



# KIAA0101 knockdown inhibits cell proliferation and induces cell cycle arrest and cell apoptosis in chronic lymphocytic leukemia cells

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**Background:** Chronic lymphocytic leukemia (CLL) is a heterogeneous disease with intense cytogenetic aberrations. Importantly, our recent report indicated that thyroid hormone receptor interactor 13 (*TRIP13*) is a potential new therapeutic target in CLL. In this study, we predicted 20 *TRIP13*-related genes and found that KIAA0101 is a novel gene that regulates cell proliferation and the cell cycle of CLL cells.

**Methods:** CD19<sup>+</sup> B cells were isolated from the peripheral blood of 26 CLL patients and 6 healthy donors through magnetic cell sorting. Cell proliferation was assessed by the CCK-8 assay. The mRNA and protein levels of genes were examined through RT-qPCR and western blot assays, respectively. Cell cycle and cell apoptosis were measured through Annexin V-based flow cytometry and the caspase 3/7 activity assay. Potential targets of *KIAA0101* were identified through microarray analysis. 20 *TRIP13* related genes was predicted by Ingenuity Pathway Analysis (IPA). *KIAA0101*-regulated functions and molecular pathways were predicted through IPA.

**Results:** *KIAA0101* knockdown had the strongest inhibitory effect on CLL cell proliferation among the 20 *TRIP13*-related genes. *KIAA0101* was highly expressed in CD19<sup>+</sup> B cells of CLL patients. *KIAA0101* knockdown induced cell cycle arrest and cell apoptosis, and inhibited *FOXO1*, *MYD88*, and *TLR4* expression in CLL cells.

**Conclusions:** Taken together, we demonstrated that *KIAA0101* plays a critical role in cell proliferation and the cell cycle of human CLL cells. *KIAA0101* knockdown induced cell apoptosis, and reduced *FOXO1*, *MYD88*, and *TLR4* expression, and may therefore be used as a therapeutic target of CLL.

**Keywords:** Chronic lymphocytic leukemia (CLL); *KIAA0101*; proliferation; apoptosis; cycle

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## Introduction

Chronic lymphocytic leukemia (CLL), a type of B-cell malignancy that is incurable, is the most common type of leukemia in the Western hemisphere (1). Over the past decades, significant advances have been made in the

treatment of CLL following the introduction of small molecule inhibitors (2-5). For instance, ibrutinib, which is currently approved for the front-line treatment of CLL, induces long-lasting responses in the majority of patients, improving outcomes with relatively limited toxicity (6).

However, small molecule inhibitors still have potential problems such as drug resistance, which restrict the efficacy of CLL treatment (7). Hence, it is imperative to explore the cellular and molecular mechanisms of CLL tumorigenesis and progression.

Thyroid hormone receptor interactor 13 (*TRIP13*) has been found to be implicated in mitotic and DNA repair processes and the pathogenesis of multiple cancers (8-11). For instance, *TRIP13* knockdown inhibited cell proliferation and invasion, and induced cell apoptosis and cell cycle arrest *in vitro*, while *TRIP13* overexpression promoted xenograft tumor growth *in vivo* in head and neck cancer (8). High genomic copy number of *TRIP13* was observed in the majority of non-small cell lung cancers (9). In addition, our previous study demonstrated that *TRIP13* knockdown inhibited CLL cell proliferation and induced cell apoptosis (10). Considering the vital roles of *TRIP13* in tumor malignant transformation, including CLL (8-11), we speculated that *TRIP13*-related genes might also play vital roles in CLL tumorigenesis and progression.

In this study, 20 *TRIP13* downstream genes (*GOLGA7B*, *HIST1H4B*, *OTUB2*, *SEZ6L2*, *SMC2*, *EPST11*, *HIST1H4C*, *OLFML2A*, *SHISA2*, *DEPDC1*, *FBXO43*, *HPS3*, *KIAA0101*, *KNL1*, *MAP9*, *PIF1*, *RFWD3*, *SAPCD2*, *SNX10*, and *UBE2T*) were identified through the Ingenuity Pathway Analysis (IPA) database (10). Next, lentiviruses that could interfere with the expression of these genes were constructed, and the effects of knockdown of these genes on B-cell lymphocytic leukemia cell (Granta-519) proliferation were examined. Among these genes, *KIAA0101* mRNA abundance was relatively high in Granta-519 cells, and *KIAA0101* knockdown had the strongest inhibitory effect on Granta-519 cell proliferation. *KIAA0101*, also known as proliferating cell nuclear antigen (PCNA)-associated factor (PAF15), is a conserved PCNA-binding protein (12). *KIAA0101* involved in DNA replication and damage repair process by mediating the interaction with DNA synthesis polymerase (12). Prior studies showed that *KIAA0101* might play vital roles in the development and progression of various cancers. For instance, *KIAA0101* has been found to be involved in microvascular invasion and epithelial-mesenchymal transition of hepatocellular cancer (13), anchorage-independent growth and migration of fibrosarcoma (14), cell cycle regulation of gastric cancer (15), and cell migration and chemoresistance of ovarian cancer (16). Nevertheless, the biological functions

of *KIAA0101* along with related molecular mechanisms of action in CLL remain to be elucidated.

In this study, downstream genes of *KIAA0101* were identified through microarray analysis. Pathway, function, and disease enrichment analyses for *KIAA0101*-regulated genes were performed using the IPA database. Effects of *KIAA0101* knockdown on cell proliferation, cell cycle progression, and cell apoptosis were examined in CLL cell models (Granta-519 and JVM-2 cell lines). Also, the effects of *KIAA0101* loss on the expression of genes of interest (*FOXO1*, *MYD88*, *TLR4*) were further assessed through western blot assays in Granta-519 cells.

We present the following article in accordance with the MDAR reporting checklist (available at <http://dx.doi.org/10.21037/atm-21-626>).

## Methods

### Clinical samples

Blood samples of 26 CLL patients and 6 healthy people were collected at the Affiliated Cancer Hospital of Zhengzhou University (Henan Cancer Hospital, Henan, China) during March 2017 to December 2018. CD19<sup>+</sup> B cells were freshly isolated from peripheral blood mononuclear cells (PBMCs) through magnetic cell sorting (MACS) of Lymphoprep-separated buffy coats based on MACS CD19 Microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany) (>98%). All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of the Henan Cancer Hospital (No. 2016098). Written informed consent was obtained from all participants.

