AKR1B10 accelerates the production of pro-inflammatory cytokines via NF-κB signaling pathway in colon cancer

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Abstract

Aldo-keto reductase family 1, member B10 (AKR1B10) has been reported to be involved in tumorigenesis of various cancer. In our studies, we evaluated the relationship between AKR1B10 expression and clinicopathological characteristics in colon cancer and showed that AKR1B10 expression was significantly correlated with TNM stage and clinical stage of colon cancer. It has been reported that colorectal cancer is closely associated with chronic inflammation and the underlying molecular mechanisms are still elusive. Here we found that knockdown of AKR1B10 significantly decreased the expression of the inflammatory cytokines, IL1\(\alpha\) and IL6, induced by lipopolysaccharide (LPS) via inhibiting NF-\(\kappa\)B signaling pathway. Furthermore, AKR1B10 depends on its reductase activity to affect the NF-\(\kappa\)B signaling pathway and subsequently affect the production of inflammatory cytokines. In addition, knockdown of AKR1B10 effectively reduced cell proliferation and clonogenic growth, indicating the biologic role of AKR1B10 in colon cancer. Collectively, our findings provided important insights into a previously unrecognized role of AKR1B10 in colon cancer.

1 Introduction

Colon cancer is the third most commonly diagnosed malignancy and the fourth leading cause of cancer-related death [1]. It was estimated that there will be more than 2.2 million new cases of colon cancer and 1.1 million death cases of colon cancer worldwide in 2030 [2]. At present, surgery remains the primary choice for the treatment of colon cancer. Meanwhile adjuvant therapy is applied to diminish the rates of local recurrence and improve the rates of survival [3]. However, the five-year survival of colon cancer patients is only about 50% [4]. More evidences have proved that the development of colon cancer is bound up with chronic inflammation and post-operative infection [5, 6].

Aldo-keto reductase 1B10 (AKR1B10) is an aldose reductase-like oxidoreductase of human origin which belongs to the aldo-keto reductase superfamily [7]. AKR1B10 typically expresses in the gastrointestinal tract, including human colon, liver, adrenal glands and small intestine. Oppositely, low expression of AKR1B10 has been identified in colon cancer, stomach cancer, head and neck cancer [8, 9], while high expression of AKR1B10 has been reported in various solid cancers, such as breast cancer, hepatocellular carcinoma, non-small cell lung carcinoma, cervical, pancreatic carcinoma and endometrial cancers [10–14]. Of note, our tissue microarray results showed that cases with low expression of AKR1B10 are predominant in the early stage of colon cancer, while the percentage of cases with high expression of AKR1B10 are substantially increased in the late stage of colon cancer.

NF-\(\kappa\)B family regulate many genes involved in immune and inflammatory responses, which consists of five members: NF-\(\kappa\)B 1 (p105/p50), NF-\(\kappa\)B 2 (p100/p52), RelA (p65), RelB and c-Rel [15]. NF-\(\kappa\)B is constitutively activated and then induces the expression of IL–1 and IL6 to exert various pro-tumorigenic functions in many kinds of tumor cells [16]. IL1\(\alpha\) was the first identified member of the IL–1 cytokine family that have pleiotropic effects in inflammation and cancer [17]. Recently, researchers has also demonstrated IL1\(\alpha\) as an apical regulator of colon inflammation and cancer, cardiovascular disease and
neural inflammation [18–20]. IL6 has both pro- and anti-inflammatory properties and play a crucial role in acute-phase and immune responses of the organism, which belongs to the family of IL (interleukin)–6-type cytokines [21]. Documented evidence has indicated that IL1α and IL6 are both found in most cell lines, including tumor cell lines [17, 21]. In our study, we found that AKR1B10 could promote the production of inflammatory cytokines IL1α and IL6 through NF-κB signaling pathway. The aldose reductase activity of AKR1B10 was responsible for its pro-inflammatory effects. In addition, we also found that AKR1B10 promoted cell proliferation and colony formation in colon cancer cells.

In a word, our study explored the clinicopathological expression pattern of AKR1B10 in colon cancer and preliminarily revealed the relationship between AKR1B10 and inflammation, proliferation and colony formation of colon cancer cells.

