

# Shikonin promoted cell death in renal cancer cells through downregulation of HIF-1 $\alpha$ /AXL pathway

Jia-Wei Hsu

Chung Shan Medical University

Li-sung Hsu

Chung Shan Medical University

Wei-Jen Chen

Chung Shan Medical University

Yi-Hsuan Chung

Chung Shan Medical University

Chih-Jung Chen

c.jchen1016@gmail.com

Taichung Veterans General Hospital

---

## Research Article

**Keywords:** shikonin, renal cancer, AXL, hypoxia-induced factor-1 $\alpha$  (HIF-1 $\alpha$ )

**Posted Date:** October 21st, 2026

**DOI:** <https://doi.org/10.21203/rs.3.rs-10916594/v1>

**License:**   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

**Additional Declarations:** No competing interests reported.

---

# Abstract

Renal cancer presents a poor prognosis due to high metastatic potential and chemoresistance. Shikonin, a natural naphthoquinone compound, exhibits potent antitumor activities. In this article, we report that shikonin induced renal cancer cells to undergo apoptosis through the downregulation of Anexelokto (AXL) expression. First, we employed human XL Oncology protein array to survey the molecular target of shikonin in renal cancer cells. Treatment with 6  $\mu\text{M}$  shikonin repressed AXL expression, whereas cotreatment with the antioxidant agent *N*-acetyl cysteine (NAC) recovered AXL expression. Furthermore, Western blot analysis indicated that AXL expression was repressed in proportion to shikonin concentration, and NAC reversed AXL expression in the presence of shikonin. The Cancer Genome Atlas database analysis revealed that AXL was significantly higher in clear and papillary renal cancer tissues than in normal tissues. AXL was also significantly correlated with advanced stage ( $p = 0.004$ ), T status ( $p = 0.005$ ), and survival ( $p < 0.001$ ). The knockdown of AXL expression by small interference RNA (siRNA) abolished AXL expression. As evidenced by flow cytometry, AXL downregulation enhanced apoptosis and necrosis induced by 2 and 4  $\mu\text{M}$  shikonin. Western blot analysis also revealed that in the presence of 2  $\mu\text{M}$  shikonin, cleavage-PARP increased in AXL siRNA cells relative to in parental cells. Moreover, shikonin dose-dependently abolished the expression of the AXL upstream transcription factor hypoxia-induced factor-1 $\alpha$ . Taken as a whole, our findings indicate that shikonin combined with AXL siRNA could be a potential therapeutic approach for the treatment of renal cancer.

## 1. Introduction

Renal cell carcinoma (RCC) is a highly prevalent malignant disease (sixth in men and 10th in women) worldwide [1]. The treatment of renal cancer depends on surgical resection, chemotherapy, and immunotherapy [2]. However, as a result of late diagnosis, recurrence and metastasis, the prognosis of renal cancer remains unsatisfactory, with a five-year survival rate of approximately 10%–18% [2, 3]. Searching for novel and effective therapies with few side effects is an urgent issue for RCC treatment.

Shikonin, a naphthoquinone isolated from the Chinese traditional medicine *Lithospermum erythrorhizon*, exhibits anti-inflammation, antioxidant, and antitumor properties [4, 5]. Shikonin is currently receiving considerable attention because of its strong cytotoxicity effects. It has been found to elevate cellular oxidative stress and induce cancer cells to undergo programmed cell death in various cancers, such as blood cancer, glioma, and oral cancer [6]. It induces reactive oxygen species (ROS) generation and eventually triggers apoptosis in adult T cell leukemia/lymphoma cells [7]. Shikonin enhances endoplasmic reticulum stress and mitochondrial membrane permeability disruption to promote the apoptosis of glioma and oral cancer cells [8, 9]. It was also found to trigger distinct cell death processes, such as apoptosis, necrosis, and autophagy, in the renal cancer cell line ACHN and Caki-1 cells through the elevation of cellular ROS [10]. Blocking LC3II expression through small hairpin RNA (shRNA) or inhibiting p38 activity with an inhibitor significantly recovers shikonin-induced cell death in renal cancer cells [10]. Kiraly et al. demonstrated that treatment with 5  $\mu\text{M}$  shikonin significantly enhances apoptosis in renal cancer cells, as evidenced by elevated caspase-3 and caspase-7 activities and Ras/MAPK and

PI3K/AKT pathway activation [11]. Shikonin regulates the expression of microRNA (miR)-15b, miR-99b, and miR-181a and their downstream targets, such as FOXO1, FOXO3, and PDCD4 and MAPK, to promote apoptosis in clear cell renal cancer cells [12].