### Cell lines

JVM-2 is an Human Epstein-Barr virus (EBV)-transformed B-lymphocytic leukemia (B-CLL) cell line (17). Granta-519 is a Human mantle cell lymphoma cell line, but functionally similar to CLL cells (18). JVM-2 and Granta-519 cell lines were supplied by the cell bank of Institute of Hematology, Chinese Academy of Medical Sciences (CAMS) (Tianjin, China) and were cultured in DMEM medium (Thermo Fisher Scientific, Rockford, IL, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, Thermo Fisher Scientific) at 37 °C in a 95% air/5% CO<sub>2</sub> atmosphere.

**Table 1** Sequences of primers (5' to 3') and shRNA used in this study

Gene	Forward primer	Reverse primer	shRNA
<i>HPS3</i>	CCACCATCCACATAAGACCAAC	TCCCGTAGACTCCAGTGTAAC	GCCTGTAGTCATTATGGCTTA
<i>SHISA2</i>	TCAAGGGCAGTATCTGCATCC	CCGTCCATGAAAGGTGGCA	CCGTGTTTGTGCGCTTTATCA
<i>MAP9</i>	CAAAAGCGGAAGTATTATGG	GAAGAGGTAGTTGTGATGGAGT	CAGTTGATCCACTACTATCTA
<i>RFWD3</i>	GACCAACTTCATCAGCGGACT	CCTGCAACTCTTCAGATACAGGA	CCCAGAGAATGATGGCAACAT
<i>UBE2T</i>	TTGATTCTGCTGGAAGGATTTG	CCTGCAACTCTTCAGATACAGGA	gaACCTCCTCAGATCCGATTT
<i>PIF1</i>	CATCCACAAGAGCCAAGGCAT	GGTGGCATAGAAGTGCAGCA	CCAGGTAAGGTACACAGATTT
<i>HIST1H4C</i>	GCAAAGGCGGAAAAGGCTTG	TAGCCGGTTTTGTAATGCCCT	CGCATTTCCGGTCTTATCTAT
<i>FBXO43</i>	CTCCGATAAGTAATCTTGTGGC	CTTGTCTTTCTTATGGTGTCCC	CGCATTTCCGGTCTTATCTAT
<i>SAPCD2</i>	GAGGTGACCGAGAAGAGTGAG	GATGAAGGTGGAATCCAGAGG	ACCTCTGGATTCCACCTTCAT
<i>KNL1</i>	TCTCAACCGACCAACCTCCAT	ACGTCTGAGTTACTGTGGAAT	CGGGAATCTAAAGGTAATGAT
<i>EPST11</i>	GGCTTCTCCTTGCTCCTCATT	CTACCCTACCAACATCACTCTAAT	GAAACTGAAGTCAGACAGAAA
<i>KIAA0101</i>	CTCTGCCACTAATTCGACATCA	TTCAGAATCTTTAGGGGACAAC	gcTTTGTGAACAGGCATTTA
<i>DEPDC1</i>	ATGCGTATGATTTCCCGAATGAG	CACAGCATAACACACATCGAGAA	GCTATCCAGTAAGGCTATCAT
<i>OLFML2A</i>	AACAGGCAGTAGAGTCAA	TTACAAGATTCCTACCAACAG	CTTCACCAAGAACATCATCAA
<i>SNX10</i>	AATTTGTAAGTGTCTGGGTTTCG	AGCAACGCATTACTTTGGAGT	CCTGTGTACGAAGAAGATATA
<i>GOLGA7B</i>	GTCTCCAGTATCTGTTCTTCT	CAGCCTGCAAAGTCTCAAAGT	CCACTATGAGAAGGTTCTCAA
<i>OTUB2</i>	AAACATCTTTCAACCTAATATCA	GTAGAAGCAGTTCCCATC	CATCCCCTACAACATCCTTT
<i>HIST1H4B</i>	AAGGCGGTAAGGTTTGGGTA	GGAAATTCGCTTAACCCACC	AGCGAATTTCCGGTTTGATTT
<i>SEZ6L2</i>	CCAAATGCGCCTTGAAGTACG	GATGGTGACCTCGCCGATAAG	GCGGGAGTATGAAGTTTCCAT
<i>SMC2</i>	ACAACACCAGAGTACAGGATC	CGGCCCTGCATGATGAGAA	CCAGATTTACTCAATGTCAAA
<i>GAPDH</i>	TGACTTCAACAGCGACACCCA	CACCCTGTTGCTGTAGCCAAA	-

### Real-time quantitative PCR (RT-qPCR)

Total RNA was extracted from cells using Trizol reagent (Thermo Fisher Scientific, MA, USA) following the manufacturer's instructions. Synthesis of cDNA was carried out through a reverse transcription reaction using M-MLV Reverse Transcriptase (Promega, Madison, WI, USA) on an Applied Biosystems 2720 Thermal Cycler (Thermo Fisher Scientific). Subsequently, cDNA was amplified and quantified using the SYBR Green kit (Toyobo, Osaka, Japan) and specific quantitative primers on the TAKARA TP800-Thermal Cycler Dice™ Real Time System (Takara, Osaka, Japan). Real-time quantitative PCR reactions were performed with the following cycling parameters: 95 °C for 30 s, 45 cycles of 95 °C for 5 s, and 60 °C for 30 s. All sequences of the primers are listed in *Table 1*.

### Western blot assay

Whole-cell lysates were prepared from CLL cells using RIPA buffer (Sigma-Aldrich, St. Louis, MO, USA). After quantification, an equal amount of proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Proteins in the gel were transferred to nitrocellulose membranes. Next, the membranes were incubated overnight at 4 °C with primary antibodies against *FOXO1*, *p53*, and *CDK6* (#2880, #2524, and #3136, respectively, Cell Signaling Technology, Danvers, MA, USA) (19); Bax, TCF4, MYD88, and TLR4 (ab7977, ab185736, ab2064, and ab13556, respectively, Abcam, Cambridge, UK), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH, sc-32233, Santa Cruz Biotechnology, Dallas, Texas, USA). The membranes

were then probed with a horseradish peroxidase-labeled secondary antibody (sc-2004/sc-2005, Santa Cruz Biotechnology) for 1 h at room temperature. Finally, protein signals were detected through chemiluminescence using ECL Western Blotting Detection Reagents (GE Healthcare, Pittsburgh, PA, USA) and visualized through the G:BOX Chemi Gel Documentation System (Syngene, Frederick, MD, USA).

