The expression of apoptosis related genes in HK-2 cells overexpressing PPM1K was determined by RNA-seq analysis

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Chronic kidney disease (CKD) is a serious disease that endangers human health. It is reported that inhibiting renal cell apoptosis can delay the progress of CKD. Our previous study found that the mice with protein phosphatase Mg2+/Mn2+ dependent 1K (PPM1K) gene deletion had obvious symptoms of glomerular vascular and interstitial vascular dilatation, congestion and hemorrhage, glomerular hemorrhage and necrosis, interstitial ﬁbrous tissue proliferation, decreased urinary creatinine clearance, and increased urinary protein level. In addition, studies have found that PPM1K is essential for cell survival, apoptosis and metabolism. However, no study has conﬁrmed that PPM1K can inhibit renal cell apoptosis. In this study, PPM1K was overexpressed in human kidney-2 cells (HK-2), and the biological process of differentially expressed genes and its effect on apoptosis were comprehensively screened by RNA sequencing (RNA-seq). Through sequencing analysis, we found that there were 796 differentially expressed genes in human renal tubular epithelial cells transfected with PPM1K gene, of which 553 were down-regulated and 243 were up-regulated. Enrichment analysis found that differentially expressed genes may play an important role in amino acid metabolism and biosynthesis. In the GO analysis functional pathway list, we also found that multiple genes can be enriched in apoptosis related pathways, such as G0S2, GADD45A, TRIB3, VEGFA, NUPR1 and other up-regulated genes, and IL-6, MAGED1, CCL2, TP53INP1 and other down-regulated genes. Then we veriﬁed these differentially expressed genes by RT-PCR, and found that only the RT-PCR results of G0S2, VEGFA and NUPR1 were consistent with the transcriptome sequencing results. We believe that G0S2, VEGFA, NUPR1 and other genes may participate in the apoptosis process of HK-2 cells induced by PPM1K. In conclusion, these ﬁndings provide some data support for the study of HK-2 cell apoptosis mechanism, and also provide a scientiﬁc theoretical basis for further study of the effect of PPM1K on kidney disease.
Introduction

Chronic kidney disease (CKD) has the characteristics of high prevalence, low awareness, poor prognosis and high medical costs. It is another disease seriously endangering human health after cardiovascular and cerebrovascular diseases, diabetes and malignant tumors (Hwang et al., 2012; Ene-Iordache et al., 2016). Renal tubules are the main part of the kidney and are vulnerable to various injuries, including hypoxia, proteinuria, toxins, metabolic disorders and aging. Studies have found that renal tubular cell injury drives the development of CKD. When suffering from injury reaction, renal tubular epithelial cells change and play a role as inflammatory cells and fibrotic cells, thus producing various bioactive molecules to drive interstitial inflammation and fibrosis (Liu et al., 2018). Therefore, it is necessary to explore the regulatory mechanism of renal tubular injury in the process of CDK, including tubular cell apoptosis and inflammatory transformation, in order to delay the progress of CKD.

Apoptosis is one of the important defense mechanisms of cells. It is a programmed death reaction, which is used to clear damaged cells and maintain the homeostasis of the host. It is closely related to cell replication, proliferation, immunity and cancer. Cell dysfunction and apoptosis are also key factors in the progress of chronic kidney disease (CDK) (Xiang et al., 2021). Excessive apoptosis may lead to disease. Lin Xie found that homocysteine promotes podocyte apoptosis by regulating epigenetic modifiers DNMT1 and EZH2, which is accompanied with glomerular damage, leading to the progress of CKD (Xie et al., 2021). Nan Yu found that podocytes are an important part of renal glomerular filtration barrier, and podocyte dysfunction and apoptosis are important factors leading to the progress of chronic kidney disease (CKD) (Yu et al., 2020). Izquierdo research found that abnormal blood lipids and lipid accumulation in the kidney may lead to changes in renal lipid metabolism, thus inducing endoplasmic reticulum (ER) stress and the production of reactive oxygen species (ROS), and ultimately leading to cytotoxicity, apoptosis and inflammation, which play an important role in advanced CKD (Izquierdo-Lahuerta et al., 2016). In addition, studies have shown that inhibiting renal tubular cell apoptosis can alleviate the progress of CKD. Xiang Cheng Xie found that relaxin inhibits human tubular epithelial cell apoptosis induced by Campbell acid by activating p38/akt signaling pathway (Xie et al., 2017); Kahori Nasu found that mint3 in epithelial cells upregulates NF-κ The anti apoptotic effect of B protects cells from apoptosis and thus inhibits fibrosis, which may be the therapeutic target of CKD (Nasu et al., 2020); Sandra Kostic found that apoptosis inducing factor (AIF) is a key factor leading to mitochondrial apoptosis, and plays an important role in apoptosis, cell function and homeostasis, and proved that AIF is a potential therapeutic target and marker of CKD progression (Kostic et al., 2020); Nan Yu confirmed that curcumin reduced podocyte injury and apoptosis by inhibiting endoplasmic reticulum stress, thereby alleviating the progress of CKD (Yu et al., 2020); The above research shows that inhibiting renal cell apoptosis is of great significance to alleviate the progress of CKD.

RNA sequencing (RNA-seq) technology is the second-generation sequencing technology with high sensitivity, high accuracy and low cost (Wang et al., 2009; Hrdlickova et al., 2017). At present, this technology is increasingly used in the analysis of diseases and drugs at the genetic level. The transcriptome sequencing data analysis using RNA-seq technology to study the expression of disease related genes mainly focuses on the analysis of differentially expressed genes, GO (Gene Ontology) function enrichment analysis and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment analysis. GO function analysis is to use the annotation results of the database to explain the functional parts of differentially expressed genes in the three categories of gene Ontology: molecular function, cellular component, and biological process (Ashburner et al., 2000). KEGG is a comprehensive database for understanding cells, organisms and ecosystems, and a database resource for practical programs of genome sequencing and other high-throughput experimental technologies generated from molecular level information, especially large molecular datasets (Kanehisa et al., 2004), which is divided into three categories: system information, genome information and chemical information. KEGG pathway is a database of system information and a collection of hand-painted metabolic pathways, including metabolism, genetic information processing, environmental information, cell process processing, biological system, human disease and drug development. At present, transcriptome sequencing technology is used in the field of kidney disease. Meeyoung Park et al. used transcriptome sequencing technology to analyze the steady-state gene expression pattern of human kidney tissue under ischemia and reperfusion conditions. Differential expression analysis showed that the metabolic pathway of cells was disordered during ischemia, and cell development, migration and immune response related pathways were out of tune during reperfusion. Cluster analysis showed that the metabolism, apoptosis and fibrosis related pathways mediated by ischemia were significantly dysregulated, while the cell growth, migration and immune response related pathways were highly dysregulated in post ischemia reperfusion. During ischemia, the expression of pro apoptotic genes and death receptors were down regulated, indicating that there was a protective mechanism against ischemic injury (Park et al., 2020). Kelly et al. Conducted
transcriptome sequencing on the rat model of diabetes nephropathy to deeply study the pathogenic mechanism of diabetes nephropathy with progressive CKD. The results showed that the pathological genes of the model led to kidney inflammation, promoted apoptosis and blocked cell cycle (Kelly et al., 2013). This study can be used to develop new clinical treatment strategies.