Receptor tyrosine kinases (RTKs) execute a wide range of cellular functions, such as cell proliferation, differentiation, metastasis, and angiogenesis [13]. Upon binding to its high-affinity ligand growth arrest-specific protein 6, anexelokto (AXL) protein, a member of the TYRO3-AXL-MER RTK family, participates in cancer progression, metastasis, and drug resistance [14]. AXL activation enhances the MAPK/ERK and PI3K/AKT pathways to stimulate cell proliferation, chemoresistance, metastasis, and regulate metabolic activities [14]. AXL has been reported to be overexpressed in several human cancers, such as pancreatic cancer, lung adenocarcinoma, renal cancer, and papillary thyroid carcinoma, and is correlated with advanced stages and poor prognosis [15]. In breast cancer cells, the high expression of AXL was found to be correlated with resistance to anti-Her2 therapies, whereas the knockdown of AXL elevates the therapeutic effects of trastuzumab [16]. Cancer proliferation is repressed in vitro and/or in vivo by using antibody-blocked AXL receptor or promoting AXL degradation. Several clinical trials have shown that the administration of AXL inhibitor markedly improves the outcomes of a range of cancers [17].

A previous report from our laboratory demonstrated that shikonin triggers different cell death processes in renal cancer cells in a ROS-dependent manner [10]. However, the molecular mechanisms of these effects still remained to be elucidated. In this study, we investigated the downstream target of shikonin and explored its cell death effects on renal cancer cells.

## **2. Materials and methods**

### **2.1. Cell culture**

Human clear cell carcinoma cells (Caki-1, ATCC HTB-46) and papillary cell carcinoma cells (ACHN, ATCC CRL-1611) were kindly gifted from professor Yi-Hsien Hsieh (Institute of Medicine, Chung Shan Medical University) and maintained in minimum essential medium (MEM) with 10% fetal bovine serum, 100 U/mL penicillin, and 50 g/mL streptomycin at 37°C with 5% CO<sub>2</sub>.

### **2.2. Human XL oncology protein array assay**

Caki-1 cells with or without 10 mM *N*-acetyl cysteine (NAC) pretreatment were treated with 0 or 6 µM shikonin for 24 h. Cell lysates were extracted by using RIPA lysis buffer (25 mM Tris-HCl, pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, and 0.1% SDS) and subjected to human XL Oncology protein array in accordance with the manufacturer's protocol. Signals were measured with a densitometer.

### **2.3. Western blot analysis**

Human Caki-1 and ACHN cells with or with 10 mM NAC pretreatment and treated with the indicated concentration of shikonin were lysed in RIPA lysis buffer. A total of 30 µg of proteins was separated on 4%–20% polyacrylamide gel, transferred, and blocked with 5% nonfat milk plus 0.1% bovine serum

albumin at room temperature for 1 h. After being washed with phosphate buffer saline containing 0.1% Tween-20, the membranes were incubated with the indicated primary antibodies at 4°C overnight.

## 2.4. Cell viability assay

ACHN or Caki-1 cells were plated in 24-well plates at a density of  $1 \times 10^4$ /mL. Cells were treated with the indicated concentration of shikonin for 24 h.

## 2.5. Statistical analysis

The chi-squared test was used to determine significant differences between clinical parameters. Kaplan–Meier analysis was conducted to measure overall survival rate.  $p < 0.05$  indicated a significant difference.

## 3. Results

### 3.1. Shikonin significantly reduced AXL expression

We performed human XL oncology array analysis to elucidate the downstream targets of shikonin. Figure 1A shows that AXL expression in ACHN cells significantly decreased under 6  $\mu$ M shikonin treatment but recovered under NAC pretreatment. We conducted Western blot analysis to confirm whether shikonin downregulated AXL expression further. AXL expression in the presence of 2, 4, and 6  $\mu$ M shikonin reduced compared with that in control ACHN cells but significantly recovered under NAC treatment (Fig. 1B). Similarly, AXL expression in Caki-1 cells was inversely related to the concentration of shikonin (Fig. 1C). These data indicate that shikonin repressed AXL expression in renal cancer cells.

### 3.2. Clinical correlation of AXL with the clinical parameters of patients with renal cancer

A previous report indicated that AXL promotes tumor progression in several cancers. We randomly selected 303 patients with renal cancer from The Cancer Genome Atlas (TCGA) database (<https://portal.gdc.cancer.gov/>) to detect whether AXL expression was correlated with the clinical parameters of patients with renal cancer and subdivided AXL expression into high (N = 152) and low (N = 151). Of the patients with low AXL expression, 67.5% were at the lower clinical stage (stage I + II) and 32.5% were at the higher stage (stage III + IV). On the other hand, of the patients with high AXL expression, approximately 51.3% were at the low clinical stage and 49.7% were at the high clinical stage. These results indicate that AXL expression is highly correlated with clinical stage ( $p = 0.04$ ). In terms of clinical TNM status, high AXL expression was significantly associated with high clinical T status (T3 + T4) ( $p = 0.005$ ). However, no association was found between AXL expression and N and M status. Survival statistics revealed that the survival rate of patients with low AXL expression (80.1%) was significantly higher than that of patients with high AXL expression (59.9%). GEPIA website analysis indicated that AXL expression was significantly higher in tumors than in normal parts of clear cell renal

carcinoma; while it was also elevated in tumors of papillary renal carcinoma, this change was not statistically significant (Fig. 2A). We found no association between the expression of AXL and the different stages of renal carcinoma on the GEPIA website (Fig. 2B). Moreover, Kaplan–Meier survival analysis showed that the five-year survival rate was significantly lower in patients with high AXL expression than in those with low AXL expression (Fig. 2C). These results indicate that AXL plays a pivotal role in renal cancer progression.

