAP44 methylation level showed inconsistent trend in other cancers including CHOL, PRAD, READ, BLCA, THCA and UCES (Fig. 3I-3N), indicating gene methylation accounts for only part of the altered expression of ARHGAP44 in cancers.

Meanwhile, similar trend was observed during the analysis of ARHGAP44 gene phosphorylation level which result indicated that phosphorylated ARHGAP44 protein was in consistent with total protein in some cancers including LUAD and LUSC, meanwhile different in BRCA and RCC (Fig. 3O-3R). The results also supports the conclusion that gene phosphorylation also accounts for maybe a part of the altered ARHGAP44 gene expression in cancers. Further deeper analysis might still be needed for uncovering more valuable mechanisms of the regulation of ARHGAP44 gene expression in cancers.

**ARHGAP44 genetic alterations and the association with HRD score in cancers**

Besides mRNA expression, the genetic alterations including mutation ratio, protein structure variant and copy number variation of ARHGAP44 gene were explored based on cBioPortal database. The results revealed that ARGFAP44 gene variation type differs in various tumors, a certain percent of gene mutation, deletion and amplification occurs in multiple human tumors (Fig. 4A). Especially, gene mutations which were mostly single nucleotide mutations were discovered in several types of cancers for instance skin cutaneous melanoma, uterine endometrioid carcinoma, colorectal adenocarcinoma, lung squamous cell carcinoma and diffuse large B-cell lymphoma (Fig. 4B).

HRR has been a major mechanism for repairing DNA deficiency, the most critical genes involved in the signaling are BRCA1 and BRCA2, and other related genes include MLH1, MSH2, ATM, FANCA/C, PALB2, RAD51C, TP53 and so on. In the study, we picked 27 commonly known HRR genes and analyzed their correlation with ARHGAP44, and the results revealed that the gene statistical significantly correlates with the 27 genes containing HRR signature in multiple cancers including BLCA, PCPG, TGCT, THCA, CHOL, KIRP, PRAD, KICH and UCS (Fig. 4C-4L). The comprehensive genetic alterations analysis shall aid more precise grasp of ARHGAP44 genetic function patterns in various cancers types.

**ARHGAP44 Interacting PPI network and related genes enrichment analysis**

To explore the detailed biological functions of ARHGAP44 gene and its interacting genes, STRING was used to construct the ARHGAP44 centering PPI network followed by genes enrichment analysis for revealing the probable signaling pathways they involved in. And the analysis result showed consistent trend as current understanding of ARHGAP44 gene which has been initially known as a GAP modulating protein for RHO GTPases that the biological processes ARHGAP44 and its interacting proteins mainly
participated in were focused on cytoskeleton organization, regulation of actin filaments as well as RHO GTPases binding (Fig. 5A). The results provided us enough support for further exploring the detailed correlation between ARHGAP44 gene and cytoskeleton relating genes.

**ARHGAP44 associated with multi cytoskeleton mobility related genes, especially Rho GTPase proteins RHOA, RAC1 and CDC42 in human cancers**

For evaluating the correlation between ARHGAP44 gene and cytoskeleton relating genes in human cancers, we firstly selected 29 genes that were reported to be related with cytoskeleton dynamics for instance actin filaments stabilization, F-actin polymerization and actin-myosin contractile force generation. The result showed that ARHGAP44 was positively correlated with the 29 genes containing signature in at least 9 cancers including THCA, PRAD, THYM, TGCT, KICH, KIRP, PCPG, LUSC and UCS (Fig. 5B-5J).

Considering RHOA, RAC1 and CDC42 have been three best characterized proteins of all the currently recognized RHO GTPases, the correlation between ARHGAP44 gene and each of the three proteins were investigated in pan-cancer. And the results displayed that ARHGAP44 was associated with RHOA in KICH, LGG, UCS, TGCT, THCA, THYM, PRAD and DLBC (Fig. 6A-6H). And it correlates with RAC1 in MESO, PRAD, THCA and THYM (Fig. 6I-6L). Meanwhile, the correlation with CDC42 was discovered in CHOL, KICH, KIRP, UCS, TGCT, THCA, THYM and PRAD (Fig. 6M-6T).