Our previous study found that mice with protein phosphatase Mg2+/Mn2+ dependent 1K (PPM1K) gene deletion significantly developed glomerular and interstitial vascular dilatation, congestion and hemorrhage, glomerular hemorrhagic necrosis, interstitial fibrous tissue proliferation with inflammatory cell infiltration, significantly decreased urinary creatinine clearance, and increased urinary protein levels. We speculate that PPM1K may play an important protective role in the development of renal fibrosis, but its molecular mechanism is not clear. Kun Lian found that the cardiac function of diabetes mice overexpressing PPM1K was improved, and the myocardial infarction area and apoptosis were reduced (Lian et al., 2020). Alfonso confirmed that PPM1K is crucial to cell survival, apoptosis and metabolism (Oyarzabal et al., 2013). Therefore, we speculate that PPM1K can alleviate the progress of CKD by inhibiting renal cell apoptosis. However, no study has confirmed that PPM1K can inhibit renal cell apoptosis. In this study, PPM1K was overexpressed in human kidney-2 cells (HK-2). As the research object, the biological process of differentially expressed genes and its effect on apoptosis were comprehensively screened by RNA sequencing (RNA-seq). It is hoped that this study will bring a new way for the treatment of CKD.

Materials and methods

Culture of human renal tubular epithelial cells

HK-2 cells were purchased from Wuhan prosai Life Technology Co., Ltd (Wuhan, China) and inoculated into a 25 cm² culture flask after resuscitation. The culture medium was DMEM medium (Gibico, Grand Island, New York, United States) containing 10% Fetal Bovine Serum (Gibico, Sandringham Ave, Thornton, NSW 2322, Australia) by mass. The culture flask was placed in a cell incubator (Thermo science, United States) with a volume fraction of 5% carbon dioxide at 37°C. Subculture when the cell fusion rate is 80%–90% under the inverted microscope.

Construction of PPM1K plasmid

Hunan Youbao biological company (Hunan, China) was entrusted to construct PPM1K plasmid. After gene sequencing experiments, the accuracy of the sequence was proved.

Plasmid transfection HK-2

The third generation human HK-2 is selected, and the cell density reaches 70%–80%. Two hours before transfection, the cells were cultured in serum-free medium, and 2.5 μG plasmid, add 100 μL incubate with opti MEM for 5min, and take 5 μL Lipofectamine™ 2000(Invitrogen, Carlsbad, CA, United States) join 100 μL opti MEM, incubate for 5min and mix the above reagents. Add the above mixture to the cell culture medium, and change the fresh medium after 4–6 h.

Western blot verifies the expression of PPM1K

Discard the culture medium, digest the cells with 0.25% trypsin solution (Thermo science, United States), collect the digested cells to 1.5 ml EP tube, centrifuge the supernatant and add 100 μL RIPA (Thermo science, United States) cracking liquid, put it on ice to crack for 30min. After lysis, centrifuge for 15min (12000r/min), and take the supernatant for protein quantification. Prepare the quantitative protein solution and cook it at 100°C for 10 min. Make 10% SDS-PAGE gel (Thermo science, United States) and add 30% for each lane μG protein. Turn on the electrophoresis apparatus (Bio-Rad, United States), the upper glue voltage is 80 V (30min), the lower glue voltage is 100 V (2H), and the film is turned for 2 h (100V). After the film transfer, 5% skimmed milk was sealed for 2 h, and tbs was cleaned three times. Cut the PVDF membrane, add 3 ml of marker antibody (PPM1K) (1:1,000, 66008-2-IG, Proteintech) antibody and 3 ml of internal reference GAPDH (1:5,000, ATPA00013Rb, AtaGenix) antibody on the membrane, shake it overnight at 4°C, take it out on the second day, wash tbs for three times, add secondary antibody (Goat anti rabbit IgG 1:5000, Abcam), incubate at room temperature for 2 h, and clean tbs for three times. After cleaning, use the enhanced chemiluminescence (ECL) reagent (Bio-Rad, 170506, United States) to avoid light and develop color. After the strip is clear, scan it with gel imaging system (Bio-Rad, United States), and calculate the gray value of the strip to be measured with image lab software.
RNA extraction and sequencing

Total RNAs were extracted from HK2 cells using TRIzol Reagent (Invitrogen, cat. NO 15596026) following the methods by Chomczynski (Chomczynski and Sacchi, 1987). DNA digestion was carried out after RNA extraction by DNaseI. RNA quality was determined by examining A260/A280 with Nanodrop™ OneCspectrophotometer (Thermo Fisher Scientific Inc). RNA Integrity was confirmed by 1.5% agarose gel electrophoresis. Qualified RNAs were finally quantified by Qubit3.0 with Qubit™ RNA Broad Range Assay kit (Life Technologies, Q10210). 2 µg total RNAs were used for stranded RNA sequencing library preparation using KCTM Stranded mRNA Library Prep Kit for Illumina (Catalog NO. DR08402, Wuhan Seqhealth Co., Ltd. China) following the manufacturer’s instruction. PCR products corresponding to 200–500 bps were enriched, quantified and finally sequenced on Novaseq 6000 sequencer (Illumina) with PE150 model.

Functional enrichment analysis

To sort out functional categories of DEGs, GO terms and KEGG pathways were identified using KOBAS 2.0 server (Chen et al., 2011). Hyper geometric test and Benjamini–Hochberg FDR controlling procedure were used to define the enrichment of each term.

RT-qPCR validation of DEGs

In order to clarify the reliability of DEGs in overexpressing PPM1K in HK-2 cells, RT-qPCR was performed on some of the up-regulated and down-regulated DEGs. The RNA samples used for RT-qPCR are the same as those used for RNA sequences. The PCR conditions were denaturation at 95°C for 10 min, then denaturation at 95°C for 15 s, annealing and extension at 60°C for 1 min, a total of 40 cycles. Then extend at 60°C for 1 min, and store the product at 4°C. All samples were amplified by PCR three times.