### **3.3. Knockdown of AXL enhanced shikonin-induced cell death and related protein expression**

We performed siRNA to knockdown AXL expression and thus investigate the effect of AXL on shikonin-induced cell death. Figure 3A shows that siAXL-574 decreased AXL expression to 53% and 27% in ACHN and Caki-1 cells, respectively, whereas siAXL-575 inhibited AXL expression to 8% and 11% in ACHN and Caki-1 cells, respectively. We selected si-AXL-575 clones for further experiments.

Next, we conducted PI/annexin V double staining to detect the effect of AXL on shikonin-induced cell death. While we found no overt alteration in necrosis in AXL-knockdown ACHN cells, we discovered that AXL knockdown significantly enhanced 2  $\mu$ M shikonin-induced apoptosis in ACHN cells (Fig. 3B). Necrosis and apoptosis were significantly elevated in AXL-knockdown Caki-1 cells treated with 2  $\mu$ M shikonin relative to in cells in the scramble group (Fig. 3B).

We conducted Western blot analysis to verify whether apoptosis-related proteins were affected by AXL knockdown. Figure 3C shows that 2  $\mu$ M shikonin significantly increased cleaved PARP in the AXL-575 knockdown group relative to in the scramble group.

### **3.4. Shikonin attenuated hypoxia-induced factor-1 $\alpha$ expression**

Previous report demonstrated that hypoxia-induced factor-1 $\alpha$  (HIF-1 $\alpha$ ) bound to the AXL promoter region and elevated AXL expression in renal cancer cells [18]. We performed Western blot analysis to determine whether shikonin affected HIF-1 $\alpha$  expression. Shikonin significantly mitigated HIF-1 $\alpha$  expression in proportion to concentration, and pretreatment with NAC recovered HIF-1 $\alpha$  expression (Fig. 4B). Next, we used MG-132, a proteinase inhibitor, to detect whether the downregulation of AXL or HIF-1 $\alpha$  by shikonin resulted from protein degradation. Western blot analysis showed that in cells pretreated with MG-132, AXL or HIF-1 $\alpha$  expression did not recover in the presence of shikonin (Fig. 4A).

## **4. Discussion**

Renal cancer is a leading cause of urinary system cancer-related deaths, causing approximately 15,000 deaths annually [19]. AXL overactivation plays critical roles in the proliferation, chemoresistance, and metastasis of human cancers [20]. Shikonin has been shown to promote cell death in several cancers [21]. In this study, we revealed that shikonin dose-dependently abolished HIF-1 $\alpha$

and its downstream target AXL, the expression of which facilitated shikonin-induced cell death via the elevation of ROS generation.

AXL is a major RTK that is involved in cancer progression, metastasis, and chemoresistance. Its overexpression has been observed in human cancers. In line with these observations, we demonstrated, by using TCGA database analysis, that in patients with renal cancer, AXL expression was significantly higher in stages III and IV than in stages I and II. Elevated AXL expression was also associated with a short overall survival rate.

Dietary flavonoids from vegetables and/or fruits exhibit several benefits, including anti-inflammation, antioxidant, and antitumor effects [22]. Yuan et al. reported that kaempferol bound to AXL through hydrogen binding, inhibited AXL activities, and then abolished NSCLC proliferation in a dose-dependent manner [23]. In cisplatin-resistant lung cancer cells, epigallocatechin gallate increased cisplatin sensitivity through the downregulation of AXL [24]. In small cell lung cancer cells, *Marsdenia tenacissima* extract significantly diminished AXL expression and promoted endoplasmic reticulum stress and immunogenic cell death [25]. Kim et al. demonstrated that quercetin decreased AXL mRNA levels, protein expression, and stability then repressed the viability of glioma cells [26]. In MDA-MB-231 breast cancer cells, ampelopsins A and C inhibited the phosphorylation of AXL then exerted antiproliferation effects [27]. In line with these observations, our results showed that shikonin dose-dependently reduced AXL expression via the elevation of ROS levels. The depletion of AXL expression by siRNA increased shikonin-induced cell death, as evidenced by the enhancement in apoptosis.

Reports have demonstrated that HIF-1 $\alpha$  regulates AXL expression. High-throughput chromatin immunoprecipitation with gene expression analysis identified AXL as a downstream target of HIF-1 $\alpha$  [18]. The depletion of HIF-1 $\alpha$  activity led to the downregulation of AXL expression [18]. Promoter assays also revealed that HIF-1 $\alpha$  bound to the promoter region of AXL and regulated AXL expression [18]. Nalwoga et al. showed that HIF-1 $\alpha$  expression is associated with AXL expression and correlated with advanced stages of breast cancer [28]. Li et al. demonstrated that treatment with shikonin diminished HIF-1 $\alpha$  activity in several human cancer cell lines [29]. In HMVEC-dLy cells, shikonin suppressed lamphangiogenesis via the downregulation of HIF-1 $\alpha$  expression [30]. Network pharmacology and molecular docking technology have recently shown that shikonin regulates the HIF-1 $\alpha$  pathway and then plays a critical role in the proliferation in oral and renal cancers [31, 32]. In line with these observations, our results revealed that shikonin significantly repressed HIF-1  $\alpha$  expression in a ROS-dependent manner.