#### ***Plasmids and lentiviral transduction***

Recombinant lentiviruses were produced by Shanghai Genechem Company (Shanghai, China). The shRNA sequence (5'-CCGGGC-target sequence-CTCGAG-reverse complementary target sequence-GCTTTTTG-3') was subcloned into the lentiviral silencing vector. A scramble shRNA (5'-GCCTAACTGTGTCAGAAGGAA-3') served as the negative control (ctrl). When cells were grown to approximately 40% confluence, cells were infected with lentiviruses at the multiplicity of infection (MOI) of 50–100. All sequences of the shRNA are listed in *Table 1*.

#### ***Cell Counting Kit-8 assay***

Infected cells were seeded into 96-well plates (2,000 cells per well). At the indicated time points after infection, 10  $\mu$ L of Cell Counting Kit-8 (CCK-8) solutions (Sigma-Aldrich) was added into each well. After 4 h of incubation at 37 °C, cell absorbance was measured at 450 nm.

#### ***Apoptosis assay***

Cell apoptotic rate was measured after Annexin V-APC staining using flow cytometry. Briefly, cells were collected on day 5 after lentivirus infection and re-suspended in binding buffer at a concentration of  $1 \times 10^6$  cells/ml. Next, 100  $\mu$ L of cell suspensions were co-incubated with Annexin V-APC staining solution for 15 min in the dark at room temperature. Finally, cell apoptotic patterns were analyzed using flow cytometry.

#### ***Caspase 3/7 activity detection***

Caspase 3/7 activity in CLL cells was detected using the Promega Caspase-Glo 3/7 assay kit (Promega). Briefly, cells infected with shctrl or shKIAA0101 lentiviruses were plated

into 96-well plates and incubated for 5 days. Next, cells were collected and cell suspensions ( $1 \times 10^4$  cells/well) were reacted with 100  $\mu$ L of Caspase-Glo reaction solution. After shaking for 30 min at 300–500 rpm, the reaction system was incubated for 2 h at room temperature. Finally, signal intensity was determined using a microplate reader.

#### ***Gene microarray analysis***

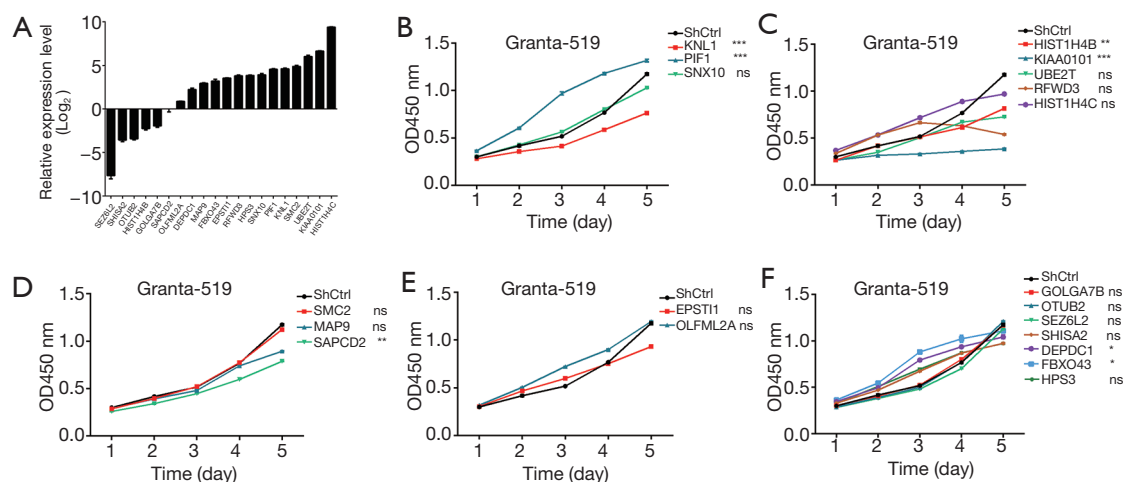
Total RNA was extracted from Granta-519 cells infected with shctrl or shKIAA0101 lentiviruses. Next, the content and quality of RNA were analyzed using the Nanodrop 2000 (Thermo Fisher Scientific) and the Agilent 2100 Bioanalyzer (Agilent, Pal Alto, CA, USA). RNA ( $1.7 < A260/A280 < 2.2$ , RNA integrity number  $\geq 7.0$  and  $28S/18S > 0.7$ ) was reverse transcribed into first-strand cDNA, which was further converted into double-stranded DNA and biotin-labeled amplified RNA (aRNA) using the GeneChip 3' IVT Express Kit (Affymetrix, Santa Clara, CA, USA). After purification, aRNA was quantified using the Nanodrop 2000 (Thermo Fisher Scientific), fragmented, and hybridized with the GeneChip Human Genome U133 plus 2.0 Array (Affymetrix). Next, hybridized chips were stained and washed using the GeneChip Hybridization Wash and Stain Kit (Affymetrix) on the Genechip Fluidics Station 450 instrument (Affymetrix). Finally, microarray signals were scanned and analyzed using the Genechip Array Scanner 3000 7G (Affymetrix).

#### ***Bioinformatics analysis***

The IPA database was used to examine the potential functions and regulatory mechanisms of differentially expressed genes in the shKIAA0101 group *vs.* the shctrl group through “disease and function”, “canonical pathway”, and “interaction network” analysis.

