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ORIGINAL ARTICLE

Propofol suppresses cell proliferation in gastric cancer cells through NRF2-mediated polyol pathway

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Abstract

Propofol, a widely used short-acting intravenous sedative agent, has gradually gained attention due to the tumour-suppressing role and non-anaesthetic effect. Dysfunction of metabolic reprogramming has been recognised as a well-documented factor for tumour progression. The aim of this study is to explore the effect of propofol on the polyol pathway in gastric cancer cells. In this study, we found that propofol treatment led to a significant downregulation of cell proliferation in BGC823 and GES-1 cells, which was attributed to the decreased AR-mediated polyol pathway. Both aldo-keto reductase family 1, member B1 (AKR1B1) and AKR1B10 were significantly reduced in BGC823 and GES-1 cells in response to propofol stimulation, leading to decreased AR activity and sorbitol level. Addition of sorbitol could reverse the inhibitory effect of propofol on cell proliferation. Mechanically, propofol treatment drastically inhibited phosphorylation and nuclear translocation of nuclear factor (erythroid-derived 2)-like 2 (NRF2), subsequently decreased the binding of NRF2 to AR promoter. Overexpression of NRF2 resulted in the recovery of AR expression in gastric cancer cell with propofol treatment. Taken together, these finding showed that propofol suppressed cell proliferation in BGC823 and GES-1 cell through NRF2-mediated polyol pathway, which would aid the selection of sedation for patients with gastric cancer.

KEYWORDS
cell proliferation, gastric cancer cell, NRF2, polyol pathway, propofol

1 | INTRODUCTION

Propofol, a short-acting intravenous sedative agent, is often used in clinical practice for intraoperative general anaesthesia and postoperative sedation.[1,2] A growing body of evidence has demonstrated that propofol has a novel function in tumour cell proliferation, invasion, migration, and apoptosis in lung cancer,[3] gastrointestinal tract,[4-6] breast cancer,[7] ovarian[5] and cervical cancer.[8] Furthermore, studies have confirmed that propofol induced a metabolic switch to glycolysis and cell death,[9] and attenuated the adhesion between tumour

Abbreviations: AMPK, adenosine monophosphate activated protein kinase; AR, aldose reductase; CD, Crohn's disease; ChIP, chromatin immunoprecipitation; CSC, cancer stem cell; DNL, de novo lipogenesis; FASN, fatty acid synthase; HMGCS2, 3-hydroxy-3-methylglutaryl-CoA synthase 2; IBD, inflammatory bowel disease; IECs, intestinal epithelial cells; IF, immunofluorescence; IHC, immunohistochemistry; JNK, c-Jun N-terminal kinases; MUC2, mucin2; NRF2, nuclear factor (erythroid-derived 2)-like 2; PKM2, pyruvate kinase muscle isoform 2; QPCR, quantitative polymerase chain reaction; SDH, sorbitol dehydrogenase; TGF-β2, transforming growth factor β2; TPA, 12-O-tetradecanoylphorbol-13-acetate; UC, ulcerative colitis; WB, western blotting.

Yajun Cao and Long Fan contributed equally to this work

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cell and endothelial cells.[10] What's more, propofol could suppress tumourigenesis through suppressing glycolysis.[3,11] In addition to glycolysis, aldo-keto reductase family 1, member B1 (AKR1B1) and aldo-keto reductase family 1, member B10 (AKR1B10), the members of the aldo-keto reductase family of enzymes that participate in the polyol pathway of aldehyde metabolism, are aberrantly expressed in colon cancer, and can be considered as a novel biomarker for colon cancer prognostication.[12] However, whether the polyol pathway is involved in propofol-induced cell biology remains to be identified.

Metabolic reprogramming has been demonstrated to play an extremely important cellular function in regulating gastrointestinal tract disease, such as inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease (CD), such as pyruvate kinase M2 (PKM2),[13,14] fatty acid synthase (FASN),[15,16] adenosine monophosphate activated protein kinase (AMPK)[17] and 3-hydroxy-3-methylglutaryl-CoA synthase 2 (HMGCS2).[18] Sugar alcohol is an alternative way to metabolise the glucose into sorbitol through polyol pathway. In general, the polyol pathway consists of two enzymes. The first enzyme aldose reductase (AR), including AKR1B1 and AKR1B10, reduces glucose to sorbitol with the aid of its co-factor NADPH, and the second enzyme, sorbitol dehydrogenase (SDH), with its co-factor NAD^+, converts sorbitol to fructose.[19] As the critical enzymes of polyol pathway, the AKR1B1 overexpression is associated with an alteration in the balance between proliferation and apoptosis of epithelial cells in the mouse lens[20] and mediates transforming growth factor β2 (TGF-β2)-induced migration and epithelial-to-mesenchymal transition.[21] Further study showed that AKR1B1 deficiency suppressed EMT and cancer cell growth, which is strictly functionally associated with cancer stem cell (CSC).[22,23] However, the regulation of the polyol pathway remains incompletely understood.

In this study, we reveal that propofol treatment led to a significant decrease in AKR1B1 and AKR1B10 expression through the NRF2-mediated polyol pathway, leading to inhibition of cell proliferation in gastric cancer cells. Addition of sorbitol, the production of the polyol pathway, could reverse the effect of propofol on cell proliferation, while overexpression of NRF2 in BGC823 cells could overcome decreased AKR1B1 and AKR1B10 expression caused by propofol. Mechanistically, propofol treatment significantly reduced NRF2 activity, leading to reduce NRF2 phosphorylation and nuclear translocation, and decrease the binding of NRF2 to the promoter of AKR1B1 and AKR1B10. These findings revealed a novel function of propofol in polyol pathway and provided novel mechanistic insights into the metabolic control of tumour cell proliferation.