## 5. Conclusion

In conclusion, our results provide the first evidence that shikonin significantly mitigates HIF-1 $\alpha$  and the expression of its downstream target AXL in a ROS-dependent manner. AXL expression was associated with advanced stages and poor survival rates. When combined with AXL siRNA, shikonin synergistically triggered cell death in renal cancer cells. Taken together, our findings indicate that the combination of shikonin with AXL siRNA could be a promising antirenal cancer agent.

# Declarations

## Ethical approval

Not applicable. Only commercial cell lines and public database were used in this study.

## Consent to participate

Not applicable.

## Consent to publish

Not applicable.

## Competing interests

The authors declare no competing interests.

## Funding

This study was supported by Taichung Veterans General Hospital, No. TCVGH-1115801C.

## Authors' contributions

J. W. H. and L.S.H. designed the experiment. L.S.H. wrote the manuscript. J. W. H., W.J.C. and Y. H. C. performed the experiments. W. J. C. and Y. H C. developed the methodology. J. W. H., W. J. C. and Y. H. C. analyzed the data and edited the Figures. L.S.H. and C. J. C. read and revised the manuscript. All Authors confirmed and approved all parts of this study.

## Clinical trial number:

Not applicable.

## Data Availability Statement

All other data are available from the corresponding author upon reasonable request.

# References

1. Siegel RL, Miller KD, Jemal A, Cancer statistics. 2019. CA Cancer J Clin. 2019; 69: 7–34.
2. Su J, Zhou L, Zhang Z, Xiao X, Qin Y, Zhou X, et al. The components of tumor microenvironment as biomarker for immunotherapy in metastatic renal cell carcinoma. Front Immunol. 2023;14:1146738.
3. Lavacchi D, Pellegrini E, Palmieri VE, Doni L, Mela MM, Di Maida F et al. Immune Checkpoint Inhibitors in the Treatment of Renal Cancer: Current State and Future Perspective. Int J Mol Sci. 2020; 21.

4. Yan C, Li Q, Sun Q, Yang L, Liu X, Zhao Y, et al. Promising Nanomedicines of Shikonin for Cancer Therapy. *Int J Nanomed*. 2023;18:1195–218.
5. Sun Q, Gong T, Liu M, Ren S, Yang H, Zeng S, et al. Shikonin, a naphthalene ingredient: Therapeutic actions, pharmacokinetics, toxicology, clinical trials and pharmaceutical researches. *Phytomedicine*. 2022;94:153805.
6. Lew CY, Tang YT, Lee AY, Chin ZJ, Chang WL, Chen CH et al. Shikonin as a Dietary Phytochemical with Multi-Target Anti-Cancer Activities: From Molecular Mechanisms to Translational Applications. *Nutrients*. 2025; 17.
7. Boonnate P, Kariya R, Okada S, Shikonin Induces. ROS-Dependent Apoptosis Via Mitochondria Depolarization and ER Stress in Adult T Cell Leukemia/Lymphoma. *Antioxid (Basel)*. 2023; 12.
8. Biswal S, Panda M, Biswal BKS. Stimulates Mitochondria-Mediated Apoptosis by Enhancing Intracellular Reactive Oxygen Species Production and DNA Damage in Oral Cancer Cells. *J Cell Biochem*. 2025;126:e30671.
9. Ding Y, He C, Lu S, Wang X, Wang C, Wang L, et al. MLKL contributes to shikonin-induced glioma cell necroptosis via promotion of chromatinolysis. *Cancer Lett*. 2019;467:58–71.
10. Tsai MF, Chen SM, Ong AZ, Chung YH, Chen PN, Hsieh YH et al. Shikonin Induced Program Cell Death through Generation of Reactive Oxygen Species in Renal Cancer Cells. *Antioxid (Basel)*. 2021; 10.
11. Kiraly J, Szabo E, Fodor P, Fejes Z, Nagy B Jr., Juhasz E et al. Shikonin Causes an Apoptotic Effect on Human Kidney Cancer Cells through Ras/MAPK and PI3K/AKT Pathways. *Molecules*. 2023; 28.
12. Vass A, Kiraly J, Szabo E, Shree N, Ramos D, Choudhury M et al. Potential Regulatory Role of miR-15b, miR-99b, and miR-181a of the Shikonin-Induced MAPK/ERK Apoptotic Signaling Pathway in Renal Carcinoma. *Biomedicines*. 2025; 13.
13. Hussain S, Mursal M, Verma G, Hasan SM, Khan MF. Targeting oncogenic kinases: Insights on FDA approved tyrosine kinase inhibitors. *Eur J Pharmacol*. 2024;970:176484.
14. Vouri M, Hafizi STAM. Receptor Tyrosine Kinases in Cancer Drug Resistance. *Cancer Res*. 2017;77:2775–8.
15. Tang Y, Zang H, Wen Q, Fan S. AXL in cancer: a modulator of drug resistance and therapeutic target. *J Exp Clin Cancer Res*. 2023;42:148.
16. Boz AB, Er I, Picher EA, Smarakan SAXL-D. Stemness and Hedgehog Signaling in HER2-Positive Breast Cancer with Acquired Trastuzumab Resistance: Synergistic Potential of AXL and HER2 Co-Targeting. *Life (Basel)*. 2026; 16.
17. Quach E, Sanginova F, Cheruku A, Stack S. Falchook GS AXL Inhibitors in Oncology Clinical Trials: A Review. *J Immunother Precis Oncol*. 2026;9:1–16.
18. Rankin EB, Fuh KC, Castellini L, Viswanathan K, Finger EC, Diep AN, et al. Direct regulation of GAS6/AXL signaling by HIF promotes renal metastasis through SRC and MET. *Proc Natl Acad Sci U S A*. 2014;111:13373–8.