Statistical analysis

The experimental data are expressed by mean ± SD, and statistical analysis is carried out by SPSS v22.0 software (IBM SPSS statistics for Windows). Using one-way ANOVA Tukey’s HSD test, p < 0.01, it is considered that there is a statistical difference.

Result

Morphological observation of HK-2 cells

HK-2 cells grow adherently, with different sizes, irregular shapes, circular and spindle shapes. They proliferate in a whirlpool. The cells in the center of the whirlpool are dense, and one to two nucleoli can be seen in the cells. Only a small number of floating cells and cell fragments are left in the culture medium; After transfection with PPM1K, the cell morphology changed little, such as round, spindle, etc., the cell proliferation rate did not change, and only a small number of floating cells and cell fragments remained in the culture medium.

The overexpression of PPM1K was verified by western blot

Western blot showed that the expression of PPM1K protein existed in HK-2 cells transfected with PPM1K gene (Figure 1B).
PCR experiment verifies the overexpression of PPM1K

PCR results showed that PPM1K gene was successfully transfected into HK-2 cells, and the transfection efficiency was 78.2% ($p < 0.01$). The transfection efficiency is high, and PPM1K is stably expressed, which can be used in subsequent experiments (Figure 1A).

Transcriptome sequencing quality evaluation

Transcriptome sequencing results show that the percentage of quality value 20 (Q20) is 100%, the percentage of quality value 30 (Q30) is more than 99%, and the proportion of GC content is 50%–50.5% (Table 1). The overall sequencing quality is good, and subsequent analysis can be carried out. According to the

| SampleID  | Raw   | Clean | Clean | Raw base(G) | Clean base(G) | Base | Q20 (%) | Q30 (%) | GC (%) | DUP(%) |
|-----------|-------|-------|-------|-------------|---------------|------|---------|---------|--------|--------|
| NC_1      | 81569682 | 79951252 | 98.02 | 12.24 | 11.62 | 94.93 | 100     | 99.15   | 50.5   | 30.9   |
| NC_2      | 84783386 | 8324756 | 98.18 | 12.72 | 12.07 | 94.89 | 100     | 99.2    | 50     | 32.7   |
| NC_3      | 85184602 | 83679342 | 98.23 | 12.78 | 12.16 | 95.15 | 100     | 99.2    | 50.5   | 30     |
| OE_PPM1K_1 | 87842902 | 86357578 | 98.31 | 13.18 | 12.53 | 95.07 | 100     | 99.2    | 50.5   | 29.5   |
| OE_PPM1K_2 | 92718702 | 91075144 | 98.23 | 13.91 | 13.26 | 95.33 | 100     | 99.15   | 50.5   | 28.8   |
| OE_PPM1K_3 | 83827288 | 82867844 | 98.25 | 12.57 | 11.97 | 95.23 | 100     | 99.2    | 50.5   | 31.1   |
TABLE 2 Mapping of clean reads on the reference genome.

| Sample         | NC_1         | NC_2         | NC_3         | OE_PPM1K_1   | OE_PPM1K_2   | OE_PPM1K_3   |
|----------------|--------------|--------------|--------------|--------------|--------------|--------------|
| Total reads    | 79951252     | 83243760     | 83679342     | 86357578     | 91075144     | 82362784     |
| Total mapped   | 75935773 (94.98%) | 78651556 (94.48%) | 79288743 (94.75%) | 81692131 (94.60%) | 86169047 (94.81%) | 77942656 (94.63%) |
| Total Uniquely mapped | 72136329 (95.06%) | 74747149 (95.04%) | 75255465 (94.91%) | 77474949 (94.84%) | 81690470 (94.81%) | 74129880 (95.11%) |
| Total Multiple mapped | 3799444 (5.00%) | 3904407 (4.96%) | 4033278 (5.09%) | 4217182 (5.16%) | 4474232 (5.19%) | 3812776 (4.89%) |
| Total Pairs    | 39975626     | 41621880     | 41839671     | 4217182 (5.16%) | 4474232 (5.19%) | 3812776 (4.89%) |
| Total Uniquely Concordant Pairs | 34795095 (87.04%) | 36016307 (86.53%) | 36312355 (86.79%) | 37343261 (86.49%) | 39443495 (86.62%) | 35760567 (86.84%) |
| Splice reads   | 40587436 (56.26%) | 39704102 (53.12%) | 41792107 (55.53%) | 44111549 (56.94%) | 47131803 (57.69%) | 42526915 (57.37%) |
| Nonsplice reads| 31548893 (43.74%) | 30540347 (46.88%) | 33463558 (44.47%) | 32365504 (43.06%) | 34563243 (42.31%) | 31602965 (42.63%) |

TABLE 3 Reads distribution across reference Genomic Regions.

| Sample          | 5'UTR         | 3'UTR         | CDS           | Nc_exon       | Introns      | Intergenic  | Antisense |
|-----------------|---------------|---------------|---------------|---------------|--------------|-------------|-----------|
| NC_1            | 4179928 (5.79%) | 773910 (10.72%) | 55093867 (76.37%) | 2015322 (2.79%) | 1997313 (2.77%) | 4841818 (0.67%) | 6348033 (0.88%) |
| NC_2            | 4149816 (5.55%) | 7941809 (10.62%) | 54569187 (73.01%) | 2235797 (2.99%) | 2977100 (3.98%) | 1403003 (1.88%) | 1670404 (1.97%) |
| NC_3            | 4248847 (6.56%) | 8330963 (11.07%) | 57092862 (73.87%) | 2192660 (2.91%) | 2112622 (2.81%) | 554408 (0.74%) | 7230999 (0.96%) |
| OE_PPM1K_1      | 4841226 (6.25%) | 8410511 (10.86%) | 59172387 (76.38%) | 2206522 (2.85%) | 1879135 (2.43%) | 4164483 (0.54%) | 5482478 (0.71%) |
| OE_PPM1K_2      | 4975662 (6.12%) | 9063648 (11.09%) | 62842610 (76.92%) | 2135777 (2.61%) | 1680215 (2.06%) | 4115265 (0.50%) | 5637016 (0.76%) |
| OE_PPM1K_3      | 4756867 (6.42%) | 7972704 (10.76%) | 56693312 (76.48%) | 1993208 (2.69%) | 1801478 (2.43%) | 3928431 (0.54%) | 5134603 (0.69%) |

FIGURE 2
The effect of overexpression of PPM1K gene on the transcriptome of HK-2 cells. (A) Scatter plot exhibiting the most enriched GO biological process results of the up-regulated DEGs. (B) Scatter plot exhibiting the most enriched GO biological process results of the down-regulated DEGs. (C) Scatter plot exhibiting the most enriched KEGG pathway results of the up-regulated DEGs. (D) Scatter plot exhibiting the most enriched KEGG pathway results of the down-regulated DEGs.
characteristics of different genomes, compare the effective sequencing data (clean reads) to the reference genome (Table 2). Statistics of the distribution of reads with unique location on the genome in each region (Table 3).