An inspiring and worth of further deeper investigating phenomenon was that ARHGAP44 was observed to correlate with all RHOA, RAC1 and CDC42 three RHO GTPases in three cancers including PRAD, THCA and THYM, indicating the collaborative network of ARHGAP44 involved genes in the modulation of RHO GTPases related cytoskeleton dynamics in the cancers. The phenomenon shall provides a promising direction for further clinical analysis of cell migration as well as cytoskeleton involved metastasis in cancers.

**ARHGAP44 correlates with ECM degradation and EMT process in metastasis process of cancers**

Besides the intensive collaboration of cytoskeleton movements, the changes of cellular microenvironment structure for instance ECM degradation was also critical and important step for cancer cell migration and metastasis. Thus, we next step analyzed the association between ARHGAP44 genes and a signature composed of 23 ECM degradation related genes, and the result showed that significant correlation was discovered in 8 cancers including THYM, HNSC, KIRC, KIRP, CHOL, SKCM, THCA and LIHC (Fig. 7A-7H). Interestingly, THYM and THCA which were previously showed positive correlations between RHO GTPases proteins related cytoskeleton dynamics and ARHGAP44 gene, also presented consistent trends when it came to ECM degradation, demonstrating that ARHGAP44 participated in cancer cell metastasis process.
Meanwhile, significant correlation were also observed between ARHGAP44 expression and EMT signature in CHOL, KIRC, LIHC and THYM (Fig. 7I-7L), the correlation with multiple critical clinical cancer traits indicating ARHGAP44 works as a potential biomarker for cancer progression.

**ARHGAP44 correlates with multiple cancers stemness index**

Cancer stemness index is an indicator that evaluates the similarity between tumor cells and stem cells, which is related to the active biological processes in cancer cells as well as the lower degree of tumor differentiation[40]. Based on TCGA pan-cancer genes expression data, we calculated the mRNAsi in each cancer and evaluated its correlation with ARHGAP44 gene expression (Fig. 8A). And the result revealed that mRNAsi index score differed significantly between high-ARHGAP44 expression and low expression groups in multiple cancers including BLCA, BRCA, CESC, HNSC, KIRC, LIHC, LUAD and LUSC (Fig. 8B-8I), even though in some cancers for instance PAAD and STAD, the score difference was not statistical significant between groups (Fig. 8J, 8K).

**ARHGAP44 negatively correlates with cancer proliferation and cell cycle**

For more comprehensive understanding ARHGAP44 gene roles in important cancers clinical traits, the association between gene expression and 14 cancer proliferation related genes and 199 cell cycle representative G2M checkpoints were analyzed. And the results revealed that ARHGAP44 negatively correlates with cancer proliferation (Fig. 9A-9H) as well as cell cycle (Fig. 9I-9M) in ACC, LGG, LUAD, MESO, OV, PAAD, THYM and TGCT, demonstrating the diversity of cancer related genes functions.

**AARHGAP44 involves in multiple cancers immune infiltration landscape and immune checkpoints expression**

To investigate the immunological roles of ARHGAP44 in the cancer environment, we firstly ran CIBERSORT algorithm to obtain 22 tumor infiltration immunocytes (TIC) correlations with ARHGAP44. And as indicted in the distribution heatmap, strong positive correlations with multiples TICs for instance T cell CD4 + memory cells, monocytes, activated mast cells and naive B cells have been observed in multiple cancers, meanwhile, negative correlations were displayed with TICs for instance T cell helper, M0 and M1 macrophages in some cancers (Fig. 10A).

Since cytotoxic T lymphocyte (CTL) have been the main affected cells during tumors immunosuppression, the association between ARHGAP44 and CD8 + T cells distribution was next step evaluated. And the result indicated that ARHGAP44 correlated with the immune cell infiltration in multiple cancers, for instance, in PCPG, LGG, KICH and THCA, statistical significant positive correlation was displayed (Fig. 10B-10E), meanwhile, in other cancers, HNSC and THYM, negative correlation were
discovered, indicating the cancer specific effect ARHGAP44 has on immune cells infiltration (Fig. 10F, 10G).

Meanwhile, we also investigated ARHGAP44 association with CTL functions based on TIDE analysis. And the result revealed that CTL dysfunction levels significantly differs between high-ARHGAP44 expression and low expression samples in multiple cancers including LUAD, LUSC, SKCM, BLCA, KIRP, BRCA and KIRC, supporting the high value meanwhile cancer specific affection ARHGAP44 gene has on CTL functions in cancers (Fig. 11A-11G).

