Expression patterns and prognostic value of \textit{RUNX} genes in kidney cancer

Ke Gao\textsuperscript{1,2,6,7}, Fang Zhang\textsuperscript{3,7}, Ke Chen\textsuperscript{6,7}, Wei Li\textsuperscript{2}, Yi-Bing Guan\textsuperscript{2}, Meng-Lu Xu\textsuperscript{5}, Tie Chong\textsuperscript{2,3} & Zhi-Ming Dai\textsuperscript{1,3}

Kidney cancer is the third most common malignancy of the urinary system, of which, kidney renal clear cell carcinoma (KIRC) accounts for the vast majority. Runt-related transcription factors (RUNX) are involved in multiple cellular functions. However, the diverse expression patterns and prognostic values of \textit{RUNX} genes in kidney cancer remained to be elucidated. In our study, we mined the DNA methylation, transcriptional and survival data of \textit{RUNX} genes in patients with different kinds of kidney cancer through Oncomine, Gene Expression Profiling Interactive Analysis, UALCAN, Kaplan–Meier Plotter, cBioPortal and LinkedOmics. We found that \textit{RUNX1} and \textit{RUNX3} were upregulated in KIRC tissues compared with those in normal tissues. The survival analysis results indicated a high transcription level of \textit{RUNX1} was associated with poor overall survival (OS) in KIRC patients. Furthermore, KIRC tumor tissues had significantly lower levels of \textit{RUNX1} promoter methylation than that in paracancerous tissues, with decreased DNA methylation of \textit{RUNX1} notably associated with poor OS in KIRC. In conclusion, our results revealed that \textit{RUNX1} may be a potential therapeutic target for treating KIRC, and \textit{RUNX1} promoter methylation level shows promise as a novel diagnostic and prognostic biomarker, which laid a foundation for further study.

Kidney cancer is the third commonest malignancy of the urinary system and has morbidity and mortality rates of 2.2% and 1.8%, respectively.\textsuperscript{1} Kidney renal clear cell carcinoma (KIRC) is the most common kidney cancer, accounting for 80–90% of total renal cell carcinoma (RCC) cases.\textsuperscript{2} Due to the lack of specific clinical symptoms in the early stage, about 30% of the patients with this disease have metastasis at the time of diagnosis, almost 40% of which are prone to postoperative recurrence.\textsuperscript{3} Meanwhile, advanced metastatic or recurrent RCC has limited treatment, since it is not sensitive to radiation nor chemotherapy. Though targeted therapy has modest improvement over previous cytokine therapies, the outlook for high-risk patients remains poor. Therefore, it is urgent to identify biomarkers for early diagnosis and prognosis of RCC. Epigenetic alterations, such as abnormal DNA methylation, play an important role in the development and progression of kidney cancer, thus, they are considered as potential biomarkers for RCC early diagnosis and for monitoring prognosis.

Runt-related transcription factors (RUNX) are named after the discovery of the developmental regulatory gene runt. They have been reported to be vital in leukemia and solid tumors derived from different organs. To date, RUNX family members (\textit{RUNX1, RUNX2, RUNX3}) have been revealed in diverse developmental process, including cell proliferation, differentiation and apoptosis.\textsuperscript{4} Abnormal methylation status of \textit{RUNX} genes has been found in several cancers including prostate cancer,\textsuperscript{5} esophageal cancer\textsuperscript{6} and breast cancer,\textsuperscript{7} which may affect their expression levels. Some studies considered \textit{RUNX1} as not only a tumor-suppressive factor but also an oncogenic factor.\textsuperscript{8–10} For instance, in lung cancer, missing \textit{RUNX1} was found to result in enhanced proliferation, migration, and invasion of tumor cells,\textsuperscript{11} while it was regarded as a tumor suppressor connected with stabilization of Axis inhibition protein 1 (\textit{AXIN1}) expression.\textsuperscript{12} Meanwhile, high \textit{RUNX1} expression level was found correlating with poor prognosis in triple-negative breast cancer.\textsuperscript{13} Furthermore, the evidence has proved that RUNX proteins...
displayed various post-translational modifications such as acetylation\textsuperscript{14}, methylation\textsuperscript{15}, phosphorylation\textsuperscript{16} and sumoylation\textsuperscript{17}. All of these contribute to the functional complexity of RUNX genes.