2 Materials And Methods

2.1 Materials

Cell counting kit 40203ES60 was made by Yeasen Biotech Co.Ltd (Shanghai, China); anti-AKR1B10 antibody ab96417 was made by abcam (Cambridge, UK), GAPDH Mouse Monoclonal antibody 60004–1-lg was made by Proteintech Group.Inc (Chicago, U.S.); Phospho-NF-κB p65(Ser536) (93H1) Rabbit mAb #3033 was made by Cell Signaling Technology (Massachusetts, U.S.); NF-κBp65(D14E12)XP Rabbit mAb #8242 was made by Cell Signaling Technology (Massachusetts, U.S.); IκB-α antibody (CST#9242); NE-PER™ Nuclear and Cytoplasmic Extraction Reagents 78835 was made by Thermo Scientific (Massachusetts, U.S.). The TMA specimens were manufactured by Shanghai Outdo Biotech (Shanghai, China).

2.2 Cell culture

Colon cancer HT–29 cells were obtained from the Cancer Research Center of Xiamen University. Cells were cultured in DMEM supplemented with 10% FBS and maintained at 37°C under an atmosphere of 95% air and 5% CO².

2.3 Oncomine database analysis

An online microarray database-oncomine (http://www.oncomine.org) was used to compare AKR1B10 mRNA levels between colon cancer tissues and normal tissues. The thresholds were set as follows: p-value: 0.0001; fold change: 2; gene rank: 10%; analysis type: cancer vs. normal analysis.

2.4 Immunohistochemical analysis

The expression of AKR1B10 in paraffin-embedded tumor samples were determined by Immunohistochemistry (IHC) staining of tissue microarrays. IHC was used a standard avidin-biotin-peroxidase method. TMAs were deparaffinized in xylene and then dehydrated in gradient concentrations of ethanol. In a microwave oven for 5min by used preheated sodium citrate buffer. After rinsed with
distilled water, in 3% hydrogen peroxide for 10min, and incubated the sections with normal goat serum for 10min at room temperature in order to eliminate nonspecific staining. The sections were incubated with rabbit anti-AKR1B10 antibody overnight at 4°C, and then incubated with secondary antibody at 37°C for 30min. Subsequently, the sections were incubated with the avidin-biotin-peroxidase complex for another 10min. Then the sections were stained with 3, 3-diaminobenzidine (DAB) for 1–5min and counterstained with hematoxylin. Negative controls were obtained by replacing the primary antibody with PBS. Images were acquired on Motic VM1 and processed with the Motic DSAssistant Lite software. Immunohistochemical staining was blindly scored by two pathologists.

2.5 AKR1B10 knockdown by stable transfection

The shRNA primers were designed according to the pLV-RNAi system and the sequences used were as follows:

shAKR1B10#1: GAACAAACCTGGACTGAAATA
shAKR1B10#2: GGTCTGATCCGTTTCCATAT

The lentivirus vector pLV-AKR1B10-Puromycin or plv-control-Puromycin was transfected into 293T cells together with auxiliary plasmids pMDLg/pRRE, pVSV-G and pRSC-Rev to package lentivirus. After infected by the lentivirus, shAKR1B10 cells and shcontrol cells were screened out through adding puromycin into the mediums.

2.6 Reverse transcription (RT)-PCR

RNA was extracted and prepared using TRIzol and cDNA was synthesized using the SuperScript III First-Strand Synthesis Supermix Kit. Quantitative analysis of each gene expression was performed on Light Cycler with SYBR Green detection with specific oligonucleotide primers. Gene expression was normalized using GAPDH as an internal control. The primers used were as shown below:

IL1α: 5/-TGTATGTGACTGCCCAAGATG–3/ (forward)
5/-TTAGTGGCCGTGAGTTCCC–3/ (reverse)
IL6: 5/-CCACTCACCTCTTCAGAACG–3/ (forward)
5/-CATCTTTGGAAGGTTCAGGTTG–3/ (reverse)
GAPDH: 5/-ACATCGCTCAGACCATG–3/ (forward)
5/- TGTAGTTGAGGTCAATGAAGGG–3/ (reverse).