Besides estimating the association with immune cells infiltration, the association between ARHGAP44 gene and clinical promising immune checkpoints including PD-L1, CTLA4, TIGIT, TIM-3 and LAG-3 were evaluated. And statistical significant correlation has been discovered with multiple immune checkpoints in various cancers. Interestingly, opposite correlation was displayed with same immune checkpoint in different cancers, foe instance, positive correlation with CTLA4 was discovered in STAD, SKCM and LIHC, meanwhile, the correlation was revealed to be negative in OV, MESO, LUAD and LGG, demonstrating the varied function modes of ARHGAP44 gene has on different cancer immune modulation (Fig. 11H).

**Tumor mutation burden (TMB) and microsatellite instability (MSI) analysis**

TMB and MSI have not only been important clinical trait of cancers immune environment, but also useful biomarkers for predicting the potential benefit of cancer patients from immune targeting therapies[41]. The detection result of TMB and MSI directly affect clinical immunotherapy strategies. As for the correlation with MSI status, moderate positive and negative correlation was discovered in ACC and KICH respectively (Fig. 12A). Meanwhile, positive correlation was found between ARHGAP44 expression and TMB count in THYM, LAML and CHOL, and the association was turned to be negative in LUAD, LGG and PAAD and so on (Fig. 12B).

**ARHGAP44 affects the drug sensitivity in certain therapies**

Drug sensitivity has been a critical and also insurmountable problem in clinic cancer treatment, to explore whether ARHGAP44 would be able to predict therapeutic responses to cancers, the clinical treatment data based on ROCplotter was accessed to evaluate the association between the therapeutic outcomes and ARHGAP44 expression in certain cancer types. In BRCA, ARHGAP44 expression was much higher in responders comparing to non-responders after endocrine therapy using tamoxifen (Fig. 12C), meanwhile, the gene expression was showed to be lower in responders after receiving anti-Her-2 therapy using lapatinib (Fig. 12D), as well as after receiving chemotherapy Taxane and Anthracycline drugs (Fig. 12E, 12F).

Meanwhile, in colorectal cancer, ARHGAP44 gene expression was higher in responders comparing to non-responders after receiving chemotherapy Bevacizumab drug (Fig. 12G). And in GBM, also the responders
when comparing to non-responders samples showed higher ARHGAP44 expression after receiving antiangi and irinotecan (Fig. 12H, 12I), although the expression was lower in responders when receiving lomustine (Fig. 12J). The area under the curve (AUC) value of above projects were between 0.61 to 0.75, demonstrating faithful results.

Besides, based on RNAactDrug platform, the top 10 compounds correlated to ARHGAP44 mRNA expression with FDR < 0.05 were also displayed (Table 1). Although deeper experiments validation and clinical trials using these drugs against ARHGAP44 gene were needed before clinical application, the results shall provide promising insights for further researches.

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<th>Spearman.fdr</th>
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**Discussion**

Cancer has been one of the major health threats for people, and the initiation of cancer involves multiple steps including the accumulation of genetic mutations and alterations that promote the transition of normal cells to a progressive neoplastic state[2]. Over the years, the ten hallmarks of cancers have been well acknowledged including the uncontrolled cell proliferation, evading growth suppressors, resisting cell death, genome instability and mutation, inducing angiogenesis, enabling replicative immortality, and more threateningly activating invasion and metastasis which has been an major cause of cancer related death, as well as reprogramming of cellular energy metabolism, evading immune surveillance for destruction and tumor promoting inflammation[36].

Despite the fact that our understanding of cancers has indeed been keeping improving over the past decades including above better understanding of the interaction between cancer cells and surrounding
microenvironments, as well as the epidemiological risk factors for cancer occurrence, the genetic alterations most importantly the “driver” genes that not only initiates the cancer development but also relates with targeting therapies, the current situation of cancer is still challenging. Current knowledge of cancer is woefully lacking comparing to the highly heterogeneous, complicated and progressive cancer development. It’s of critical significance to keeps investigating the collaborative genetic networks in cancers and identifying potential disease causing gene alterations as well as promising drug targets.

Rho GTPases are a family of small G proteins that contributes to nearly all hallmarks of cancers including sustaining cell morphology and polarity, regulating cytoskeleton dynamics and cell motility, modulating cell growth and cell cycle progression, as well as affecting stem cell differentiation, cell survival and new gene expression. Although it has been emerging as a novel therapeutic target, the role of Rho GTPases proteins in different types of cancers are controversial[13]. Initially, Rho GTPase family proteins were proposed to activate cancer formation and progression, as RhoA, Rac1, and Cdc42 which have been the best characterized proteins were shown to be important for cell migration by controlling cell contraction and membrane protrusion, suggesting an crucial role in cancer cell invasion and metastasis[42, 43].