The effect of overexpression of PPM1K gene on the transcriptome of HK-2 cells

RNA-seq results showed that 31369 genes were co expressed in overexpression group (OE) and control group (NC) cells, and 796 genes were specifically expressed, of which 243 genes were up-regulated and 553 genes were down regulated (Figure 1E). The down regulated genes were significantly more than the down regulated genes (FC ≥ 2 or ≤0.5, FDR <0.05). The heat map shows that NC group and OE group are different, and the two biological replication are highly correlated (Figure 1F).

By extracting the up-regulated epitope genes after PPM1K overexpression, GO analysis showed that the up-regulated genes were significantly enriched in the cellular amino acid biosynthetic process, tRNA aminoacylation for protein translation, intrinsic apoptotic signaling pathway in response to endoplasmic reticulum stress, apoptotic process, response to virus, response to endoplasmic reticulum stress, response to toxic substance, calcium ion transport, nervous system development, cellular calcium ion homeostasis and other biological processes (Figure 2A).

GO analysis showed that the down-regulated genes were significantly enriched in extracellular matrix organization, positive regulation of endothelial cell proliferation, acute–phase response, angiogenesis, cell adhesion, transport across blood–brain barrier, Notch signaling pathway, negative regulation of endopeptidase activity, female pregnancy, epithelial cell differentiation and other biological processes (Figure 2B).

KEGG analysis showed that the up-regulated genes were significantly enriched in biological processes such as cytokine and methionine metabolism, biosynthesis of amino acids, glycine, serine and threonine metabolism, transcriptional misregulation in cancer, vitamin B6 metabolism, metabolic pathways, VEGF signaling pathway, insulin resistance, glycosphingolipid biosynthesis – globo and isoglobo series, endocrine and other factor–regulated calcium reabsorption and so on (Figure 2C).

KEGG analysis showed that the down-regulated genes were significantly enriched in biological processes such as pantothenate and CoA biosynthesis, pathways in cancer, breast cancer, Rap1 signaling pathway, AGEC–RAGE signaling pathway in diabetic complications, regulation of actin cytoskeleton, protein digestion and absorption, IL–17 signaling pathway, axon guidance, complement and coagulation cascades (Figure 2D).

PPM1K regulated expression of apoptosis associated genes in HK2 cells

In the list of functional pathways by GO analysis, we also found that many genes can be enriched in biological processes related to apoptosis, and the up-regulated genes are significantly enriched in biological processes such as apoptotic process, intrinsic apoptotic signaling pathway in response to endoplasmic reticulum stress, positive regulation of apoptotic process, negative regulation of apoptotic process, cell cycle, positive regulation of transcription, DNA–templated, negative regulation of transcription by RNA polymerase II, signal transduction and so on (Figure 3A).

The down-regulated genes are significantly enriched in biological processes such as apoptotic process, positive regulation of apoptotic process, negative regulation of apoptotic process, regulation of apoptotic process, positive regulation of endothelial cell proliferation, negative regulation of neuron apoptotic process, activation of cysteine–type endopeptidase activity involved in apoptotic process, positive regulation of gene expression, positive regulation of transcription DNA–templated, neuron differentiation (Figure 3B).

RT-PCR verifies the reliability of sequencing

In order to verify the expression of apoptosis gene in HK-2 cells overexpressing PPM1K, we used RT-PCR to verify. In the GO functional pathway list, we performed RT-PCR verification on the up-regulated genes such as G0S2, GADD45A, TRIB3, VEGFA and NUPR1, and RT-PCR verification on the down-regulated genes such as IL-6, MAGED1, CCL2 and TP53INP1. The results showed that the RT-PCR expression of G0S2, GADD45A, VEGFA and NUPR1 genes increased in HK-2 cells overexpressing PPM1K gene, which was consistent with the transcriptome sequencing results. The RT-PCR results of G0S2, VEGFA and NUPR1 were statistically significant; The RT-PCR expression of TRIB3 gene decreased, which was statistically significant, but was inconsistent with the transcriptome sequencing results. In HK-2 cells overexpressing PPM1K gene, the RT-PCR expression of IL-6, MAGED1, CCL2 and TP53INP1 genes increased, and the RT-PCR results of IL-6, CCL2 and TP53INP1 were statistically significant; The RT-PCR results of MAGED1 were not statistically significant, but the RT-PCR results of IL-6, MAGED1, CCL2 and TP53INP1 were inconsistent with the transcriptome sequencing results (Figure 4).
Discuss

The single nucleotide polymorphism (SNP) of *PPM1K* leading to protein function defects is associated with many human diseases, such as cardiovascular disease, maple diabetes disease (MSUD), type 2 diabetes and nervous system diseases (Rostaminasab et al., 2018). However, the research on the mechanism of the important role of *PPM1K* in kidney disease is still limited. In this study, we overexpressed *PPM1K* in HK-2 cells, trying to analyze the biological process of differentially expressed genes and their effects on apoptosis through transcriptome.

Apoptosis occurs through two pathways, endogenous and exogenous apoptosis, both of which induce caspase cascade (Kroemer et al., 2007). Apoptosis is considered to be an important process of cell death (Elmore, 2007). In the GO analysis functional pathway list, we also found that multiple genes can be enriched in apoptosis related pathways, such as G0S2, GADD45A, TRIB3, VEGFA, NUPR1 and other up-regulated genes, and IL-6, MAGED1, CCL2, TP53INP1 and other down-regulated genes. Then we verified these differentially expressed genes by RT-PCR, and found that only the RT-PCR results of G0S2, VEGFA and NUPR1 were consistent with the transcriptome sequencing results, while the RT-PCR results of TRIB3, IL-6, MAGED1, CCL2 and TP53INP1 were inconsistent with the transcriptome sequencing results. Therefore, we believe that G0S2, VEGFA, NUPR1 and other genes may participate in the apoptosis process of HK-2 cells induced by *PPM1K*.

G0S2 is a small protein with a length of 103 residues, which is involved in a variety of cellular processes (Russell and Forsdyke, 1991). In lipolysis, G0S2 interacts specifically with triglyceride lipase to inhibit its activity, resulting in down-regulation of lipolysis. Studies have found that the function of G0S2 is to act directly with key proteins. In a similar way, G0S2 is also involved in the regulation of apoptosis, cell proliferation and oxidative phosphorylation (Páez-Pérez et al., 2020). In mitochondria, G0S2 interacts specifically with Bcl-2 to prevent its interaction with Bax. In this way, G0S2 inhibits the formation of anti-apoptotic heterodimer complex bcl-2/bax, which is conducive to apoptosis (Welch et al., 2009).