2.7 Overexpression and mutant plasmids establishment

Human AKR1B10 cDNA was used to design PCR primers. PCR primers used were as follows:
AKR1B10-N-F: AGAGAATTCGGATCCGCCACGTTTGTGGAGCTCAGTACCAA
AKR1B10-N-R: TGGCTCGAGCCCGGGTCAATATTCTGCATTGAAGGGATAGT

The eukaryotic expression plasmid pLV-n-Flag-AKR1B10 of AKR1B10 was obtained by LIC ligation. Using this plasmid as a template, pLV-n-Flag-AKR1B10 (K125L) was obtained by using the AKR1B10 enzyme active point primers:

AKR1B10-K125L-F: CCTTTTCCCCTTAGATGATAAAGG
AKR1B10-K125L-R: CCTTTATCATCTAAGGGGAAAAGG

The plasmids established above were transfected into the AKR1B10 knockdown cell line.

2.8 Western blot analysis

Colon cancer HT–29 cells were lysed in RIPA lysis buffer supplemented with a protease inhibitor PMSF to extraction total protein. Nuclear lysates were isolated using the NE-PER™ Nuclear and Cytoplasmic Extraction Reagents (Thermo Fisher Scientific). Protein lysates were separated on 10% or 13% SDS-PAGE gels. And then transferred to PVDF membranes and blocked by 5% BSA for 1h. Afterwards, incubation was carried out with the primary antibodies overnight at 4°C. After that, washed with TBST 10min, repeated this at least three times. Next, incubation was carried out with secondary antibodies for 1h at room temperature. The membranes were washed 10min and repeated for three times. Immunoreactive bands were detected using electrochemiluminescence (ECL).

2.9 Cell proliferation assay and cell colony formation assay

The viability of cells was measured by using cell counting kit (CCK8) assay. For cck8, 5000 cells with 100μl of medium were seeded into each well of 96-well plate. Cells were cultured for 24, 48 and 72 h at 37˚C. Finally, 10ul cck8 sodium was added to each well and incubated for 2 h at 37 ℃. Then the optical density (OD) was measured at a wavelength of 450nm.

For cell colony formation assay, 50,100,200 cells with 5000μl of medium were seeded into 6cm plate, respectively. The culture medium was changed every three days. When visible clones appeared after 2 to 3 weeks, the medium was removed, washed twice with PBS, and air-dried for 10min. Then 100% formaldehyde was fixed at room temperature for 15–20min. Finally, stained with Giemsa stain for 10min. Took pictures and counted the number of colonies formed.

2.10 Statistical analyses

Immunohistochemical AKR1B10-stainings were compared with subgroups by use of the Kaplan-Meier. P-values of <0.05 were considered statistically significant. For statistical analyses, we used R for Windows.

3 Results
3.1 AKR1B10 is downregulated in colon cancer

The oncomine database was used to compare AKR1B10 mRNA levels between colon cancer tissues and normal tissues. The Hong colorectal cancer dataset indicated that AKR1B10 mRNA levels were significantly reduced in colon cancer (Fig. 1A). This is in accordance with previous reports that AKR1B10 is under-expressed in colon cancer and gastric cancer [2]. Tissue Microarray analysis of 80 colon cancer samples confirmed that AKR1B10 expression was considerably reduced in colon cancer tissues than in normal tissues (P<0.01, Fig. 1B-C). In addition, AKR1B10 localized mainly in the cytoplasm (Fig. 1B). After analyzing the correlation of AKR1B10 mRNA expression in colon cancer tissues with clinicopathological characteristics (Table 1), we found that AKR1B10 mRNA expression was significantly associated with TNM stage and clinical stage of colon cancer, but not apparently with other clinicopathologic variables, such as age, gender, and position and differentiated degree of the cancer. In our study, it is a new finding that, although low-expression of AKR1B10 is predominant in colon cancer, the proportion of high-expression of AKR1B10 increased with the progression of TNM stage and clinical stage of colon cancer. The results might indicated the role of AKR1B10 in oncogenesis and development of colon cancer.