However, although several Rho GTPases expressions were indeed reported to be up-regulated in human cancers, activating mutations of Rho GTPases appear to be rare in human cancers, moreover, animal mice models also revealed that deletion of certain Rho GTPase genes do not promote a general tumor growth effect[44–46]. The fact that not all data obtained from culturing cells, animal models and patients samples coincide easily suggesting a complex and potentially diversified functions of Rho GTPases and modulating proteins in various human cancers emerges. A comprehensive and pan-cancer analysis of targeting gene involving multidimensional biological aspects of functions shall aid better understanding of the gene roles in cancers development.

In the study, we mainly focused on a GAP protein of Rho GTPases, namely ARHGAP44 which was first exposed to us from our previous studies that the gene was repeatedly suggested when we analyzed the different expressed genes in primary cancers comparing to normal control samples in several cancers[47–49]. And also we further realized that up to now, only very limited functions have been reported about the gene, considering its potential value, TCGA pan-cancer profiles and various bioinformatic analyzing tools were combine used to preliminary explore the roles of ARHGAP44 gene in cancers.

After the basic understanding of the physiochemical property of ARHGAP44 gene which supporting ARHGAP44 works as a cellular unstable and hydrophilic protein, the gene expression patterns as well as prognosis correlation were comprehensively investigated. We observed that the gene expression various in different types of cancers, the expression seemed to be down regulated in multiple cancers including BLCA, CESC, GBM, KIRP, LGG, LUAD, LUSC, OV, SKCM and UCEC. Interestingly, in LUAD and RIRP, high expression of ARHGAP44 was associated with better OS and longer RFS, although in BLCA, LUSC and CESC, the gene was related with worse OS and RFS survival. The seemingly “inconsistent” expression trend
and survival association indicates that a gene that is up regulated in cancer comparing to normal tissues doesn’t necessary mean that it’s also higher expressed in more advancing cancers, and also a gene’s gain/loss of expression in cancer vs normal samples should not be equal to a tumor promoter/inhibitor role. Genes’ functions and roles should be highly specific in different types of cancers.

For investigating the probable reasons for the altered expression of ARHGAP44 in different types of human cancers, common post transcription modulation including methylation and phosphorylation levels of ARHGAP44 gene in cancers were explored. And the analysis result supported that gene methylation and phosphorylation account for at least part of the altered expression of ARHGAP44 in cancers. Meanwhile, given that others types of genetic alterations including mutation ratio, protein structure variant and copy number variations also affect genes cellular activity and functions, we analyzed the ARHGAP44 gene alterations in cancers and discovered that a certain percentage of gene mutation which were mostly single nucleotide mutations, deletion and amplification have been observed in several types of cancers including SKCM, LUSC and READ. More importantly, not only ARHGAP44 gene itself occasionally mutated in cancers, but also the gene was related with the cellular DNA repair system.

DNA repair system was an important cellular adaption and response system to recover the DNA replication errors that were caused by different types of external stimuli and damages for instance environmental toxins, UV light, radiation therapy, chemotherapy and so on. The main methods for DNA repair system composed of base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MRR), homologous recombination repair (HRR) and non homologous end joining (NHEJ). Once the repair system started, the repair related proteins were recruited in the nucleus to repair deficient DNA which process could prevent cells in the S, G2, and M phases of the cell cycle. In the study, we discovered that ARHGAP44 correlated with MSI and HRR system in certain cancers including BLCA, PCPG, TGCT, THCA, CHOL, KIRP, PRAD and KICH, and negatively correlated with cell proliferation as well as G2M checkpoints related cell cycles in multiple cancers. The results partly support that ARHGAP44 as a crucial member of DNA repair system, which also assistants the maintaining of cancer stemness.