So far, a few studies have investigated the expression profiles of RUNX genes in RCC\textsuperscript{18,19}. However, the relationship between the expression of RUNX genes and the onset, progression and prognosis of RCC remains controversial. Therefore, our study aimed to analyze the connection between the expression levels of RUNX genes and clinicopathological parameters of RCC especially KIRC patients by multi-dimensional analysis methods, and conducted a preliminary analysis of their regulation and potential functions.

Materials and methods

Pan-cancer analysis of expression levels of RUNX genes by Oncomine and gene expression profiling interactive analysis (GEPIA). Initially, pan-cancer analysis was performed to assess the transcriptional levels of RUNX genes in different types of cancer and corresponding normal tissues using Oncomine, including 460, 450 and 447 total unique analyses for RUNX1, RUNX2 and RUNX3 respectively. Oncomine (https://www.oncomine.org/) is a platform that provides solutions for individual researchers and multinational companies with robust peer-reviewed analysis methods as well as a powerful set of analysis functions that compute gene expression signatures, clusters and gene-set modules and that allow extracting biological insights from the data automatically. This database contains 715 datasets, 86,733 normal and tumor samples\textsuperscript{20}. Then, we utilized GEPIA in this study to verify the relationship between the expression of RUNX genes and kidney cancer. GEPIA (http://gepia.cancer-pku.cn/) is an interactive web server for analyzing the RNA sequencing expression data of 9736 tumors and 8587 normal samples from the TCGA and the GTEx projects by using a standard processing pipeline\textsuperscript{21}. In prognostic analysis, we grouped the patients by the median as a cut-off value.

UALCAN, cBio cancer genomics portal (cBioPortal) and CPTAC analyses. Then we obtained and downloaded the clinical and FPKM-standardized RNA-seq data and the clinical information of 531 KIRC patients from the TCGA database. The clinical data were preprocessed by removal of samples without survival status and patients with survival time less than 30 days were also excluded because they might die of non-cancer-related diseases. Furthermore, we performed univariate and multivariate Cox regression analysis to demonstrate the correlations between OS and clinical variables.

After the pan-cancer analysis, we explored the relationship between promoter methylation status and its mRNA expression of RUNX1 gene, using UALCAN and cBioPortal database. UALCAN (http://ualcan.path.ual.edu/index.html) is an interactive web resource for analyzing cancer data and providing gene expression analysis by using TCGA datasets (https://www.cancer.gov)\textsuperscript{22}. The Beta value indicates level of DNA methylation ranging from 0 (unmethylated) to 1 (fully methylated). Different beta value cut-off has been considered to indicate hypermethylation (Beta value: 0.7–0.5) or hypo-methylation (Beta-value: 0.3–0.25)\textsuperscript{23,24}. The cBioPortal (http://www.cbioportal.org/) is an open access for interactive exploration of multiple cancer genomics data sets, currently covering 282 cancer researches\textsuperscript{25}. The National Cancer Institute's Clinical Proteomic Tumor Analysis Consortium (CPTAC, https://proteomics.cancer.gov/) is a public data portal of proteomic sequence and corresponding genomic sequence datasets. Using CPTAC, we mined the protein expression of RUNX genes.

LinkedOmics analysis and gene set enrichment analysis (GSEA). After confirming that RUNX1 was associated with prognosis of KIRC, we preliminarily analyzed its specific cytopathological function and molecular mechanism. LinkedOmics (http://linkedomics.org/login.php) is a publicly available portal that includes multi-omics data from all 32 TCGA cancer types\textsuperscript{26}. LinkedOmics was used to investigate the transcription networks of RUNX1 in KIRC. Data from the LinkFinder results were signed and ranked, meanwhile GSEA was used to perform Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways\textsuperscript{27}, transcription factor-target enrichment and kinase-target enrichment. The statistical analyses were based on the Molecular Signatures Database (MSigDB)\textsuperscript{28}, 1000 simulations were performed and False discovery rate (FDR < 0.05) was used to select significantly enriched gene sets.