3.2 AKR1B10 promotes the production of inflammatory cytokines

To further explore the role of AKR1B10 in human colon cell, we developed two AKR1B10- knockdown HT–29 cell lines named shAKR1B10#1 and shAKR1B10#2 (Fig. 2 A, left panel) by lenti virus-based shRNA technique using two distinct targeting shRNAs and thereafter complemented shAKR1B10#1 with AKR1B10 (Fig. 2A, right panel) to test the AKR1B10-rescue effect. Knock down of AKR1B10 significantly decreased the mRNA level of IL1α and IL6 and replenishment of AKR1B10 rescued the expression of IL1α and IL6 (Fig. 2B), precluding any off-target effect of the AKR1B10 shRNA. Accumulated studies have confirmed that LPS can not only induce the expression of inflammatory cytokines in macrophage but also in tumor cells [22, 23]. Due to intestinal mucosal permeability and postoperative bacterial displacement, the patient's LPS content increased. It was indicated that LPS-induced inflammatory cytokines are critical for tumor development. Therefore, we also determined the effect of AKR1B10 on LPS induced-inflammatory cytokines expression (Fig. 2C). As shown, compared to the control cells, AKR1B10 knockdown obviously inhibited mRNA level of cytokines IL1α and IL6 when stimulated with LPS. Moreover, AKR1B10 rescue can reverse the inhibition of the expression of the inflammatory factors in shAKR1B10 cells stimulated with LPS. Collectively, these results suggested that AKR1B10 promoted the expression of IL1α and IL6 in HT–29 cells.

3.3 The aldose reductase activity of AKR1B10 is required for the promotion of inflammatory cytokines production.

Growing evidences have revealed that aldose reductase activity of AKR1B10 links with its physiological function [24]. In order to explore whether the aldose reductase activities of AKR1B10 is essential for the production of inflammatory cytokines, Oleanolic Acid (OA), an specific and potent inhibitor of AKR1B10, was used to inhibit the aldose reductase activity of AKR1B10. As shown, the mRNA levels of IL1α were declined in LPS-induced HT–29 cells after treatment of OA (Fig. 3A). To further ensure this hypothesis,
the AKR1B10 K125L mutant stably overexpressed in shAKR1B10 cells were used (Fig. 3B). Lys–125 site of AKR1B10 is a key residue to attain a high catalytic efficiency with all-trans-retinaldehyde [24]. The results showed that replenishment of the AKR1B10 K125L mutant couldn't effectively rescue the mRNA expression of IL1α and IL6 compared to the replenishment of wild-type AKR1B10 (Fig. 3C). These results indicated that aldose reductase activities of AKR1B10 played an important role in regulating the production of IL1α and IL6.

3.4 AKR1B10 promotes the production of inflammatory cytokines via NF-κB pathway

A large number of studies showed that NF-κB and STAT3 played an important role in the regulation of certain inflammatory cytokines expression [25, 26]. We explored whether AKR1B10 affected NF-κB or STAT3 to regulate the expression of inflammatory cytokines. Our results showed that the phosphorylation level of STAT3 protein was not affect greatly by AKR1B10 (Fig. 4A). However, we found that knockdown of AKR1B10 led to the phosphorylation level of the total P65 protein decreased and rescue of AKR1B10 reversed this change (Fig. 4A). Nuclear localization of P65 was dramatically decreased in shAKR1B10 cells and increased in Flag-AKR1B10 (Rescue) cells, especially when the cells were treated with the LPS (Fig. 4B-C). Replenishment of wild-type AKR1B10 could reverse the effect of shAKR1B10 and promote the shift of p65 from the cytoplasm to the nuclear while replenishment of AKR1B10 K125L mutant could not (Fig. 4D), which indicated the aldose reductase activity of AKR1B10 is essential for the activating of the NF-κB signaling pathway. To test the effect of NF-κB on the production of IL1α and IL6 in HT–29 cells, we treated the cells with BAY 11–7082, an inhibitor of NF-κB, to block the phosphorylation of IκBα, and found that the production of IL1α and IL6 was inhibited when NF-κB was inactivated (Fig. 4E). Taken together, the data demonstrated that AKR1B10 induced the production of inflammatory cytokines of IL1α and IL6 via activating the NF-κB signaling pathway.

3.5 AKR1B10 promotes cell proliferation and clonogenic growth

To clarify the relationship of AKR1B10 and cell growth in human colon cancer, CCK8 and clonogenic assays were performed in HT–29 cells. As shown, AKR1B10 knockdown inhibited cell proliferation and AKR1B10 rescue promoted cell proliferation in HT–29 cells (Fig. 5A). Cell proliferation and tumorigenesis were further determined and the results revealed that the efficiency of colony formation was significant reduced in AKR1B10 knockdown cells and clearly increased in AKR1B10 rescue cells (Fig. 5B).