19. Hsieh JJ, Purdue MP, Signoretti S, Swanton C, Albiges L, Schmidinger M, et al. Renal cell carcinoma. *Nat Rev Dis Primers*. 2017;3:17009.
20. Zhang WN, Li XP, Wang PF, Zhu L, Xiao XH, Dai YJ Comprehensive analysis of the novel omicron receptor AXL in cancers. *Comput Struct Biotechnol J*. 2022;20:3304–12.
21. Gong K, Li W. Shikonin, a Chinese plant-derived naphthoquinone, induces apoptosis in hepatocellular carcinoma cells through reactive oxygen species: A potential new treatment for hepatocellular carcinoma. *Free Radic Biol Med*. 2011;51:2259–71.
22. Kopustinskiene DM, Jakstas V, Savickas A, Bernatoniene J. Flavonoids as Anticancer Agents. *Nutrients*. 2020; 12.
23. Yuan Y, Guo Y, Guo ZW, Hao HF, Jiao YN, Deng XX, et al. Marsdenia tenacissima extract induces endoplasmic reticulum stress-associated immunogenic cell death in non-small cell lung cancer cells through targeting AXL. *J Ethnopharmacol*. 2023;314:116620.
24. Kim KC, Lee C. Reversal of Cisplatin resistance by epigallocatechin gallate is mediated by downregulation of axl and tyro 3 expression in human lung cancer cells. *Korean J Physiol Pharmacol*. 2014;18:61–6.
25. Yang L, Ren A, Wang X, Chen S, Ni S, Wang Y, et al. Marsdenia tenacissima extract potentiates the anti-lung cancer efficacy of gefitinib through dual regulation of the Wnt pathway and ferroptosis. *J Ethnopharmacol*. 2026;359:121121.
26. Kim HI, Lee SJ, Choi YJ, Kim MJ, Kim TY, Ko SG. Quercetin Induces Apoptosis in Glioblastoma Cells by Suppressing Axl/IL-6/STAT3 Signaling Pathway. *Am J Chin Med*. 2021;49:767–84.
27. Huang C, Huang YL, Wang CC, Pan YL, Lai YH, Huang HC, Ampelopsins A. Induce Apoptosis and Metastasis through Downregulating AxL, TYRO3, and FYN Expressions in MDA-MB-231 Breast Cancer Cells. *J Agric Food Chem*. 2019;67:2818–30.
28. Nalwoga H, Ahmed L, Arnes JB, Wabinga H, Akslen LA. Strong Expression of Hypoxia-Inducible Factor-1alpha (HIF-1alpha) Is Associated with Axl Expression and Features of Aggressive Tumors in African Breast Cancer. *PLoS ONE*. 2016;11:e0146823.
29. Li MY, Mi C, Wang KS, Wang Z, Zuo HX, Piao LX, et al. Shikonin suppresses proliferation and induces cell cycle arrest through the inhibition of hypoxia-inducible factor-1alpha signaling. *Chem Biol Interact*. 2017;274:58–67.
30. Prangsaengtong O, Jantaree P, Lirdprapamongkol K, Svasti J, Koizumi K. Shikonin Suppresses Lymphangiogenesis via NF-kappaB/HIF-1alpha Axis Inhibition. *Biol Pharm Bull*. 2018;41:1659–66.
31. Gong WW, Yang Y, Wang SM, Zha X, Ruan JH, Zhang L et al. Screening the Mechanism of Shikonin Against Renal Cell Carcinoma via Network Pharmacology, Molecular Docking, and Cellular Experimental Verification. *J Vis Exp*. 2026.
32. Hou L, Wang K, Wang Y, Li J, Guo L, Zhao Q. Exploring the Mechanism of Oral Cancer With Shikonin Based on the Network Pharmacology and Molecular Docking Technology. *Int Dent J*. 2026;76:103954.

Table 1

Table 1 is available in the Supplementary Files section.