Ethical approval. No ethical approval was obtained because this study did not involve a clinical evaluation, did not involve laboratory animals and invasive procedures.

Results

Analysis of expression level of RUNX genes in pan-cancer. As shown in Fig. 1, pan-cancer analysis results showed that RUNX1 increased significantly in 58 datasets, especially those of leukemia, head and neck cancer, colorectal cancer, kidney cancer, and breast cancer. As for RUNX2, 24 datasets displayed increased expression, whereas 14 datasets showed the opposite results. Similarly, higher expression of RUNX3 was found in 19 datasets, while lower expression was found in 10 datasets. Overall, the obtained results indicated significantly elevated expression of RUNX1 (with fold changes of 6.58 to 6.51) and RUNX3 (with fold changes of 2.7 to 8.32) in kidney cancer (Table 1).

Verification of expression of RUNX genes in kidney cancer. To further verify differential expression of RUNX genes in kidney cancer, we compared expression profiles of each gene between different kinds of kidney cancer samples and paired normal tissues by GEPIA. The results indicated that RUNX1 and RUNX3 were significantly overexpressed in KIRC (Fig. 2A, C). For RUNX2, no significant expression difference was found (Fig. 2B). Furthermore, the relationship between expressions of RUNX1, RUNX2 and RUNX3 and prognosis of KIRC were performed by GEPIA and the results revealed that higher expression of RUNX1 had poorer overall
Figure 1. Oncomine analysis of the mRNA expression levels of RUNX genes in different cancers. The differences in expression levels of genes between cancer and normal tissues are concluded. The thresholds (p-value < 0.01, fold change > 2; gene rank < 10%; data type: mRNA) are indicated in the colored cells. Red cells represent overexpression of the target gene in tumor tissues compared to normal tissues, while blue cells indicate downregulation of the gene. Gene rank is depicted by the color depth in the cells.

Table 1. Differential expression level analysis of RUNX genes in different types of kidney cancer. KIRC kidney renal clear cell carcinoma, KIRP kidney renal papillary cell carcinoma, NHRCC non-hereditary renal clear cell carcinoma, HRCC hereditary renal clear cell carcinoma, KICH kidney chromophobe renal cell carcinoma.
survive (OS) in kidney cancer patients ($p < 0.001$, Fig. 2D). Especially, higher expression of RUNX1 was also found correlated to poor prognosis in KIRC ($p < 0.001$, Fig. 2E) and KIRP patients ($p = 0.024$, Fig. 2G), whereas there was no significant relation between RUNX1 and OS in KICH patients ($p = 0.27$, Fig. 2F). Besides, RUNX2 expression showed significant correlation to the prognosis of KIRC patients ($p = 0.046$, Fig. 2H), while RUNX3 did not ($p = 0.77$, Fig. 2I).

Table 2. The clinical characteristics about RUNX1 expression in KIRC patients. FPKM: Fragments per Kilobase Million.

| Characteristic | RUNX1 FPKM | % |
|----------------|------------|---|
| Numbers of patients, n | 531 | |
| Median age, years | 61 (26–90) | |
| Gender, n | | |
| Male | 344 | 64.78 |
| Female | 187 | 35.22 |
| Age, n | | |
| < 65 years | 332 | 62.52 |
| ≥ 65 years | 199 | 37.48 |
| Race, n | | |
| White | 462 | 87.01 |
| Non-white | 64 | 12.05 |
| Not known | 5 | 0.94 |
| TNM Stage, n | | |
| Stage I + II | 324 | 61.02 |
| Stage III + IV | 207 | 38.98 |
| Grade, n | | |
| Grade I + II | 243 | 45.76 |
| Grade III + IV | 282 | 33.11 |
Stage and age were significantly associated with OS in KIRC patients. (A) Univariate Cox regression analysis of correlations between OS and clinical variables. (B) Multivariate Cox regression analysis of correlations between OS and clinical variables.