#### ***Statistical analysis***

Data analysis was performed using GraphPad Prism 7 software (GraphPad Software, La Jolla, CA, USA). The results are shown as mean  $\pm$  standard deviation (SD) ( $n=3$ ). Statistical significance was determined by the *t* test between data from two groups, and Two-way ANOVA was used to evaluate the differences among multiple groups. Results were regarded as statistically significant when the P value



**Figure 1** Effects of knockdown of 20 TRIP13-related genes on Granta-519 cell proliferation. (A) The mRNA abundances of 20 TRIP13-related genes predicted by the IPA database in Granta-519 cells were examined through RT-qPCR. The expression levels of all genes were normalized to SAPCD2. (B,C,D,E,F) Granta-519 cells were infected with lentiviruses targeting the above-mentioned 20 genes or control lentiviruses (shctrl). The above-mentioned 20 genes were divided into 5 groups (i.e., chromosome maintenance (B), DNA repair (C), cell division (D), macrophage polarization (E), and others (F) according to their molecular functions. Cell proliferative ability was assessed through the CCK-8 assay at the indicated time points after infection. <sup>ns</sup>P>0.05, \*P<0.05, \*\*P<0.01, \*\*\*P<0.001. CLL, chronic lymphocytic leukemia; BTK, Bruton's tyrosine kinase; TRIP13, thyroid hormone receptor interactor 13; IPA, Ingenuity Pathway Analysis; RT-qPCR, real-time quantitative PCR; MACS, magnetic cell sorting; NC, negative control; CCK-8, Cell Counting Kit-8; aRNA, amplified RNA; MYD88, myeloid differentiation factor 88; TLR, toll-like receptor; FOXO, Forkhead box O.

was lower than 0.05.

## Results

### Effects of TRIP13-related genes on CLL cell proliferation

Our previous study showed that TRIP13 knockdown inhibited cell proliferation and facilitated cell apoptosis in CLL (10). To further explore potential crucial genes implicated in the pathogenesis of CLL, 20 putative TRIP13-related genes were predicted through IPA, and the mRNA abundances of these genes in JVM-2 cells were measured using RT-qPCR. As presented in *Figure 1A*, *KIAA0101* and *HIST1H4C* were relatively highly expressed among these genes in JVM-2 cells. Next, the effects of knockdown of these genes on JVM-2 cell proliferation were examined using the CCK-8 assay. Results showed that knockdown of *PIF1*, *KNL1*, *HIST1H4B*, *KIAA0101*, *SAPCD2*, *DEPDC1*, and *FBXO43* could markedly influence JVM-2 cell proliferation, while the loss of other genes did not have a significant effect on JVM-2 cell proliferation (<sup>ns</sup>P>0.05, \*P<0.05, \*\*P<0.01, \*\*\*P<0.001, *Figure 1B,C,D,E,F*). Considering that *KIAA0101*

knockdown had the strongest inhibitory effect on JVM-2 cell proliferation, the functions of *KIAA0101* in the development of CLL cells along with related molecular mechanisms were further investigated in subsequent experiments.

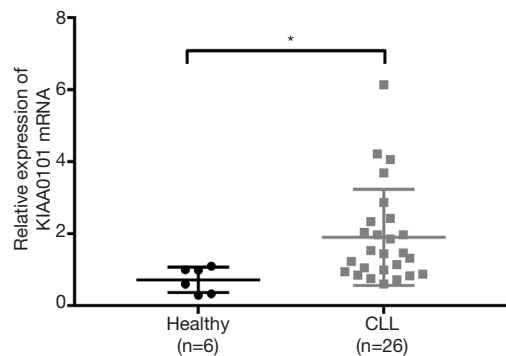
### *KIAA0101* expression was markedly up-regulated in CD19<sup>+</sup> B cells isolated from the peripheral blood of CLL patients

Next, the mRNA expression pattern of *KIAA0101* in CD19<sup>+</sup> B cells isolated from the peripheral blood of 6 healthy donors and 26 CLL patients was examined through RT-qPCR. Results showed that the mRNA level of *KIAA0101* was markedly increased in the CLL group compared to the healthy control group (\*P<0.05, *Figure 2*), suggesting the close link between *KIAA0101* and CLL progression.

### Potential functions and molecular mechanism investigations of *KIAA0101*

To further explore the functions and molecular mechanisms of *KIAA0101* in CLL, Affymetrix GeneChip analysis was





**Figure 2** *KIAA0101* expression was markedly up-regulated in CD19<sup>+</sup> B cells isolated from the peripheral blood of CLL patients. The mRNA expression level of *KIAA0101* was examined through RT-qPCR in CD19<sup>+</sup> B cells isolated from the peripheral blood of 6 healthy people and 26 CLL patients. \* $P < 0.05$ . CLL, chronic lymphocytic leukemia; RT-qPCR, real-time quantitative PCR.

performed to identify differentially expressed genes in Granta-519 cells infected with sh*KIAA0101* lentiviruses versus cells infected with shctrl lentiviruses. As presented in Figure 3A, 785 genes were differentially expressed (253 up-regulated, 532 down-regulated) in the sh*KIAA0101* group versus the shctrl group ( $|\text{fold change}| > 1.5$ ,  $P < 0.05$ ). Next, the IPA database was used to analyze the enrichment patterns of these differentially expressed genes in pathway, disease, and function categories. Disease and function enrichment analysis revealed that these differentially expressed genes were significantly enriched in cancers, and were mainly involved in the regulation of cell death, survival, and cell cycle (Figure 3B), suggesting that *KIAA0101* might influence CLL tumorigenesis and progression principally through regulating the expression of genes related to cell cycle progression, cell survival, and cell death. IPA pathway enrichment analyses demonstrated that these differentially expressed genes were significantly enriched in multiple molecular pathways, especially in the p53/ATM signaling pathway and pathways associated with cancer, DNA damage/repair, cell cycle, and cell apoptosis (Figure 3C). These outcomes suggested that *KIAA0101* might exert its functions through regulating the expression of genes implicated in the aforementioned pathways.