Being defined as a GAP for modulating the activity of RHO GTPases, for comprehensively analyzing the correlation between ARHGAP44 gene and the main RHO GTPase proteins in different types of cancers, we analyzed the association between the gene and each of the three main proteins including RHOA, RAC1 and CDC42, and discovered that ARHGAP44 gene correlates with different of the three proteins in individual cancer type, although in some cancers including PRAD, THCA and THYM, ARHGAP44 gene was revealed to correlate with all RHOA, RAC1 and CDC42 three RHO GTPases, indicating the complex meanwhile collaborative network of ARHGAP44 regulation on cancers cytoskeleton dynamics. Although comparing to the gene expression, it would be a lot be meaningful to evaluating the GTP or GDP bound state of the RHO GTPases and identifying the effect ARHGAP44 gene has on the state transition of the genes which would require extensively designed cell lines experiments, current results shall provide an useful direction for further experiments and researches design.
Moreover, besides the evaluation of ARHGAP44 gene correlation with cancer metastasis including above cytoskeleton related genes and ECM degradation associated genes analysis, the potential effect ARHGAP44 has in cancer microenvironment modulation, more specifically, the immune infiltration landscape was explored. Tumor microenvironment was able to exert great influence on tumor proliferation, metastasis, and even environment angiogenesis, especially, evading immune surveillance and tumor promoting inflammation have been well accepted cancer hallmarks. However, to date, the role of ARHGAP44 gens in cancers immune environment modulation has not been elucidated. Our results indicated ARHGAP44 has strong correlations with multiples TICs for instance T cell CD4 + memory cells, monocytes, activated mast cells and naive B cells in multiple cancers, and also CTL dysfunction levels significantly differs between high-ARHGAP44 expression and low expression samples. The results indicate the potential regulation ARHGAP44 gene has on cancers immune environment modulation.

For preliminary evaluating the potential of ARHGAP44 gene as a probable drug target, we explored the gene expression association with cancer patients response to certain therapies, and discovered that ARHGAP44 gene expression was indeed different in the responders and non-responders after receiving certain drugs. Although deeper in vitro experiments validation and clinical trials using these drugs against ARHGAP44 gene were needed before clinical application, the results shall provide promising insights for further researches.

**Conclusion**

In conclusion, our research unveiled the complex and comprehensive roles of ARHGAP44 gene in cancer progression and clinical outcome that shall benefit further researches. ARHGAP44 gene expression various in different cancers and correlates with the survival of certain cancers patients. Meanwhile, the gene was also supported to participate in the cellular DNA repair system via MMR or HRR and assistant maintaining cancer genome stability and stemness. Significantly, ARHGAP44 was involved in not only the modulation of cytoskeleton related cancer metastasis, but also cancer immunity. The results shall provide meaningful insights into better understanding of the molecular mechanism behind ARHGAP44 regulation on cancers development, even though comprehensive studies and biological experiments are needed to confirm the findings before promoting the clinical utility of the gene in further cancers treatment.

**Abbreviations**

TCGA cancer types: Bladder urothelial carcinoma (BLCA), Breast invasive carcinoma (BRCA), Cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), Cholangio carcinoma (CHOL), Colon adenocarcinoma (COAD), Lymphoid neoplasm diffuse large B-cell lymphoma (DLBC), Esophageal carcinoma (ESCA), Glioblastoma multiforme (GBM), Head and neck squamous cell carcinoma (HNSC), Kidney clear cell carcinoma (KIRC), Kidney renal papillary cell carcinoma (KIRP), Brain lower grade glioma (LGG), Liver hepatocellular carcinoma (LIHC), Lung adenocarcinoma (LUAD), Lung squamous cell carcinoma (LUSC), Thymoma (THYM), Ovarian serous cystadenocarcinoma (OV), Mesothelioma (MESO),
Skin cutaneous melanoma (SKCM), Pancreatic adenocarcinoma (PAAD), Pheochromocytoma and paraganglioma (PCPG), Prostate adenocarcinoma (PRAD), Rectum adenocarcinoma (READ), Stomach adenocarcinoma (STAD), Thyroid carcinoma (THCA), Uterine corpus endometrial carcinoma (UCEC), Uterine carcinosarcoma (UCS).

Other abbreviations: Protein-protein interaction network (PPI), Search Tool for the Retrieval of Interacting Genes (STRING), Overall survival rate (OS), Recurrence free survival rate (RFS), Extra cellular matrix (ECM), Homologous Recombination Deficiency (HRD), Homologous Recombination Repair (HRR), Epithelial Mesenchymal Transition (EMT), Tumor infiltrating cell (TIC), Cytotoxic T lymphocytes (CTLs), Tumor mutation burden (TMB), Microsatellite instability (MSI).