Figures

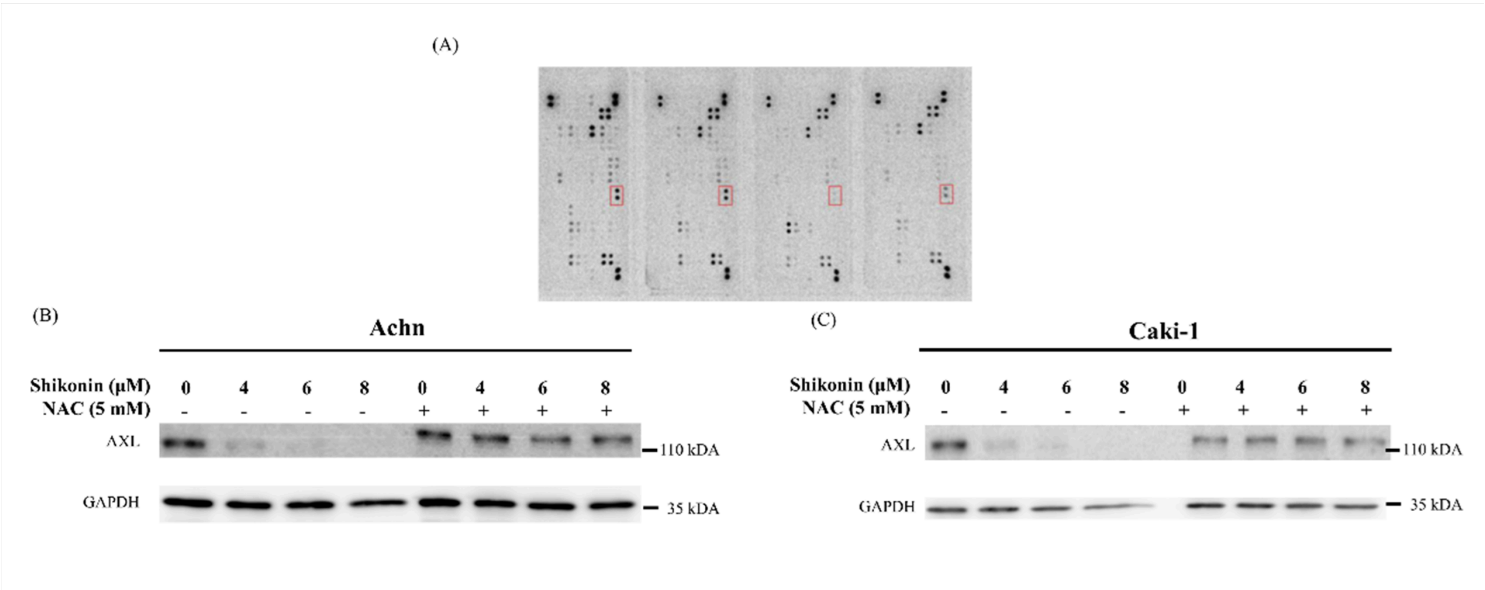


Figure 1

**Shikonin mitigated AXL expression in renal cancer in a dose-dependent manner.** (A) Proteins extracted from Caki-1 cells with or without NAC pretreatment were treated with 6 mM shikonin and subjected to human XL oncology array analysis. Red boxes indicate AXL expression. (B and C) Cell lysates obtained from ACHN or CAKi-1 were subjected to the indicated treatment for 24 h. AXL expression was detected through Western blot analysis.