#### ***Knockdown of KIAA0101 inhibited cell proliferation and induced cell cycle arrest in Granta-519 and JVM-2 cells***

Knockdown efficiency analysis revealed that the infection

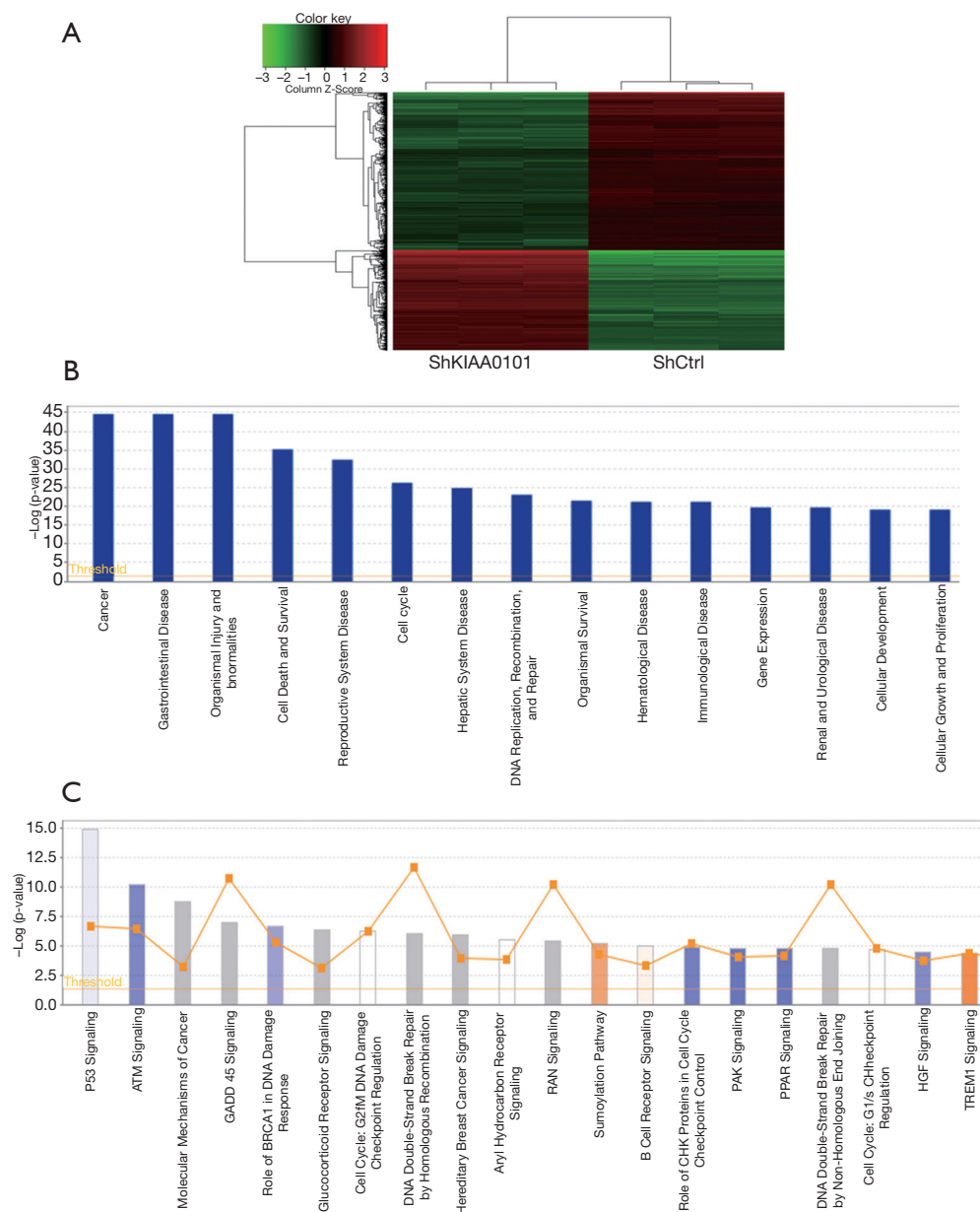
of sh*KIAA0101* lentiviruses triggered the marked down-regulation of *KIAA0101* mRNA and protein expression in Granta-519 and JVM-2 cells compared to the shctrl group ( $P < 0.001$ , Figure 4A,B), suggesting that sh*KIAA0101* lentiviruses could be used for subsequent loss-of-function experiments. Functional analyses revealed that *KIAA0101* knockdown strikingly inhibited cell proliferation in both Granta-519 and JVM-2 (Figure 4C,D) cells. Moreover, the percentages of cells in the G1 and G2/M phases were notably reduced and the proportion of cells in S phase was noticeably increased in Granta-519 and JVM-2 infected with sh*KIAA0101* lentiviruses relative to the shctrl group, suggesting that *KIAA0101* knockdown induced cell cycle arrest at the S phase in Granta-519 and JVM-2 cells (\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , Figure 4E,F).

#### ***KIAA0101 knockdown induced cell apoptosis in Granta-519 and JVM-2 cells***

Next, the effect of *KIAA0101* knockdown on cell apoptosis was further investigated in Granta-519 and JVM-2 cells. Our outcomes revealed that *KIAA0101* knockdown led to significant up-regulation of caspase 3/7 activity in Granta-519 and JVM-2 cells compared with the shctrl group ( $P < 0.001$ , Figure 5A,B). Also, the expression level of the anti-apoptotic protein Bcl-2 was strikingly reduced in Granta-519 and JVM-2 cells infected with sh*KIAA0101* lentiviruses compared to the shctrl group ( $P < 0.001$ , Figure 5C,D). Additionally, the percentage of apoptotic cells was notably elevated in the sh*KIAA0101* group ( $12.12\% \pm 0.3\%$  in Granta-519 and  $7.47\% \pm 0.81\%$  in JVM-2) compared to the shctrl group ( $4.65\% \pm 0.14\%$  in Granta-519 and  $4.71\% \pm 0.71\%$  in JVM-2) (Figure 5E,F). In summary, these outcomes showed that *KIAA0101* knockdown could induce cell apoptosis in Granta-519 and JVM-2 cells.