Nucleoprotein-1 (NUPR1) was initially found to be expressed as a small protein in rats and as a new gene activated during the acute phase of induced pancreatitis and pancreatic development (Mallo et al., 1997). NUPR1 regulates cell injury and death in different forms, depending on the cellular environment and the type of stress induction. NUPR1 is involved in D9 tetrahydrocannabinol (THC) - induced cancer cell death through downstream targeted death inducing telomere repeat binding factor 3 (TRB3) protein (Martin et al., 2021). NUPR1 knockout can regulate autophagy by interfering with FoxO3 and promoting
BNIP3 transcription to control autophagy, which is a stress-dependent self-defense mechanism that helps cells eliminate toxic microenvironment (Kong et al., 2010). It is reported that NUPR1 silences PI3K/AKT mammalian targets through the rapamycin (mTOR) pathway, inhibits autophagy activity of multiple myeloma (mm) cells and induces autophagy mediated apoptosis (Li et al., 2020).

Vascular endothelial growth factor (VEGF) is an endothelial cell specific protein, which can regulate the activity of endothelial cells, promote the formation of new blood vessels, and chemotactic vascular endothelial cells and monocytes, and mediate angiogenesis (Almawi and Saldanha., 2013). Vascular endothelial growth factor A (VEGFA) is a highly effective angiogenesis factor, which can increase vascular permeability and prevent apoptosis. Ting Liu found that metformin can protect kidney by activating Hif-2 α- VEGF signal pathway can reduce the proteinuria of hypertensive rats (Liu et al., 2020). Some studies have found that VEGFA signal can maintain the activity of glomerular endothelial cells, but when the concentration of VEGFA is too high, it may lead to endothelial cell damage (Brosius and Coward, 2014). In the disease of endothelial hyperplasia, excessive release of VEGFA leads to endothelial cell proliferation and swelling, but too little release of VEGFA will lead to endothelial damage and apoptosis, resulting in glomerulosclerosis (Sivaskandarajah et al., 2012). Fan found that inhibiting VEGFA can inhibit cell proliferation, promote cell apoptosis, and thus inhibit cell migration and invasion (Zhengyan et al., 2016). Therefore, in order to maintain the structure and function of the glomerulus, it is necessary to regulate VEGFA signal transduction and regulate the concentration of VEGFA. In our study, although we found

**FIGURE 4**
Related expression level (FPKM) and RT-qPCR measurement of apoptotic-related DEGs. ****p-value < 0.0001. ***p-value < 0.001. **p-value < 0.01.
that VEGFA may participate in the apoptosis process of HK-2 cells induced by PPM1K, we also speculated that HK-2 cells expressing PPM1K may activate VEGFA signals. In order to verify this assumption, we will conduct more in-depth research.

Gadd45a gene is a member of the Gadd45 family, which is involved in the regulation of cell cycle, immunity and apoptosis (Fang et al., 2018). Under the induction of hypertonic pressure, renal medulla cells secrete Gadd45 protein. Hypertonic stress beyond a certain threshold will strongly induce apoptosis (Mak and Kultz, 2004). TRIB3 is a pseudokinase in mammals, which can control cell proliferation, apoptosis, migration and invasion (Ohoka et al., 2005; Salazar et al., 2009; Quan et al., 2016). Huo up-regulated the expression of TRIB3 level in mice. The results showed that there was obvious ER stress reaction in the lungs and kidneys of mice (Lingling et al., 2015). Although apoptosis was found in the kidney, liver and lungs of mice, and apoptosis was found in the lungs and kidneys of mice (Lingling et al., 2015). Although our sequencing results show that GADD45A and TRIB3 are involved in the process of cell apoptosis, the PCR results do not support it. Later, we will further verify it through protein detection and other experiments.

IL-6 is a typical cytokine, which also has pro-inflammatory and anti-inflammatory effects (Lu et al., 2016). IL-6 can activate JAK-STAT signal pathway through membrane-bound IL-6 receptor (classical pathway) or soluble IL-6 receptor (cross signal) (Hebenstreit et al., 2005). IL-6 connected to the receptor can start cascade reaction through JAK activation, activated JAK kinase phosphorylation induces dimer formation through STAT3 phosphorylation (Rose-John, 2012; Brooks et al., 2014), and this signal pathway promotes cell proliferation, differentiation, migration and apoptosis (Rawlings and Harrison, 2004). Some studies have found that IL-6 can play an anti-apoptotic role by regulating the expression of apoptosis related proteins such as Bcl-2, Bax, etc (Meng et al., 2006; Isomoto, 2009). MAGE-D1 is a member of the type II melanoma associated antigen (MAGE) family. MAGE-D1 plays an important role in cell cycle, cell differentiation and apoptosis (Zhang et al., 2016). MAGE-D1 has long been considered as an apoptosis promoting gene. MAGE-D1 is involved in the apoptosis of gastric cancer cells, adrenal pheochromocytoma, Hela cells and other cancer cells (Kendall et al., 2005; Salehi et al., 2006; Cheng et al., 2008). Recent studies have found that MAGE-D1 may play an anti-apoptotic role. Kumar et al. proposed that MAGE-D1 can inhibit the anoikis of lung cancer and breast cancer cells, and promote the anoikis sensitivity (Kumar et al., 2011). Yang et al. found that MAGE-D1 can promote the proliferation of esophageal cancer cells, so they suspected that MAGE-D1 can inhibit the apoptosis of esophageal cancer cells. However, they did not find the difference in apoptosis between the esophageal cancer cells with MAGE-D1 knocked out and the control group (Yang et al., 2014), and other researchers also found that MAGE-D1 has no effect on apoptosis (Wen et al., 2004; Xue et al., 2005; Du et al., 2009).