Declarations

Ethnic approval and consent to participate

All of the local hospital patients samples that were used in QPCR experiment were obtained from our hospital biobank (Second Hospital of ShanXi Medical University). Informed consent of the potential scientific application of the surgery samples have been obtained from patients at the same time they donated the samples to hospital Biobank, The paper agreement signed with the donors’ signatures were kept by the biobank committee. And the biobank cancers samples that were used in this research was approval by both the biobank committee and Hospital Institutional Board (Second Hospital of ShanXi Medical University, ShanXi Province, China). All methods were carried out in accordance with relevant guidelines and regulations or declaration of Helsinki.

Consent for publication

Not applicable

Availability of data and materials

TCGA pan-cancer profiles were analyzed in the study, which was downloaded from UCSC Xena. All data generated or analyzed during this study are included in this published article.

Competing interests

All of the authors agreed the publication of the paper and declare no conflicts of interests.

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Authors’ contributions

NS and HY designed the study and drafted the manuscript, contributed equally to the whole study. XW, JD, ZY, LM and SL performed the data collecting and analysis. LG and WM participated in data interpretation and study design, WM and CW were involved in the drafting and critical revision of manuscript. As the corresponding author, both WM and CW have full access to all data of the manuscript, CW made the eventual decision to submit the article for publication. All authors read and approved the final manuscript.

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References


**Figures**
Figure 1

ARHGAP44 gene expression pattern in pan-cancer and association with clinical pathological parameters

(A) UALCAN prediction of ARHGAP44 gene expression in human pan-cancers comparing to corresponding normal control samples based on TCGA profiles. The various expression of ARHGAP44 in different subtypes of a single cancer, including in (B) LUAD, (C) BRCA and (D) KIRC. Expression trends of
ARHGAP44 based on the advancing of cancer stages in (E) BLCA, (F) LUAD, (G) READ, (H) BRCA, (I) LUSC, (J) KIRC. (*p<0.05, **p<0.01, ***p<0.001. The first layer * which is right above the error bar representing comparison to normal group, and the above layers *which were above a secondary line represent the comparison between corresponding groups that were covered by the line).
The association between ARHGAP44 and pan-cancer patients survival

The association between ARHGAP44 gene and patients overall survival (left graphic) as well as recurrence free survival (right graphic) in (A) LUAD, (B) READ, (C) PAAD, (D) KIRP, (E) LUSC, (F) CESC, (G) BRCA and (H) ESCA. (p<0.05 was considered statistical significant.)
The promoter methylation and phosphorylation modulation of ARHGAP44 gene expression in cancers

The promoter methylation level of ARHGAP44 gene in (A) BRCA, (B) KIRC, (C) COAD, (D) GBM, (E) CESC, (F) HNSC, (G) PCPG, (H) THYM, (I) CHOL, (J) PRAD, (K) READ, (L) THCA, (M) BLCA and (N) UCEC comparing to in corresponding normal samples. The total protein expression (left graphic) and phosphorylated protein expression (right graphic) of ARHGAP44 gene in (O) BRCA, (P) LUAD, (Q) LUSC and (R) KIRC. (*p<0.05, **p<0.01, ***p<0.001)
Figure 4

ARHGAP44 gene variation and association with HRR related gene signature in pan-cancer

(A) Different type of ARHGAP44 gene variations in human cancers, and (B) the mutation detection results revealed by cBioPortal dataset. The association between ARHGAP44 gene and HRR gene signature in (C) PCPG, (D) TGCT, (E) THCA, (F) CHOL, (G) KIRP, (H) UCS, (I) SARC, (J) PRAD, (K) KICH and (L) BLCA. (R>0.30 was considered correlated, R between 0.30~0.59 was considered preliminary correlated, and 0.60~0.79 was moderate correlated, meanwhile, R>0.80 was thought as strongly correlated).
Figure 5

ARHGAP44 interacting genes enrichment analysis and association with cytoskeleton related gene signature

(A) The PPI network which is centered on ARHGAP44 gene (left graphic) for analyzing the main biological functions ARHGAP44 and its interacting genes participated in (right table). The association
between ARHGAP44 and cytoskeleton related gene signature in (B) TGCT, (C) THCA, (D) THYM, (E) KICH, (F) KIRP, (G) LUSC, (H) PCPG, (I) PAAD and (J) UCSC. (R>0.30 was considered correlated, R between 0.30~0.59 was considered preliminary correlated, and 0.60~0.79 was moderate correlated, meanwhile, R>0.80 was thought as strongly correlated).
Association between ARHGAP44 and three main Rho GTPases in cancers

ARHGAP44 association with Rho GTPase RHOA in (A) KICH, (B) LGG, (C) UCS, (D) TGCT, (E) THCA, (F) THYM, (G) PRAD and (H) DLBC.