#### ***KIAA0101 knockdown triggered the notable down-regulation of FOXO1, MYD88, and TLR4 protein levels in Granta-519 cells***

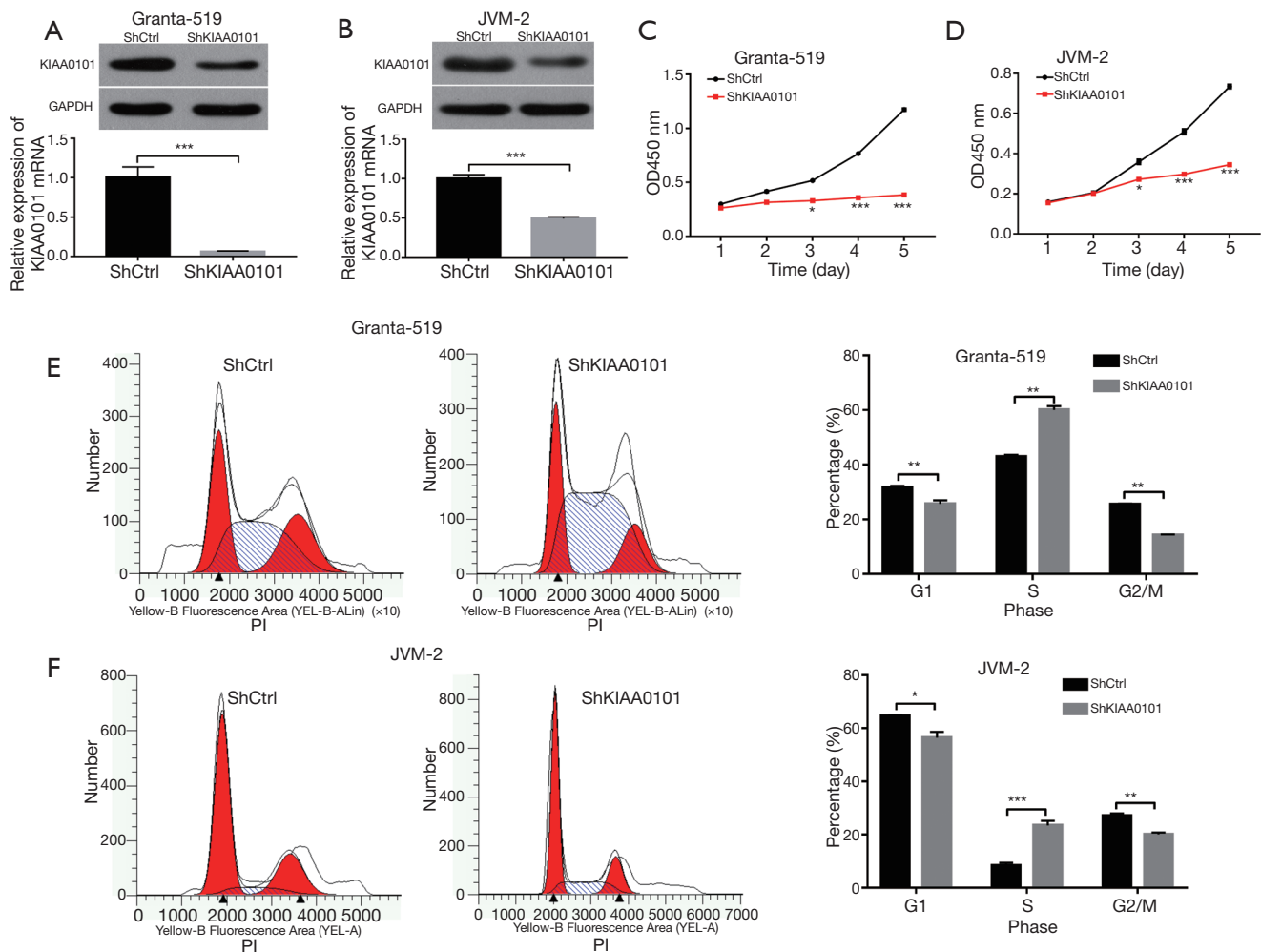
It is well known that the p53 signaling pathway is involved in the regulation of the cell cycle, cell apoptosis, and tumorigenesis. The interaction networks of *KIAA0101* (*PCLAF*) and some *KIAA0101* downstream genes of interest (*BAX*, *FOXO1*, *CCND1*, *BRCA1*, *CDKN1A*, *CAST*, *CDK6*, *MYD88*, *TCF4*, *TLR4*, and *p53*) were established using IPA interaction analysis (Figure 6A). Moreover, the effects of *KIAA0101* knockdown on the expression of *FOXO1*,



**Figure 3** Potential functions and molecular mechanism investigations of *KIAA0101*. (A) Heatmap of differentially expressed genes (1fold change  $>1.5$ ,  $P < 0.05$ ) in Granta-519 cells infected with *sbKIAA0101* lentiviruses versus cells infected with shctrl lentiviruses (green: down-regulated; red: up-regulated). *sbKIAA0101* and shctrl groups contained 3 replicates. Genes and samples were displayed in rows and columns, respectively. (B) The top 15 disease and molecular function terms significantly enriched by differentially expressed genes. (C) The top 20 molecular functional pathways significantly enriched by differentially expressed genes.

*MYD88*, *TCF4*, and *TLR4* were further examined through western blot assays. Consistent with our microarray data, western blot outcomes validated that *KIAA0101* loss led to the notable down-regulation of *FOXO1*, *MYD88*,

and *TLR4* protein levels in Granta-519 cells (Figure 6B). These outcomes suggested that *KIAA0101* might exert its functions partly through regulating *FOXO1*, *MYD88*, and *TLR4* expression in Granta-519 cells.