We believe that the effect of MAGE-D1 on apoptosis is a complex process, which needs a lot of research to confirm. C-C Motif chemokine ligand 2 (CCL2) is a member of the G protein coupled receptor family (Kuang et al., 1996; Bose and Cho, 2013). CCL2 can express chemokine C-C motif receptor 2 (CCR2) chemotactic cells, making monocytes, T lymphocytes and natural killer cells migrate and infiltrate into the inflammatory region (Semple et al., 2010). Fan found that angiopoiitin-1 can reduce the expression of chemokine C-C motif ligand 2 (CCL2) in endothelial cells of fibrotic kidney, thereby inhibiting macrophage migration and reducing cell apoptosis. Nucleoprotein 1 (TP53INP1) induced by tumor protein 53 is a p53 inducible gene that encodes two protein subtypes and regulates p53 biological activity (Jiang et al., 2006). Overexpression of TP53INP1 induces cell cycle arrest in G1 phase and enhances p53 mediated apoptosis (Tomasini et al., 2003). Many studies have shown that TP53INP1 is the target gene of many miRNAs (Ma et al., 2010; Jiang, 2011; Cai et al., 2017; Dan et al., 2018), including miR-221, miR-30a and mir-205 (Chen et al., 2016; Wang et al., 2016; Xu et al., 2016). In addition, TP53INP1 is a regulator of autophagy, which may interact with autophagy related molecules, including light chain three and autophagy related protein eight family proteins, indicating that it can not only regulate, but also promote autophagy (Seillier et al., 2012; Saadi et al., 2015). In our study, we sequenced the transcriptome of HK-2 cells overexpressing PPM1K and found that down-regulated genes such as IL-6, MAGED1, CCL2 and TP53INP1 were enriched in apoptosis related pathways. However, we did not find any reliable basis in the subsequent PCR validation, and we will conduct more in-depth research later.

To sum up, based on RNA seq and bioinformatics analysis, this study identified and screened differentially expressed genes overexpressing PPM1K in HK-2 cells. Through GO and KEGG analysis, combined with PCR validation, it was further screened that G0S2, VEGFA and NUPR1 might participate in the apoptosis process of HK-2 cells induced by PPM1K. This study provides some data support for the study of the mechanism of HK-2 cell apoptosis, and also provides a scientific theoretical basis for the further study of the impact of PPM1K on kidney disease.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/geo; GSE212681.
Author contributions

LZ and XS conceived and designed the study. LZ, YH, AA, ME and ZM collected the data. YH, AA and SK performed the research, analyzed data. LZ, XS and YH wrote the paper. WY and CL reviewed and modified the article. All authors discussed the results and contributed to the final article. All authors read and approved the final article.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Almawi, W. Y., Saldanha, F. L., Mahmood, N. A., Al-Zaman, I., Sater, M. S., and Mustafa, F. E. (2013). Relationship between VEGFPA polymorphisms and serum VEGF protein levels and recurrent spontaneous miscarriage. Hum. Reprod. 28, 2628–2635. doi:10.1093/humrep/det308

Ashburner, M., Ball, C. A., Blake, J. A., Botstein, D., Butler, H., Cherry, J. M., et al. (2000). Gene ontology: Tool for the unification of biology. The Gene Ontology Consortium. Nat. Genet. 25 (1), 25–29. doi:10.1038/75556

Bose, S., and Cho, J. (2013). Role of chemokine CCL2 and its receptor CCR2 in neurodegenerative diseases. Arch. Pharm. Res. 39, 1039–1050. doi:10.1007/s12272-013-0161-z

Brooks Andrew, J., Wei, D., O’Mara, M. L., Chhabra, Y., Pelekanos, R. A., et al. (2014). Mechanism of activation of protein kinase JAK2 by the growth hormone receptor. Science 344 (6185), 710. doi:10.1126/science.1249783

Brosius, F. C., and Coward, R. J. (2014). Podocytes, signaling pathways, and vascular factors in diabetic kidney disease. Adv. Chronic Kidney Dis. 21 (3), 304–310. doi:10.1053/j.ackd.2014.03.011

Cai, Z., Dai, W., Ma, W., and Wu, J. (2017). miR-504 promotes tumour growth and metastasis in human osteosarcoma by targeting TP53INP1. Oncol. Rep. 38 (5), 2995–3000. doi:10.3892/or.2017.5983

Chen, Q., Zhou, Y., Richards, A. M., and Wang, P. (2016). Up-regulation of miRNA-221 inhibits hypoxia/reoxygenation-induced autophagy through the DDRT4/ATM/ологии pathways. Biochem. Biophys. Res. Commun. 474 (1), 168–174. doi:10.1016/j.bbrc.2016.04.090

Chen, X., Mao, X., Huang, J., Yang, D., Wu, J., Dong, S., et al. (2011). KOBAS 2.0: A web server for annotation and identification of enriched pathways and diseases. Nucleic Acids Res. 39, 316–322. doi:10.1093/nar/gkr483

Cheng, C., Huang, X., Ding, Y., Zhang, W., University, H., and Kaifeng (2008). Effect of shRNA against NRAGE gene on proliferation and apoptosis of SGCC791 cells. J. Zhengzhou Univ. (Med. Sci.) (02), 304–306. doi:10.13705/jissn.1671-6825.2008.02.076

Chomczynski, P., and Sacchi, N. (1987). Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. 162 (1), 156–159. doi:10.1006/abio.1987.9999

Dan, L., Tong, L., Ye, C., Zeng, L., Li, H., Pu, X., et al. (2018). miR-221 inhibits autophagy and targets TP53INP1 in colorectal cancer cells. Exp. Ther. Med. 15 (2), 1712–1717. doi:10.3892/etm.2017.5522

Du, Q., Zhang, Y., Tian, X. X., Li, Y., and Fang, W. G. (2009). MAGED-D1 inhibits proliferation, migration and invasion of human breast cancer cells. Oncol. Rep. 22 (3), 659–665. doi:10.3892/or_0000846

Elmore, S. (2007). Apoptosis: A review of programmed cell death. Toxicol. Pathol. 35, 495–516. doi:10.1080/01926230701320337

Ense-Iordanche, B., Perico, N., Bikov, B., Carminati, S., Remuzzi, A., Perna, A., et al. (2016). Chronic kidney disease and cardiovascular risk in six regions of the world (ISN kddc): A cross-sectional study. Lancet. Glob. Health 4 (5), e307–e319. doi:10.1016/S2214-109X(16)00071-1

Fang, Y., Xu, X. Y., Shen, Y., and Li, J. (2018). Molecular cloning and functional analysis of Growth arrest and DNA damage-inducible 45 aa and ab (Gadd45aa and Gadd45 ab) in Ctenopharyngodon idella. Fish. Shellfish Immunol. 77, 187–193. doi:10.1016/j.fsi.2018.03.051

Hebenstreit, D., Horejsi-Hocek, J., and Duschl, A. (2005). JAK/STAT-dependent gene regulation by cytokines. Drug News Perspect. 18 (4), 243–249. doi:10.1588/dnp.2005.18.4.90865

Hrdlickova, R., Toloue, M., and Tian, B. (2017). RNA-Seq methods for transcriptome analysis. WIREs RNA 8 (1). doi:10.1002/wrna.1364

Hwang, S.-J., Lin, M.-Y., and Chen, H.-C. (2012). Prevalence of chronic kidney disease in China. Lancet. Glob. Health 10 (1), 214–216. doi:10.1016/S2214-109X(12)61207-0