**Correlation analysis between RUNX1 and clinicopathological characteristics in KIRC.** Table 2 showed the basic clinical characteristics. In total, we identified 531 KIRC patients with RUNX1 expression data and clinical information. The results showed that RUNX1 was highly expressed in female patients and white race. Higher RUNX1 expression was associated with advanced TNM stage and poor histological grade stage. The results were consistent with that RUNX1 might be an unfavorable factor for KIRC patients. Furthermore, we performed Cox regression analysis, and results showed stage and age were significantly associated with OS in KIRC patients (Fig. 3A). Then Multivariate Cox regression analysis showed that stage was an independent factor influencing KIRC prognosis (Fig. 3B).

**Analysis of promoter methylation status and protein expression of RUNX1 gene in KIRC.** To explore the hinge of RUNX1 expression, we investigated the promoter methylation level of RUNX1 in KIRC by UALCAN. Twelve probes in RUNX1 promoter were used for detecting DNA methylation level of RUNX1 (Fig. 4A). Notably, primary tumor tissues had obviously lower promoter methylation levels than normal tissues (p < 0.001, Fig. 4B). Meanwhile, it was a significant inverse correlation between DNA methylation level of RUNX1 gene and its mRNA expression in KIRC samples (Spearman = −0.69, p = 1.33e−46; Pearson = −0.60, p = 1.9e−32, Fig. 4C) based on cBioPortal analysis. The results indicated that upregulated expression of RUNX1 was associated with DNA hypomethylation, and it could be considered as a risk factor for KIRC. Surprisingly, the prognosis analysis showed that patients with promoter hypomethylation of RUNX1 had a worse OS (p < 0.001, Fig. 4D). Furthermore, we compared the protein expression of RUNX1 in normal and KIRC tissues, verifying that the expression level of RUNX1 protein was indeed significantly elevated in KIRC (p < 0.001, Fig. 4E).

**Enrichment analysis of RUNX1 gene functional networks in KIRC.** As shown in the volcano plot using LinkedOmics analysis (Fig. 5A), red spots represented genes positively correlated with RUNX1 in KIRC, while green spots represented genes with a negative correlation. Furthermore, the top 50 significant gene correlated positively or negatively with RUNX1 were shown in the heat maps (Fig. 5B, C), which suggested a widespread impact of RUNX1 in the transcription. In addition, GSEA showed varying expression of RUNX1 gene mainly in the receptor complex, cell-substrate junction and apical part of cell, which primarily participated in adaptive immune response, leukocyte migration and protein targeting. They played a key role in receptor-ligand activity, protein tyrosine kinase activity, transferase activity, and transferring acyl groups (Fig. 6A–C). KEGG pathway analysis showed that the differentially expressed genes were mainly enriched in proteoglycans in cancer, microRNAs in cancer, focal adhesion and peroxisome (Fig. 6D–F). To further explore the targets of RUNX1 gene in KIRC, we analyzed the transcription factors and kinase targets of positively correlated gene sets generated by GSEA (Table 3). The top 3 most significant kinase target networks related to LYN proto-oncogene, p21 activated kinase 1 (PAK1) and HCK proto-oncogene 3. The transcription factor target network was mainly related with ETS1, NFKAPPAB, MZF1, IRF, SRF.