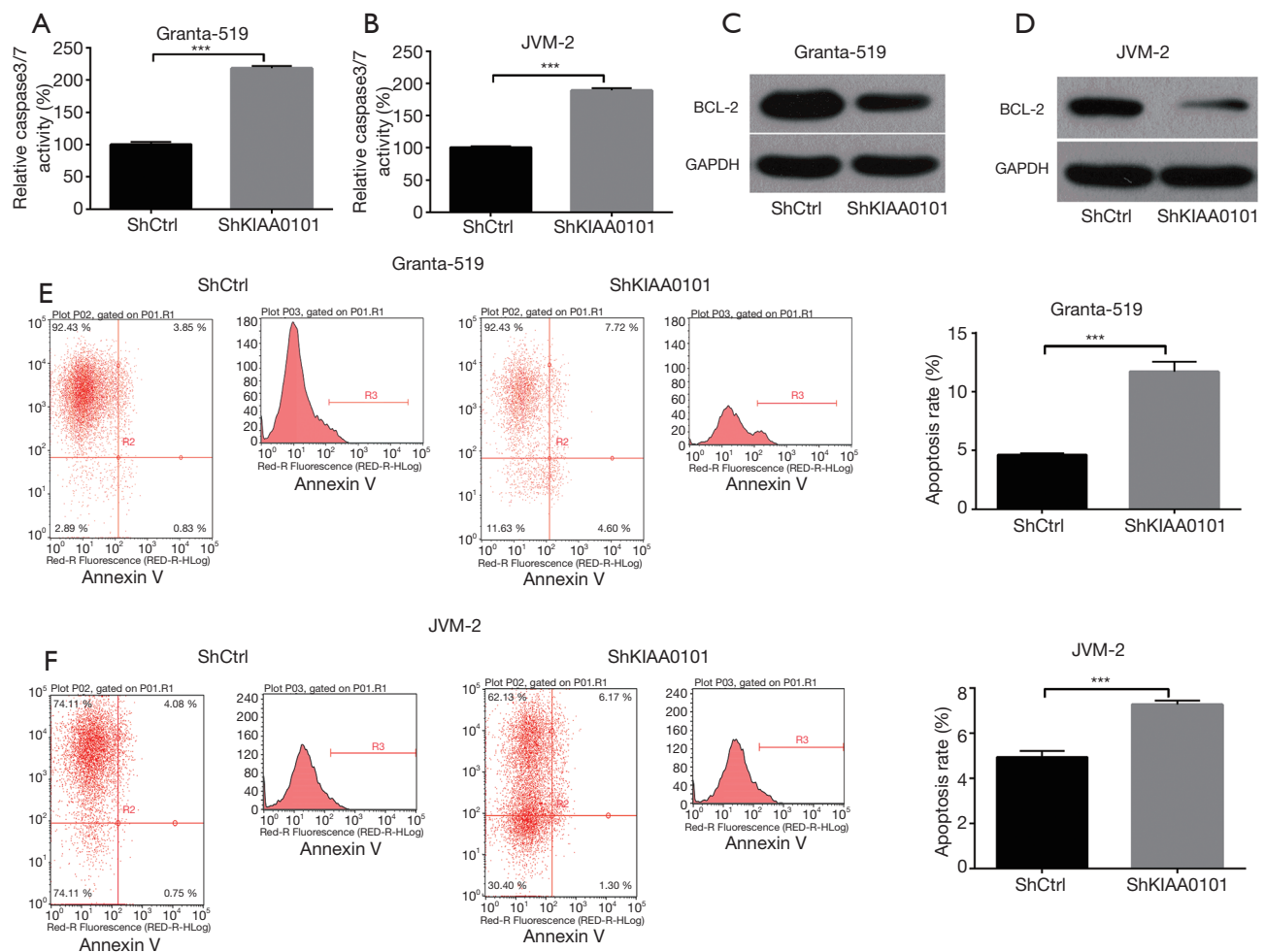
**Figure 4** Knockdown of *KIAA0101* inhibited cell proliferation and induced cell cycle arrest in Granta-519 and JVM-2 cells. (A,B,C,D,E,F) Granta-519 and JVM-2 cells were infected with shctrl or *shKIAA0101* lentiviruses. (A,B) On day 3 after infection, mRNA and protein levels of *KIAA0101* were measured through RT-qPCR and western blot assays, respectively. (C,D) At the indicated time points (1, 2, 3, 4, or 5 days) after infection, cell proliferative activity was detected by the CCK-8 assay. (E,F) On day 3 after infection, cell distribution patterns in different phases of the cell cycle were measured by flow cytometry after PI staining. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . RT-qPCR, real-time quantitative PCR; PI, propidium iodide.

## Discussion

Considering the vital roles of *TRIP13* in the development of CLL as shown in our previous study (10), we speculated that *TRIP13*-related genes might also be implicated in the pathogenesis of CLL. In this study, 20 *TRIP13*-related genes were identified by IPA. Further investigations showed that *KIAA0101* mRNA abundance was higher in Granta-519 cells, and *KIAA0101* knockdown had the strongest inhibitory effect on Granta-519 cell proliferation. Moreover, a notable increase in *KIAA0101* mRNA

expression was observed in  $CD19^+$  B cells isolated from CLL patients compared to the healthy control group. These data suggest the close link between *KIAA0101* and CLL progression. To gain a deeper insight into the functions and molecular mechanisms of *KIAA0101* in CLL, gene microarray analysis was performed in Granta-519 cells infected with *shKIAA0101* or shctrl lentiviruses. Through gene microarray analysis, we identified 785 potential downstream targets of *KIAA0101*. IPA database analyses further revealed that these possible targets of