Isomoto, H. (2009). Epigenetic alterations in cholangiocarcinoma-sustained IL-6/STAT3 signaling in cholangiocarcinoma due to SOCS3 epigenetic silencing. Digestion 79 (1), 2–8. doi:10.1159/0001067859

Izquierdo-Lahuerta, A., Martinez-Garcia, C., and Medina-Gomez, G. (2016). Lipotoxicity as a trigger factor of renal disease. J. Nephrol. 29 (5), 603–610. doi:10.1007/s40620-016-0278-5

Jiang, F., Liu, T., He, Y., Yan, Q., Chen, X., Wang, H., et al. (2011). MiR-125B promotes proliferation and migration of type II endometrial carcinoma cells through targeting TP53INP1 tumor suppressor in vitro and in vivo. BMC Cancer 11 (1), 425. doi:10.1186/1471-2407-11-425

Jiang, P. H., Motoo, Y., Garcia, S., Iovanna, J. L., Pébusque, M., and Sawabu, N. (2006). Down-regression of tumor protein p53-induced nuclear protein 1 in human gastric cancer. World J. Gastroenterol. 12 (5), 691–696. doi:10.3748/wjg.v12.i5.691

Ju, H. J., Byun, Y. S., Mok, J. W., and Joo, C. K. (2016). The blockade of IL6 counterparts the osmolar stress-induced apoptosis in human conjunctival epithelial cells. J. Ophthalmol. 2016 (3), 835034–835037. doi:10.1155/2016/835034

Kanehsa, M., Goto, S., Kawashima, S., Okuno, Y., and Hattori, M. (2004). The KEGG resource for deciphering the genome. Nucleic Acids Res. 32, D277–D280. doi:10.1093/nar/gkh063

Kelly, K. J., Liu, Y., Zhang, J., Gowami, C., Lin, H., and Dominguez, J. H. (2013). Comprehensive genomic profiling in diabetic nephropathy reveals the predominance of proinflammatory pathways. Physiol. Genomics 45 (5), 710–719. doi:10.1152/physiogenomics.00028.2013

Kendall, S. E., Battelli, C., Irwin, S., Mitchell, J. G., Glackin, C. A., and Verdi, J. M. (2005). NRAGE mediates p38 activation and neural progenitor apoptosis via the bone morphogenetic protein signaling cascade. Mol. Cell. Biol. 25 (17), 7711–7724. doi:10.1128/MCB.25.17.7711-7724.2005

Kim, D., Langmoad, B., and Saalberg, S. L. (2015). Fisat: A fast spliced aligner with low memory requirements. Nat. Methods 12 (4), 357–360. doi:10.1038/nmeth.3317

Kong, D. K., Georgescu, S. P., Kyrilis, J. M., Goruppi, S., Iovanna, J. L., Patten, R. D., et al. (2010). Deficiency of the transcriptional regulator p6 results in increased autophagy and apoptosis, and causes impaired heart function. Mol. Biol. Cell 21 (5), 1333–1349. doi:10.1091/mbc.e09-09-0818
Kostic, S., Haske, T., Ghabramani, N., Filipovic, N., and Vukojevic, K. (2020). Expression pattern of apoptosis-inducing factor in the kidneys of streptozotocin-induced diabetic rats. Acta Histochem. 122 (8), 151655. doi:10.1016/j.acthis.2020.151655

Kroemer, G., Galluzzi, L., and Brenner, C. (2007). Mitochondrial membrane permeabilization in cell death. Physiol. Rev. 87, 99–163. doi:10.1152/physrev.00013.2006

Kuang, Y., Wu, Y., Jiang, H., and Wu, D. (1996). Selective G protein coupling by C-C chemokine receptors. J. Biol. Chem. 271 (8), 3975–3978. doi:10.1074/jbc.271.8.3975

Kumar, S., Park, S. H., Cipriy, B., Schupp, J., Killiam, E., Zhang, F., et al. (2011). A pathway for the control of anoikis sensitivity by E-cadherin and epithelial-to-mesenchymal transition. Mol. Cell. Biol. 31 (19), 4036–4051. doi:10.1128/mcb.01342-10

Li, A., Li, X., Chen, X., Zeng, C., Wang, Z., Li, Z., et al. (2020). NUP1 silencing induces autophagy-mediated apoptosis in multiple myeloma cells through the PI3K/AKT/mTOR pathway. DNA Cell Biol. 39 (3), 368–378. doi:10.1089/dna.2019.5196

Lian, K., Guo, X., Wang, Q., Liu, Y., Wang, R. T., Gao, C., et al. (2020). PP2Cm overexpression alleviates MI/R injury mediated by a BCAA catabolism defect and oxidative stress in diabetic mice. Eur. J. Pharmacol. 866, 172796. doi:10.1016/j.ejphar.2019.172796

Lingling, H., Zhao, L., Long, D., Shi, X., Bai, R., Chen, R., et al. (2015). Silver nanoparticles activate endoplasmic reticulum stress signaling pathway in cell and mouse models. The role in toxicity evaluation. Biomaterials 61, 307–315. doi:10.1016/j.biomaterials.2015.05.029

Liu, B. C., Tang, T. T., Li, L. L., and Lan, H. Y. (2018). Renal tube injury: A driving force toward chronic kidney disease. Kidney Int. 93 (5), 568–579. doi:10.1016/j.kidint.2017.09.033

Liu, T., Hong, L., Yang, Y., Qiao, X., Fu, Y., Zhong, M., et al. (2020). Metformin reduces proteinuria in spontaneously hypertensive rats by activating the HIF-2α-VEGF-A pathway. Eur. J. Pharmacol. 891, 173731. doi:10.1016/j.ejphar.2020.173731

Love, M. I., Huber, W., and Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15 (12), 550. doi:10.1186/s13059-014-0559-8

Ma, S., Tang, K. H., Chan, Y. P., Lee, T. K., Kwan, P. S., Castillo, A., et al. (2010). miR-130b Promotes CD133+ liver tumor-initiating cell growth and self-renewal via tumor protein 53-induced nuclear protein 1. Cell Stem Cell 6 (7), 694–707. doi:10.1016/j.stem.2010.11.010

Mak, S. K., and Kultza, D. (2004). Gadd45 proteins induce G2/M arrest and modulate apoptosis in kidney cells exposed to hyperosmotic stress. J. Biol. Chem. 279, 39705–3987. doi:10.1074/jbc.M301979200

Mallo, G. V., Fiedler, F., Calvo, E. L., Ortiz, E. M., Vasseur, S., Keim, V., et al. (2012). Transcript assembly and quantification of unannotated transcripts and isoform switching during cell differentiation. Nat. Biotechnol. 28 (39), 32360–32369. doi:10.1038/nbt.2337