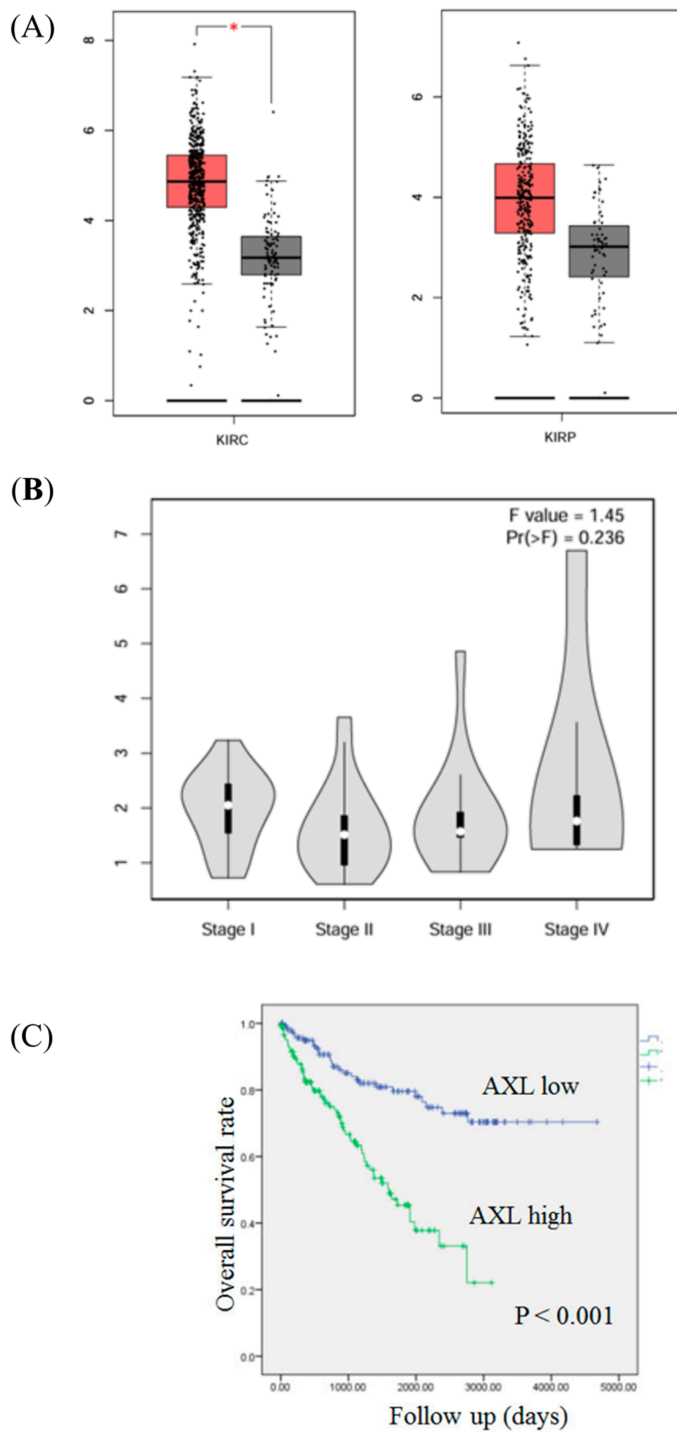


**Figure 2**

**AXL expression in (A) different types and (B) stages of renal carcinoma. (C) Kaplan–Meier survival analysis of patients with or with high AXL expression.** A total of 303 patients with renal cancer were classified as AXL high (AXL mRNA levels above average) and AXL low (AXL mRNA levels below average), and their survival rate was calculated through Kaplan–Meier analysis. \*:  $p < 0.05$ .

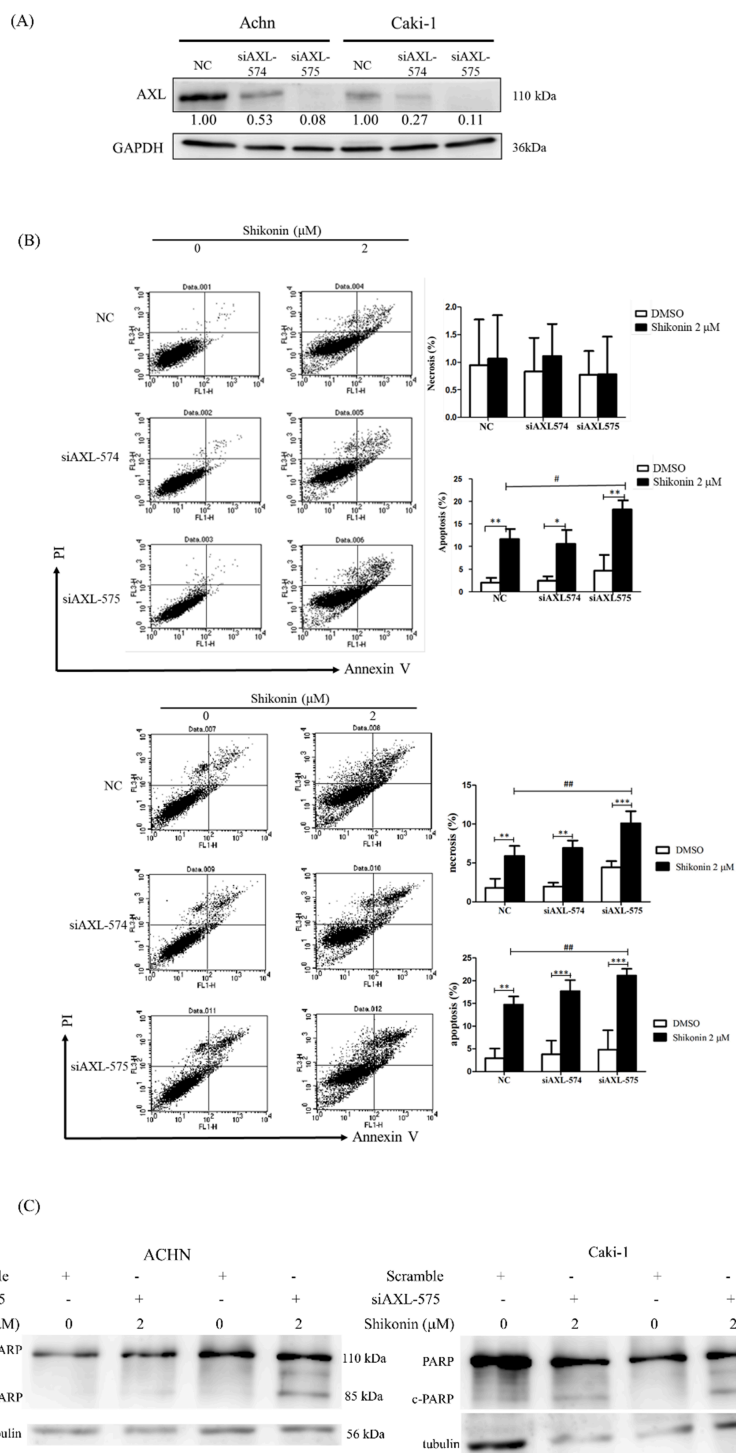


**Figure 3**

**Knockdown of AXL enhanced shikonin-induced cell death.** (A) ACHN and Caki-1 cells were treated with scramble or AXL shRNA for 24 h. Cell lysates were then subjected to Western blot analysis by using AXL-specific antibodies. (B) ACHN or Caki-1 cells treated with scramble or AXL-575 shRNA were treated with 2 mM shikonin for 24 h. Cells were stained with PI/Annexin V and subjected to flow cytometry. (C) Cell

lysates derived under the indicated ACHN and CAki-1 treatment were subjected to Western blot analysis. DAPDH was used as the internal control.