**Discussions**
The RUNX family has been noticed to play an important role in leukemia and solid tumors. Known as one of the most frequently mutated genes in human leukemia, RUNX1 is originally identified to have a role in hematopoiesis29. Increasingly, it has been implicated in cancers of ovary30, prostate31 and stomach32, which was associated with either gain or loss of RUNX1 function. Recently, Yang et al. revealed that higher RUNX1 expression may be associated with poorer survival in RCC33; this was later confirmed by Rooney et al. who utilized a genetically engineered mouse (GEM) model18. According to a recent research from Kamikudo et al., as the RUNX family has a mechanism to compensate for loss among the family members, it is difficult to individually inhibit RUNX family proteins, RUNX family cluster regulation might be a cancer treatment strategy34. However, As Lie-a-ling mentioned, although it has become evident that expression level of RUNX1 can be used as a marker of tumor progression35, it is not yet fully uncovered how the alteration contributes to tumorigenesis, since both the amount and activation status of proteins can have effects. Therefore, we performed comprehensive analyses of the expression levels of RUNX genes in kidney cancer.
Our study showed that compared to normal kidney tissues, the expression levels of \textit{RUNX1} and \textit{RUNX3} were increased in KIRC cases. Prognostic studies further suggested that higher expression of \textit{RUNX1} gene was significantly associated with poorer OS in KIRC. Interestingly, the lower promoter methylation level of the \textit{RUNX1} gene was found to be significantly related to its higher mRNA and protein expression and, consequently, to the poorer OS of KIRC. In a research reported by Matsumura T et al., defection of \textit{RUNX1} methylation in hematopoietic stem cells (HSCs) was found to inhibit apoptosis and provide cells with a growth advantage, which is one

**Figure 4.** DNA methylation aberration of \textit{RUNX1} in KIRC. (A) Probes for detecting DNA methylation of \textit{RUNX1} promoter. (B) UALCAN analysis about the promoter methylation levels of \textit{RUNX1} in KIRC and normal samples. (C) The correlation analysis between the promoter methylation level of \textit{RUNX1} and its expression level based on cBioPortal database. (D) Correlation analysis between the promoter methylation level of \textit{RUNX1} and overall survival in KIRC patients by LinkedOmics. The blue line indicated low methylation level of \textit{RUNX1}, while the red line indicated high level. (E) CPTAC analysis about the comparison of \textit{RUNX1} protein expression between normal and KIRC tissues. KIRC: kidney renal clear cell carcinoma.

**Figure 5.** Genes differentially expressed in correlation with \textit{RUNX1} in KIRC by LinkedOmics. (A) Volcano plots in analyzing differential expression genes correlated with \textit{RUNX1} in KIRC. (B, C) Heat maps showing genes positively and negatively correlated with \textit{RUNX1} in KIRC (TOP 50). Red indicates positively correlated genes and blue indicates negatively correlated genes.
Figure 6. Significantly enriched GO annotations and KEGG pathways of RUNX1 in KIRC were analyzed using GSEA. (A) Cellular components. (B) Biological processes. (C) Molecular functions. (D) KEGG pathway analysis (www.kegg.jp/kegg/kegg1.html). KEGG pathway annotations of the cell cycle pathway. (E) Peroxisome biogenesis, (F) MicroRNAs in cancer. Red marked nodes are associated with the Leading Edge Gene.
cos et al. found that RUNX3 methylation-mediated expression regulation has been observed to play a role in leukemia and solid tumors. Mar-

cos et al. found a connection between higher level of RUNX3 methylation and poorer OS in KIRC

Table 3. Targets transcription factor and kinase of RUNX1 gene in KIRC from Linkedomics. TF transcription factor, FDR false discovery rate, *: FDR < 0.05.

of the important mechanisms to prevent the proliferation of damaged cells and maintain the genomic integrity after DNA damage. Also, hypomethylation in some sites of RUNX1 can influence the transcription activity, though it remained unclear how promoter methylation of RUNX1 affects the expression status. For RUNX3, the methylation-mediated expression regulation has been observed to play a role in leukemia and solid tumors. Mar-

Recent studies have found overexpression of RUNX1 in mouse models of kidney fibrosis, which is related to KIRC, indicating RUNX1 has a regulation of TGFβ-driven epithelial-to-mesenchymal transition (EMT). Recently, Young et al. indicated that RUNX1, which had a relationship with multiple signaling pathways including JAK/STAT, MAPK, p53 and VEGF, could be recognized as a novel therapeutic target and prognostic factor. Kamikudo et al. found that moderate inhibition of RUNX1 most significantly increased the total level of RUNX1 family through "genetic compensation of RUNX family transcription factors", emphasized the role of RUNX1 in tumorigenesis. Furthermore, Zhao et al. manifested that PRMT1-dependent methylation of RUNX1 likely contributed to its inhibitory activity. Considering our findings, patients with higher transcription levels of RUNX1 had worse prognosis, which corresponded to lower levels of promoter methylation. The results suggested that the promoter methylation of RUNX1 occurred in KIRC cases and deserved to be seen as a potential diagnostic and prognostic marker.