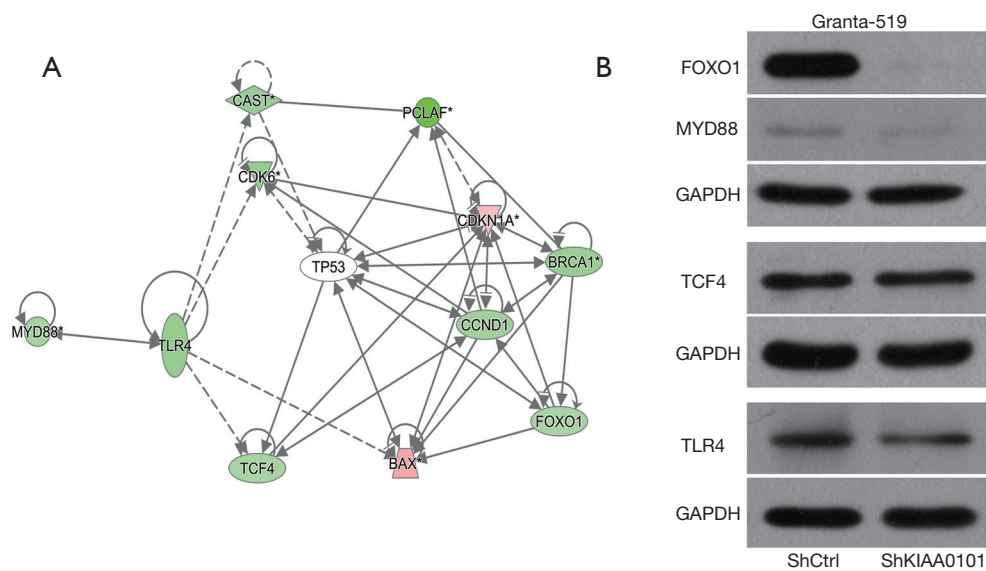


**Figure 5** *KILAA0101* knockdown induced cell apoptosis in Granta-519 and JVM-2 cells. (A,B,C,D,E,F) Granta-519 and JVM-2 cells were infected with shctrl or *shKILAA0101* lentiviruses. (A,C) On day 3 post lentivirus infection, relative caspase 3/7 activity was determined by ELISA. (B,D) On day 3 post lentivirus infection, Bcl-2 protein level was detected by western blot assay. (E,F) On day 3 post lentivirus infection, cell apoptotic rate was assessed by flow cytometry. \*\*\* $P < 0.001$ .

*KILAA0101* were mainly involved in the regulation of cancer progression, cell death/survival/cycle, and DNA damage/repair. Hence, the effects of *KILAA0101* knockdown on CLL cell proliferation, cell cycle progression, and cell apoptosis were investigated in subsequent experiments. Results showed that *KILAA0101* loss weakened cell proliferative ability, hindered cell cycle progression, and induced cell apoptosis in Granta-519 and JVM-2 cells. Consistent with our outcomes, *KILAA0101* has been found to be a potential oncogene in multiple cancers such as hepatocellular cancer (13), ovarian cancer (14), and breast cancer (20).

The toll-like receptor 4 (TLR4)/myeloid differentiation factor 88 (MYD88) signaling pathway has been found to

be implicated in the tumorigenesis and progression of multiple cancers, including CLL (21,22). For instance, MYD88 knockdown inhibited cell proliferation, migration, and invasion *in vitro*, and inhibited subcutaneous xenograft tumor growth *in vivo* in colorectal cancer (23). TLR4 and MYD88 were highly expressed in highly invasive human MDA-MB-231 breast cancer cells and breast cancer tissues, and TLR4 and MYD88 expression was positively associated with the histological grade and metastatic potential of breast cancer (24). In addition, previous studies showed that the inhibition of the MYD88 downstream signaling pathway induced CLL cell death and inhibited tumor development in CLL E $\mu$ -TCL1 adoptive transfer models (22). In this



**Figure 6** *KIAA0101* knockdown triggered the notable down-regulation of FOXO1, MYD88, and TLR4 protein levels in Granta-519 cells. (A) The interaction networks of *KIAA0101* (PCLAF) and some *KIAA0101* downstream genes of interest (*BAX*, *FOXO1*, *CCND1*, *BRCA1*, *CDKN1A*, *CAST*, *CDK6*, *MYD88*, *TCF4*, *TLR4*, and *P53*) were established using the Ingenuity Pathway Analysis (IPA) database. (B) The effects of *KIAA0101* knockdown on the expression of *FOXO1*, *MYD88*, *TCF4*, and *TLR4* were examined through western blot assays on day 3 after lentivirus infection in Granta-519 cells infected with shctrl or *shKIAA0101* lentiviruses.

study, our microarray and western blot data revealed that *KIAA0101* knockdown led to the notable down-regulation of TLR4 and MYD88 protein levels in Granta-519 cells. These findings suggested the molecular mechanism of *KIAA0101*-mediated regulation of cell proliferation may be realized through TLR4/MYD88 signaling pathway and this phenomenon is worthy of clarification in the future.

The Forkhead box O (FOXO) family have also been found to be crucial players in cell fate decisions and cancer development (25). In addition, previous studies showed that *FOXO1* functioned as a tumor suppressor in multiple cancers such as breast cancer (26), non-small cell lung cancer (27), and gastric cancer (28). For example, ectopic expression of *FOXO1* curbed cell proliferation, hindered cell cycle progression, and induced cell apoptosis in cervical cancer (29). Also, previous studies showed that FOXO1 expression was markedly up-regulated in freshly isolated CLL cells from peripheral blood and lymph node biopsies from CLL patients with poor prognosis (30), suggesting the close link between *FOXO1* and CLL progression. Our present study also demonstrated that *FOXO1* expression was markedly down-regulated in CLL cells following *KIAA0101* knockdown. Interestingly, the functions of *KIAA0101* were similar to an oncogene in CLL based on

promoted cell proliferation, silencing of *KIAA0101* leads to dramatic CLL cells apoptosis, cell cycle arrest and inhibition of cell proliferation. However, knock down of *KIAA0101* also decreased the expression of *FOXO1* which served as tumor suppressor genes as reported. According to these findings, *FOXO1* expression might be independent on cell proliferation in *KIAA0101*-depleted CLL cells.

## Conclusions

Taken together, our data revealed that *KIAA0101* knockdown hindered cell proliferation, and induced cell cycle arrest and cell death in CLL cells, suggesting the vital roles of *KIAA0101* in CLL progression. Also, our study disclosed the main functions and regulatory pathways of *KIAA0101*, and identified 785 potential targets of *KIAA0101* in CLL, which has potential value in elucidating the functions and regulatory mechanisms of *KIAA0101* in CLL. In addition, microarray analysis and western blot assays demonstrated that *KIAA0101* knockdown led to the notable reduction of *FOXO1*, *MYD88*, and *TLR4* levels in CLL cells. *KIAA0101*-mediated regulation of cell proliferation may be realized through TLR4/MYD88 signaling pathway and *FOXO1* expression might be

independent on cell proliferation in *KIAA0101*-depleted CLL cells. It is imperative to further investigate the underlying mechanism *KIAA0101* on CLL tumorigenesis and progression. Also, other pivotal targets of *KIAA0101* need to be further discovered.

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**Ethical Statement:** The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of the Henan Cancer Hospital (No. 2016098). Written informed consent was obtained from all participants. A copy of the written consent is provided to the editors of this journal for review.

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