4 Discussion

AKR1B10 is a NADPH-dependent monomeric reductase, which can reduce a variety of aldehydes. Moreover, the retinoid-binding sites, especially at position 125, are determining factors for the all-trans-retinaldehyde specificity of AKR1B10 [24]. AKR1B10 is overexpressed in normal colon tissue, whereas it has limited expression in colon cancer tissues. Of note, our results demonstrated that, despite the low expression of AKR1B10 in colon cancer, the expression of AKR1B10 increased with the progression of colon cancer. In the intestine and adrenal gland, the biological function and the role of AKR1B10 in tumor development and progression were reported as the following points. On one hand, AKR1B10 may act as
an important cell survival protein via modulating lipid synthesis, mitochondrial function and oxidative status, as well as carbonyl levels [27]. On the other hand, as an important protective enzyme of the colonic epithelial cells, AKR1B10 protects host cells from DNA damage induced by electrophilic carbonyl compounds in colon cells [28].

The AKR1B10 gene contains several putative regulatory motifs of NF-κB [29]. NF-κB dimers are retained in the cytoplasm by specific inhibitors, IkBs, in most types of cells. NF-κB could be activated by exposure of cells to LPS or other inflammatory cytokines [30]. Phosphorylation of P65 and translocation of NF-κB dimers from the cytoplasm to the nucleus are favorable evidences for the activation of NF-κB. Meanwhile, the activation of NF-κB has long been linked to the production of inflammatory cytokines [31]. The expression of inflammatory factors plays an important role in the development of colon cancer [32]. IL1α has an anti-tumor effect via boosting anti-tumor immunity when it is at low level. However, increased release of IL1α keeps a close link with pro-tumorigenic effect, which can promote survival and proliferation of cancer cells [33]. Previous studies also have shown that elevated IL6 activates early immunity to exert an anti-tumor effect [34]. Dysregulation of IL6 cytokine was considered as an important factor for inducing and maintaining of several diseases, such as rheumatoid arthritis, inflammatory bowel disease, osteoporosis and various types of cancer. Besides, LPS is the main component of the outer membrane of Gram-negative bacteria that can induce severe sepsis systemic inflammation as well as postoperative infectious complications after colon surgery [35].

In conclusion, we have shown for the first time that expression of AKR1B10 is significantly correlated with TNM stage and clinical stage in human colon cancer. Based on above premises, this study utilized colon cancer HT–29 cells as a model system to reveal the relationship between AKR1B10 and the production of inflammatory cytokines. In the present study, we showed that AKR1B10 activates NF-κB and promotes the production of IL1α and IL6 in colon cancer cells. Such effects are tightly associated with the aldose reductase activities of AKR1B10 which was evidenced by the decreased expression of IL1α and IL6, and the down-regulation of nuclear localization of P65 in AKR1B10 K125L mutant rescue cells. This work also focused on studying the effects of AKR1B10 on cell proliferation as well as clonogenic growth, further supporting the role of AKR1B10 in colon cancer. Additional studies are needed to further reveal the role of AKR1B10 in inflammatory microenvironment of colon cancer in our future work.

5 Declarations

Acknowledgements

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Conflict of interest:

We have no conflicts of interest to declare.
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Tables
Table 1. Correlation between AKR1B10 expression and clinicopathological features of colon cancer patients (n=80).

| Characteristic                  | N   | AKR1B10 immunohistochemical staining | P       |
|--------------------------------|-----|-------------------------------------|---------|
|                                |     | Low expression                      | High expression |
| Gender                         |     |                                     |          |
| Male                           | 43  | 31(72.1%)                           | 12(27.9%) | 0.3443  |
| Female                         | 37  | 23(62.16%)                          | 14(37.84%)|
| Age (years)                    |     |                                     |          |
| ≤68                            | 43  | 26(60.5%)                           | 17(39.5%) | 0.1475  |
| >68                            | 37  | 28(75.68%)                          | 9(24.32%) |
| Position                       |     |                                     |          |
| Left colon                     | 39  | 24(61.54%)                          | 15(38.46%)| 0.2668  |
| Right colon                    | 41  | 30(73.2%)                           | 11(26.8%) |
| Differentiated degree          |     |                                     |          |
| High or Middle                 | 61  | 41(67.21%)                          | 20(32.79%)| 0.4933  |
| Low                            | 19  | 13(68.42%)                          | 6(31.58%) |
| Invasion depth                 |     |                                     |          |
| T1-T2                          | 8   | 8(100%)                             | 0(0%)    | 0.038a  |
| T3-T4                          | 72  | 46(63.88%)                          | 26(36.11%)|
| Lymph node metastasis          |     |                                     |          |
| N (-)                          | 53  | 38 (71.7%)                          | 15(28.3%) | 0.2613  |
| N (+)                          | 27  | 16(59.26%)                          | 11(40.74%)|
| Distant metastasis             |     |                                     |          |
| M0                             | 76  | 53(69.74%)                          | 23(30.26%)| 0.0626  |
| M1                             | 4   | 1(25%)                              | 3(75%)   |
| Clinical stage                 |     |                                     |          |
| Stage I-II                     | 52  | 38(73.1%)                           | 14(26.9%) | 0.1466  |
| Stage III-IV                   | 28  | 16(57.14%)                          | 12(42.86%)|