Martín, T. A., Li, A. X., Sanders, A. J., Ye, L., Frewer, K., Harget, R., et al. (2021). NUPR1 and its potential role in cancer and pathological conditions (Review). Int. J. Oncol. 58 (5), 21. doi:10.3892/ijo.2021.5201

Meng, F., Yamagishi, Y., Ueno, Y., and Patil, T. (2006). Over-expression of inter-alpha-trypsin enhances cell survival and transformed cell function in human malignant cholangiocytic. J. Hepatol. 44 (6), 1055–1065. doi:10.1016/j.jhep.2005.10.030

Nasu, K., Kawakami, T., Shinohara, A., Sakamoto, T., and Nanaguki, M. (2020). Munc18-1-interacting protein 3 mitigates renal fibrosis through protection of tubular epithelial cells from apoptosis. Nephrol. Dial. Transpl. 35 (6), 576–587. doi:10.1093/ndt/gfaa177

Ohoka, N., Yoshi, S., Hattori, T., Ono, Kazuki, and Hayashi, H. (2005). TRB3, a novel ER stress-inducible gene, is induced via ATF4-CHOP pathway and is involved in cell death. Biochem. Biophys. Res. Commun. 323 (39), 37722–37729. doi:10.1016/j.bbrc.2005.11.012

Pellet, S., Meisel, P., Gayet, O., Gauthier, C., N’Guessan, P., Monte, M., et al. (2012). TPS3NIP1, a tumor suppressor, interacts with LC3 and ATG-family proteins through the LC3-interacting region (LIR) and promotes autophagy-dependent cell death. Cell Death Differ. 19 (9), 1525–1535. doi:10.1038/cdd.2012.30

Seillier, M., Páez-Pérez, E., Llamas, M., Benitez-Cardoza, C. G., Montero-Moran, G. M., and Morganti-Kossmann, M. C. (2010). Role of chemokines in CNS health and pathology: A focus on the CCL2/CCL22 and CXCL8/CXCR2 networks. J. Cell. Biochem. 110 (30), 459–473. doi:10.1002/jcb.20940

Semple, B. D., Kossmann, T., and Morganti-Kossmann, M. C. (2010). Role of chemokines in CNS health and pathology: A focus on the CCL2/CCL22 and CXCL8/CXCR2 networks. J. Cell. Biochem. 110 (30), 459–473. doi:10.1002/jcb.20940

Swaskendarajah, G. A., Jeannesson, M., Maezawa, Y., Fermina, V., and Quagg, S. E. (2012). Vga protects the glomerular microvasculature in diabetes. Diabetes 61 (11), 2986–2986. doi:10.2337/db11-1655

Tomasini, R., Simari, A. A., Carrier, I., Isard, D., Cuccinelli, R., Soda, S., et al. (2003). TPS3NIP1 and homodomain-interacting protein kinase-2 (HIPK2) are partners in regulating p53 activity. J. Biol. Chem. 278 (39), 37722–37729. doi:10.1074/jbc.M301979200

Trappnell, C., Williams, B. A., Pertea, G., Mortazavi, A., Kwan, G., van Baren, M. J., et al. (2010). Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. Nat. Biotechnol. 28 (5), 511–515. doi:10.1038/nbt.1621

Wang, W., Liu, J., and Wu, Q. (2016). MI-R253 suppresses apoptosis and enhances radiosensitivity of prostate cancer cells by targeting TPS3NIP1. Eur. Rev. Med. Pharmacol. Sci. 20 (1), 92–100.

Wang, Z., Gerstein, M., and Snyder, M. (2009). RNA-seq: A revolutionary tool for transcriptomics. Proc. Natl. Acad. Sci. U. S. A. 106 (1), 57–63. doi:10.1073/pnas.0810783106

Welch, C., Santra, M. K., El-Assaad, W., Zhu, X., Huber, W. E., Keys, R. A., et al. (2009). Identification of a protein, GOS2, that lacks bcl-2 homology domains and interacts with and antagonizes bcl-2. Cancer Res. 69 (17), 6782–6789. doi:10.1158/0008-5472.CAN-09-0128

Xia, Y., Xie, Y., Lin, L., Zhang, Y., Zhao, H., and Quan, H. (2016). Inhibition of TRB3 protects photoepithelial basement membrane from retinal detachment. Curr. Eye Res. 41, 240–248. doi:10.3109/02713683.2015.1086371

Xue, L., Xie, X. C., Zhou, N., Xue, Q. H., Yang, X., Xie, W. K., Chen, Q., et al. (2017). Relaxon attenuates aristolochic acid induced human tubular epithelial cell apoptosis in vitro by activation of the PI3K/Akt signaling pathway. Apoptosis 22 (6), 769–776. doi:10.1007/s10495-017-1369-z
Xu, C. G., Yang, M. F., Fan, J. X., and Wang, W. (2016). MiR-30a and miR-205 are downregulated in hypoxia and modulate radiosensitivity of prostate cancer cells by inhibiting autophagy via TP53INP1. *Eur. Rev. Med. Pharmacol. Sci.* 20 (8), 1501–1508.

Xue, B., Wen, C., Shi, Y., Zhan, D., and Li, C. (2005). Human NRAGE disrupts E-cadherin/beta-catenin regulated homotypic cell-cell adhesion. *Biochem. Biophys. Res. Commun.* 336 (1), 247–251. doi:10.1016/j.bbrc.2005.08.069

Yang, Q., Ou, C., Liu, M., Xiao, W., Wen, C., and Sun, F. (2014). NRAGE promotes cell proliferation by stabilizing PCNA in a ubiquitin-proteasome pathway in esophageal carcinomas. *Carcinogenesis* 35 (7), 1643–1651. doi:10.1093/carcin/bgu084

Yu, N., Yang, L., Ling, L., Liu, Y., Yu, Y., Wu, Q., et al. (2020). Curcumin attenuates angiotensin II-induced podocyte injury and apoptosis by inhibiting endoplasmic reticulum stress. *FEBS Open Bio* 10 (10), 1957–1966. doi:10.1002/2211-5463.12946

Zhang, G., Zhou, H., and Xue, X. (2016). Complex roles of NRAGE on tumor. *Tumour Biol.* 37 (9), 11535–11540. doi:10.1007/s13277-016-5084-0

Zhengyan, T., Fan-Chang, Z., Ming-Qiang, Z., Liang, H., Yong-Lin, L., Ben-Min, G., et al. (2016). Downregulation of VEGFA inhibits proliferation, promotes apoptosis, and suppresses migration and invasion of renal clear cell carcinoma. *Onco. Targets. Ther.* 9, 2131–2141. doi:10.2147/OTT.S98002