Previous studies suggested that RUNX1 was critical in a variety of genes transcription and played a role in cellular regulation. It has been recognized that genomic instability and mutagenesis are essential features of cancer cells, and kinases and their associated signaling pathways help stabilize and repair genomic DNA. Ballissimo et al. noticed that cells with inefficient RUNX1 showed defects in DNA repair, including base excision, homologous recombination and DNA interstrand crosslink repair. Sanoji et al. also proved that RUNX1 was involved in cell cycle arrest and apoptosis, as a potential factor in cancer formation. In order to clarify the role of RUNX1 in KIRC, we tried to locate its target kinases and transcription factors by LinkedOmics, and found an extensive connection with them, indicating that RUNX1 was rather involved in cellular regulation. Furthermore, GSEA was performed to identify significantly enriched or depleted groups of genes. The results showed that RUNX1 was mainly responsible for adaptive immune response, leukocyte migration and protein targeting. In addition, RUNX1 mainly participated in peroxisome, microRNA and proteoglycans in cancers. We assume that these participations are likely to make RUNX1 an initiator of KIRC.

In conclusion, we performed an integrated analysis about the expression and prognostic value of RUNX genes in kidney cancer. Our results showed that RUNX1 and RUNX3 were upregulated in the tissues of KIRC compared to the normal ones. Furthermore, the results revealed that RUNX1 was a potential therapeutic target for KIRC, and that lower promoter methylation level of RUNX1 indicated poorer survival. Generally, we assumed RUNX1 may be a potential diagnostic and prognostic marker for KIRC, and its abnormal promoter methylation may participate in tumorigenesis, which lay a foundation for further study.

Data availability
Patients data were acquired from Oncomine (https://www.oncomine.org/), GEPIA2 (http://geopia2.cancer-pku.cn), cBioportal (https://www.cbioportal.org/), UALCAN (http://ualcan.path.uab.edu), KM Plotter (http://kmplot.com/analysis/), LinkedOmics (http://linkedomics.org) and KEGG (www.kegg.jp/kegg/kegg1.html) database tool. We have referred expression profiling, DNA methylation level and protein expression profile of RUNX genes.