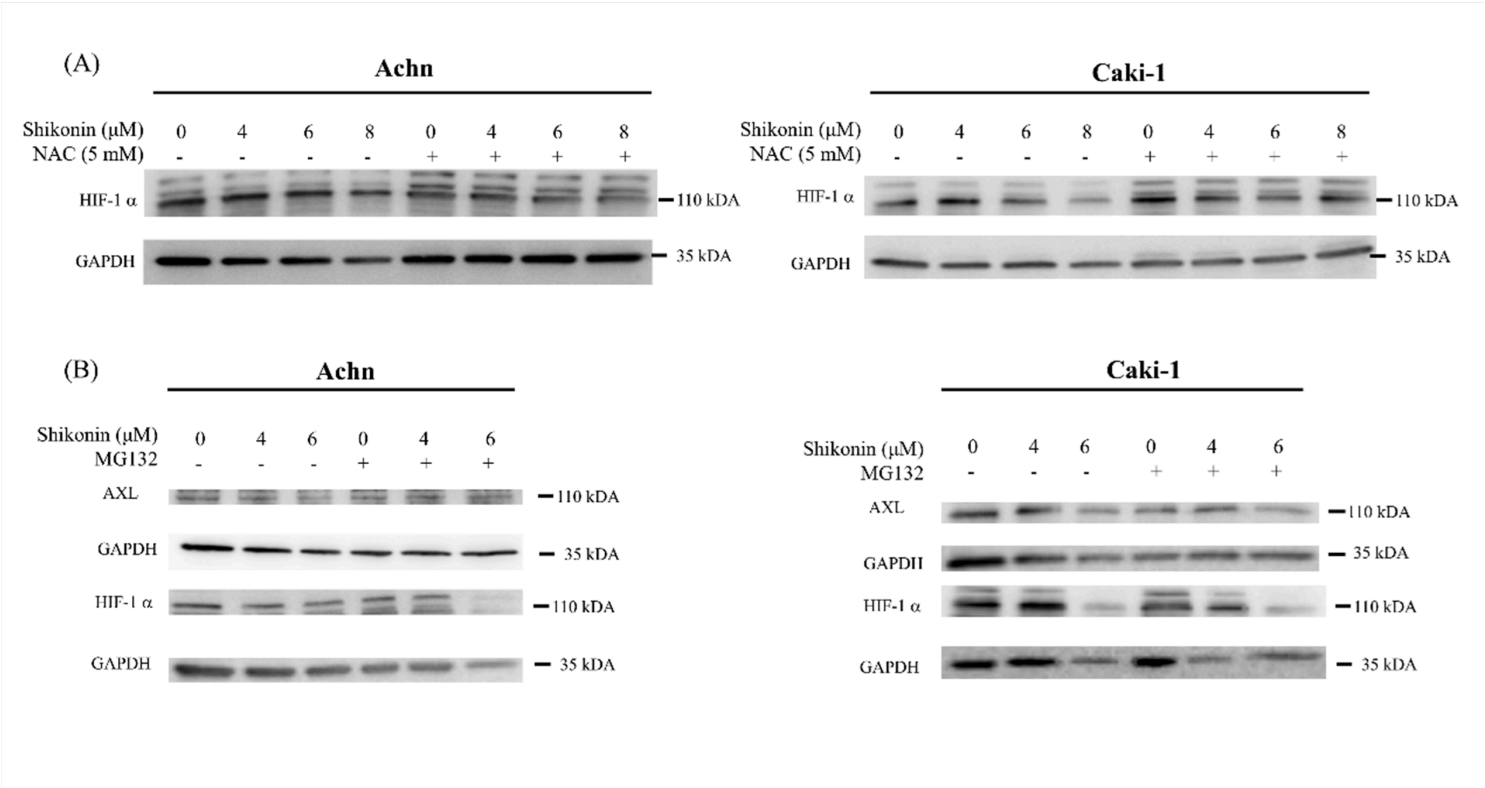


Figure 4

**Shikonin downregulated HIF-1 a expression.** (A) ACHN or Caki-1 cells with or without NAC pretreatment were treated with the indicated concentration of shikonin for 24 h. Cell proteins were subjected to Western blot analysis. (B) ACHN or Caki-1 cells with or without MG-132 pretreatment were treated with the indicated concentration of shikonin for 24 h. Cell proteins were subjected to Western blot analysis.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [RESULTORIGINAL.pptx](#)
- [Table1.docx](#)