aStatistically significant
Figures

A

Hong Colorectal Statistics

Fold Change: 30.671
Gene Rank: 29 (in top 1%)

P-value: 5.61E-29

Colon (N=12)
Colorectal Carcinoma (N=70)

B

AKR1B10 staining intensity

Noncancerous Colon cancer tissues

% of cases

Strong
Moderate
Weak
P<0.01

C

Colon cancer tissues
Low AKR1B10

High AKR1B10

Noncancerous tissues
Low AKR1B10

High AKR1B10

Figure 1
Reduced expression of AKR1B10 in colon cancer. A. AKR1B10 expression in Hong Colorectal cancer dataset from oncomine. B. AKR1B10 expression was analysed by immunohistochemical staining based on a TMA containing 80 colon cancer specimens and 80 noncancerous tissue specimens. C. Representative images of immunohistochemical staining of AKR1B10.

Figure 2

Effects of AKR1B10 on inflammation cytokine expression in HT-29 cells. The mRNA levels were quantitated by RT-PCR normalized to GAPDH. (*: P<0.05 **: p<0.01 ***: p<0.001 ****: p<0.0001). A. The AKR1B10 knock down cell lines (shAKR1B10#1, shAKR1B10#2) and AKR1B10 rescue cell lines (FlagAKR1B10-Rescue). B. The mRNA levels of IL1α and IL6 in shctrl, shAKR1B10 (labeled as #1 and #2, respectively) and FlagAKR1B10 (Rescue) cells without any stimulation. C. The mRNA levels of IL1α and IL6 in shctrl, shAKR1B10 (labeled as #1 and #2, respectively) and FlagAKR1B10 (Rescue) cells stimulated with LPS (100μg/ml, 1h).
Figure 3

Aldose reductase activity of AKR1B10 is essential for the production of inflammatory cytokines IL1α and IL6. The mRNA levels of IL1α and IL6 were quantitated by RT-PCR normalized to GAPDH. A. HT-29 cells were incubated with 100μg/ml LPS and/or 10μM OA for 1h. B. Replenish the AKR1B10 K125L mutant into shAKR1B10 cells. This cell line was named FlagAKR1B10 (K125L) (Rescue). C. FlagAKR1B10 (K125L) (Rescue) cells were treated with or without 100μg/ml LPS for 1h.
AKR1B10 induced the production of inflammatory cytokines via NF-κB signaling pathway. A. Western blot analysis of STAT3 expression and p65 expression in whole cell lysates in shctrl, shAKR1B10#1 and FlagAKR1B10 (Rescue) cells. B. Western blot analysis of p65 expression in nuclear and cytosolic lysates in shctrl, shAKR1B10#1 and FlagAKR1B10 (Rescue) cells. C. Western blot analysis of p65 expression in nuclear and cytosolic lysates in shctrl and shAKR1B10#1 cells after treatment with 100μg/ml LPS for 1h.
D. Westernblot analysis of p65 expression in nuclear and cytosolic lysates in shctrl, FlagAKR1B10 (Rescue) and FlagAKR1B10 (K125L) (Rescue) cells after treated with 100μg/ml LPS for 1h. E. The mRNA levels of IL1α and IL6 in HT-29 cells. HT-29 cells were stimulated with LPS (100μg/ml, 1h), in the presence or absence of 100μM BAY 11-7082, an inhibitor of NF-κB signaling.

Figure 5
Effect of AKR1B10 on cell proliferation and clonogenic growth in HT-29 cells. OD, optical density. A. Cell proliferation in shAKR1B10 and FlagAKR1B10 (Rescue) cells after 24, 48 and 72h with CCK8 assay. B. Colony formation in shAKR1B10 and FlagAKR1B10 (Rescue) cells.