Received: 21 January 2021; Accepted: 7 July 2021
Published online: 22 July 2021

References
1. Freddie, B. et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: Cancer J. Clin. 68(6), 394–424 (2018).
2. Aristotle. B. et al. Current clinical practice guidelines for the treatment of renal cell carcinoma: A systematic review and critical evaluation. Oncology 22(6), 667 (2017).
3. Choueiri, T. K. et al. Preliminary results for avelumab plus axitinib as first-line therapy in patients with advanced clear-cell renal-cell carcinoma (JAVELIN Renal 100): An open-label, dose-finding and dose-expansion phase 1b trial. Lancet Oncol. 19(4), 451–460 (2018).
4. Woosuk, S. & Ichiro, T. The roles of RUNX family proteins in development of immune cells. Mol. Cells 43(2), 107 (2020).
5. Richardi, L. et al. Promoter methylation in APC, RUNX3, and GSTP1 and mortality in prostate cancer patients. J. Clin. Oncol.: Off. J. Am. Soc. Clin. Oncol. 27(19), 3161–3168 (2009).
6. Lu, Y., Zabihula, B., Yibulayin, W. & Liu, X. Methylation and expression of RECK, P53 and RUNX genes in patients with esophageal cancer. Oncol. Lett. 14(5), 5293–5298 (2017).
7. Zheng, J. et al. DNA methylation affects metastasis of renal cancer and is associated with TGF-β/RUNX3 inhibition. Cancer Cell Int. 18(1), 56 (2018).
8. Blyth, K., Cameron, E. R. & Neil, J. C. The RUNX genes: Gain or loss of function in cancer. Nat. Rev. Cancer 5(32), 376–387 (2005).
9. Yoshiaki, I., Suk-Chul, B. & Huey, C. L. S. The RUNX family: Developmental regulators in cancer. Nat. Rev. Cancer 15(2), 81–95 (2015).
10. Neil, J. C. et al. The RUNX genes as conditional oncogenes: Insights from retroviral targeting and mouse models. Adv. Exp. Med. Biol. 962, 247–264 (2017).
11. Shimizu, M. et al. Loss of RUNX1/AML1 arginine-methylation impairs peripheral T cell homeostasis. Br. J. Haematol. 170(6), 859–873 (2015).
12. Mercado-Matos, J., Matthew-Onabanjo, A. N. & Shaw, L. M. RUNX1 and breast cancer. Oncotarget 8(23), 36934 (2017).
13. Nym-Osor, C. et al. RUNX1 prevents oestrogen-mediated AXIN1 suppression and β-catenin activation in ER-positive breast cancer. Nat. Commun. 7, 1–12 (2016).
14. Bai, S. C. Transforming growth factor-β Stimulates p300-dependent RUNX3 acetylation, which inhibits ubiquitination-mediated degradation. J. Biol. Chem. 279(28), 29409–29417 (2004).
15. Zhao, X. et al. Methylation of RUNX1 by PRMT1 abrogates SIN3A binding and potentiates its transcriptional activity. Cold Spring Harbor Lab. Press 22(5), 640–653 (2008).
16. Hong, G. & Friedman, F. A. D. Phosphorylation of RUNX1 by cyclin-dependent kinase reduces direct interaction with HDAC1 and HDAC3. J. Biol. Chem. 286(1), 208–215 (2011).
17. Kim, J. H. et al. RUNX family members are covalently modified and regulated by PIAS1-mediated sumoylation. Oncogenesis 3, e101 (2014).
18. Rooney, N. et al. RUNX1 is a driver of renal cell carcinoma correlating with clinical outcome. Cancer Res. 80(11), 2325–2339 (2020).
19. Feifei, C. et al. RUNX3 regulates renal cell carcinoma metastasis via targeting miR-6780a-5P/E-cadherin/EMT signaling axis. Oncotarget 8(60), 101042 (2017).
20. Rhodes, D. R. et al. Oncomine 3.0: Genes, pathways, and networks in a collection of 18,000 cancer gene expression profiles. Neoplasia 9(2), 166–180 (2007).
21. Zafar, T. et al. GEIPA: A web server for cancer and normal gene expression profiling and interactive analyses. Nucl. Acids Res. 45(W1), W98–W102 (2017).
22. Chandrahaske, D. S. et al. UALCAN: A portal for facilitating tumor subgroup gene expression and survival analyses. Neoplasia 19(8), 649–658 (2017).
23. Men, C. et al. Identification of DNA methylation associated gene signatures in endometrial cancer via integrated analysis of DNA methylation and gene expression systematically. J. Gynecol. Oncol. 28(6), e83 (2017).
24. Shimavi, T. et al. DNA methylation profiles of long- and short-term glioblastoma survivors. Epigenetics 8(2), 149–156 (2013).
25. Cerami, E. et al. The cBio cancer genomics portal: An open platform for exploring multidimensional cancer genomics data. Cancer Discov. 2(5), 401–404 (2012).