ARHGAP44 association with Rho GTPase RAC1 in (I) MESO, (J) PRAD, (K) THCA and (L) THYM.

ARHGAP44 association with Rho GTPase CDC42 in (M) CHOL, (N) KICH, (O) KIRP, (P) UCS, (Q) THCA, (R) THYM, (S) PRAD and (T) TGCT. (R between 0.30~0.59 was considered preliminary correlated, and 0.60~0.79 was moderate correlated, meanwhile, R>0.80 was thought as strongly correlated).
Figure 7

**ARHGAP44 association with ECM degradation and EMT transition related gene signature in cancers**

ARHGAP44 association with ECM degradation related gene signature in (A) THYM, (B) HNSC, (C) KIRC, (D) KIRP, (E) SKCM, (F) CHOL, (G) THCA and (H) LIHC.

ARHGAP44 association with EMT transition related gene signature in (I) KIRC, (J) CHOL, (K) LIHC and (L) THYM. (R between 0.30~0.59 was considered preliminary correlated, and 0.60~0.79 was moderate correlated, meanwhile, R>0.80 was thought as strongly correlated).
Figure 8

Cancers mRNAsi stemness score distribution in different ARHGAP44 gene expressed samples

(A) ARHGAP44 association with mRNAsi score in different cancers. Comparison of mRNAsi score in high-ARHGAP44 and low expression groups in (B) BLCA, (C) BRCA, (D) CESC, (E) HNSC, (F) KIRC, (G) LIHC, (H) LUAD, (I) LUSC, (J) PAAD and (K) STAD. (*p<0.05, **p<0.01, ***p<0.001)
ARHGAP44 association with tumor proliferation and G2M checkpoints related gene signature in cancers

ARHGAP44 association with tumor proliferation related gene signature in (A) ACC, (B) LGG, (C) LUAD, (D) MESO, (E) PAAD, (F) THYM, (G) UVM and (H) OV. ARHGAP44 association with G2M checkpoints related gene signature in (I) LGG, (J) PAAD, (K) LUAD, (L) MESO and (M) THYM. (R between 0.30~0.59 was
considered preliminary correlated, and 0.60~0.79 was moderate correlated, meanwhile, R>0.80 was thought as strongly correlated).

Figure 10

ARHGAP44 association with TIC distribution and CD8+T cells infiltration in cancers
(A) Association between ARHGAP44 gene expression and 22 TICs distribution in pan-cancer. Association between ARHGAP44 gene and CD8+T cell infiltration in (B) PCPG, (C) LGG, (D) KICH, (E) THCA, (F) HNSC and (G) THYM. (R between 0.30~0.59 was considered preliminary correlated, and 0.60~0.79 was moderate correlated, R>0.80 was thought as strongly correlated).

Figure 11
ARHGAP44 association with CTL dysfunction and immune checkpoints expression in cancers

Association between ARHGAP44 gene CTL function status in (A) LUAD, (B) LUSC, (C) BLCA, (D) KIRC, (E) KIRP, (F) BRCA and (G) SKCM. (H) Association between ARHGAP44 gene expression and immune checkpoints expression in pan-cancer. (p<0.05 was considered statistical significant.)

Figure 12
ARHGAP44 association with cancers MSI and TMB as well as certain drugs sensitivity

ARHGAP44 association with tumor (A) MSI and (B) TMB status in cancers. ARHGAP44 gene expression difference between breast cancer responders and non-responders patients as well as the generated AUC curve after using (C) endocrine therapy Tamoxifen, (D) anti-Her-2 therapy Lapatinib, (E) chemotherapy Taxane, (F) Anthracycline. ARHGAP44 gene expression difference between colorectal cancer responders and non-responders patients as well as the generated AUC curve after using (G) Bevacizumab. ARHGAP44 gene expression difference between GBM responders and non-responders patients as well as the generated AUC curve after using (H) antiangio, (I) irinotecan and (J) lomustine. (*p<0.05, **p<0.01, ***p<0.001)

Supplementary Files

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- Supplementarytables.doc