26. Vasaikar, S. V., Peter, S., Jing, W. & Ring, Z. LinkedOmics: Analyzing multi-omics data within and across 32 cancer types. Nucl. Acids Res. 46(D1), D956–D963 (2018).
27. Kanehisa, M., Furumichi, M., Sato, Y., Ishiguro-Watanabe, M. & Tanabe, M. KEGG: Integrating viruses and cellular organisms. Nucl. Acids Res. 49(D1), D545–D551 (2021).
28. Libezon, A. et al. Molecular signatures database (MSigDB) 3.0. Bioinformatics 27(12), 1739–1740 (2011).
29. Okuda, T., Van Deursen, J., Hiebert, S. W., Grosveld, G. & Downing, J. R. AML1, the target of multiple chromosomal translocations in human leukemia, is essential for normal fetal liver hematopoiesis. Cell 84(2), 321–330 (1996).
30. Myriam, A. et al. Acute myeloid leukemia fusion proteins deregulate genes involved in stem cell maintenance and DNA repair. J. Clin. Investig. 112(11), 1751–1761 (2003).
31. Ken-ichi, T. et al. RUNX1, an androgen- and EZH2-regulated gene, has differential roles in AR-dependent and -independent prostate cancer. Oncotarget 6(4), 2263 (2015).
32. Yoshihide, M. et al. RUNX1 positively regulates the ErbB2/HER2 signaling pathway through modulating SOS1 expression in gastric cancer cells. Sci. Rep. 8(1), 1–13 (2018).
33. Fu, Y., Sun, S., Man, X. & Kong, C. Increased expression of RUNX1 in clear cell renal cell carcinoma predicts poor prognosis. PeerJ 7, e7854 (2019).
34. Kamikubo, Y. CROX (cluster regulation of RUNX) as a potential novel therapeutic approach. Mol. Cells 43(2), 198–202 (2020).
35. Trieu, A. L. M. et al. RUNX1 dosage in development and cancer. Mol. Cells 43(2), 126–138 (2020).
36. Matsumura, T. et al. Hematopoietic stem cells acquire survival advantage by loss of RUNX1 methylation identified in familial leukemia. Blood 116(7), 1919–1923 (2020).
37. Estecio, M. R. H. et al. RUNX3 promoter hypermethylation is frequent in leukemia cell lines and associated with acute myeloid leukemia inv(16) subtype. Br. J. Haematol. 169(3), 344–351 (2015).
38. Yucebas, M., Gunduz, C., Sigva, Z. O. D., Caglar, H. O. & Akalin, T. Promoter hypermethylation-mediated down-regulation of RUNX3 gene in human brain tumors. Ir. J. Med. Sci. 183(2), 259 (2013).
39. Cen, D. et al. Renal cell carcinoma: Predicting RUNX3 methylation level and its consequences on survival with CT features. Eur. Radiol. 29(10), 5415–5422 (2019).
40. Wang, H. Runt-related transcription factor 1 (RUNX1) promotes TGF-β-induced renal tubular epithelial-to-mesenchymal transition (EMT) and renal fibrosis through the PI3K subunit p110δ. EBioMedicine 31, 217–225 (2018).
41. Cheng-Cao, S. et al. Expression and prognosis analyses of runt-related transcription factor family in human leukemia. Mol. Ther. Oncol. 12, 103–111 (2019).
42. Kamikubo, Y. Genetic compensation of RUNX family transcription factors in leukemia. Cancer Sci. 109(8), 2358–2363 (2018).
43. Yoshosawa, S. & Yoshida, K. Tumor suppressive role for kinases phosphorylating p53 in DNA damage-induced apoptosis. Cancer Sci. 109(11), 3376–3382 (2018).
44. Anezar, X., Yasin, A. & Bahman, Y. Multiple functions of p21 in cell cycle, apoptosis and transcriptional regulation after DNA damage. DNA Repair 42, 63–71 (2016).
45. Bellissimo, D. C. & Speck, N. A. RUNX1 mutations in inherited and sporadic leukemia. Front. Cell Dev. Biol. 5, 111 (2017).
46. Sanoji, S. A., Nah-Young, S. & Cantor, A. B. Role of RUNX family transcription factors in DNA damage response. Mol. Cells 43(2), 99 (2020).

Author contributions
K.G. and Z.-M.D. developed the idea and designed the research. K.C., M.-L.X. and F.Z. analyzed the data. K.G., Y.-B.G. and W.L. drafted the manuscript. T.C. and Z.-M.D. revised the writing. All authors read and approved the submitted version.

Funding
The Talent Fund Project of the Second Affiliated Hospital of Xian Jiaotong University (No. RC-XM-201802).

Competing interests
The authors declare no competing interests.

Additional information
Correspondence and requests for materials should be addressed to T.C. or Z.-M.D.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2021