2 | RESULTS

2.1 | Propofol induced cell proliferation suppression

To explore the effect of propofol on cell proliferation in BGC823 and GES-1 cells, we analyse the cell viability in response to various concentration of propofol stimulation. As shown in Figure 1A, cell viability was decreased in BGC823 and GES-1 cells in a dose-dependent manner. In line with this, compared with control group, the data from real-time PCR and western blotting analysis indicated that PCNA and Ki-67 was significantly reduced in BGC823 and GES-1 cells at a concentration of 300 μM (Figure 1B-E). These findings suggested the inhibitory effect of propofol on cell proliferation.

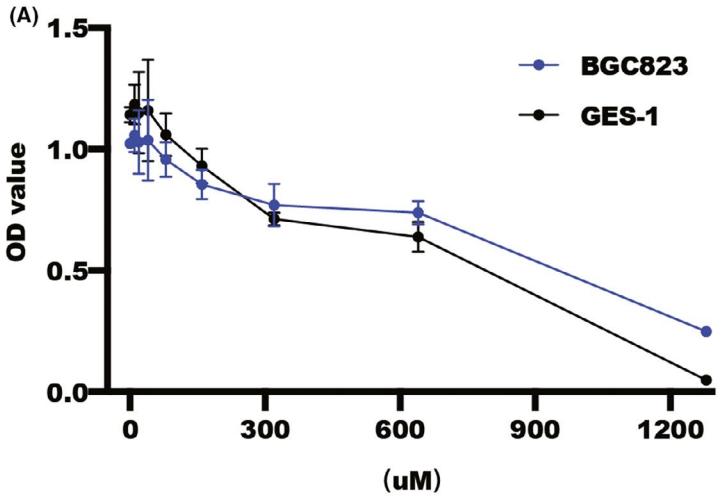
2.2 | Sorbitol reversed the effect of propofol-induced cell proliferation suppression

Tumour cells altered their metabolism to support their malignant properties,[23,24] the connection between sucrose/fructose consumption with increased cancer risk has been demonstrated by epidemiological and experimental studies.[25-28] We tried to ask whether the glucose-transforming polyol pathway involved in the effect of propofol on cell proliferation. As expected, propofol treatment in BGC823 and GES-1 cells led to abolish sorbitol level (Figure 2A) compared with the control group. CCK-8 result showed that sorbitol (10 mmol/L) treatment alone increased, while propofol stimulation alone decreased cell proliferation in BGC823 and GES-1 cells in a time course, and sorbitol stimulation could reverse the inhibitory effect of propofol on cell proliferation by CCK-8 assay (Figure 2B-C). Consistent with this, WB results revealed both PCNA and Ki-67 expression were enhanced in response to sorbitol stimulation (Figure 2D). Collectively, these results showed that propofol suppressed cell proliferation is dependent of sorbitol.

2.3 | Propofol suppressed AKR1B1 and AKR1B10 expression

To further determine the effect of propofol in polyol pathway, we sought to identify the genes responsible for propofol-induced polyol pathway. Our results demonstrated that both AKR1B1 and AKR1B10 were drastically inhibited in BGC823 and GES-1 cells with propofol

FIGURE 1 Propofol stimulation led to proliferation inhibition in gastric cancer cells. (A) BGC823 and GES-1 cells were digested and reseeded in 96-well plate at a density of 0.8×10^5. Serials of different concentration of propofol were used to treat BGC823 and GES-1 cells for 72 h. The medium was replaced with 100 μL fresh medium containing 10 μL CCK-8 to incubate further 2 h. Data were presented as mean ± SD $n = 3$ in each group. (B) BGC823 and GES-1 cell was treated with or without 300 μmol/L propofol for 72 h, and the total protein were subjected to SDS-PAGE to detect PCNA and Ki-67 expression. (C-D) the band intensity was quantified and analysed by *t*-test. Data represented the mean ± SD of three independent experiments and were analysed by *t*-test for significance vs. the control group, $^{***}p < 0.001$, $^{**}p < 0.01$. (E) Real-time PCR was performed to detect Ki-67 and PCNA expression in BGC823 and GES-1 cells treated with or without propofol stimulation for 72 h. Data represented the mean ± SD of three independent experiments and were analysed by *t*-test for significance vs. the control group, $^{***}p < 0.001$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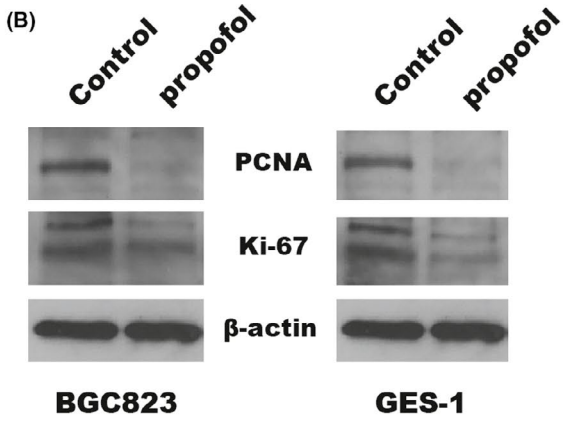
GES-1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propofol
Control
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PCNA
Ki-67
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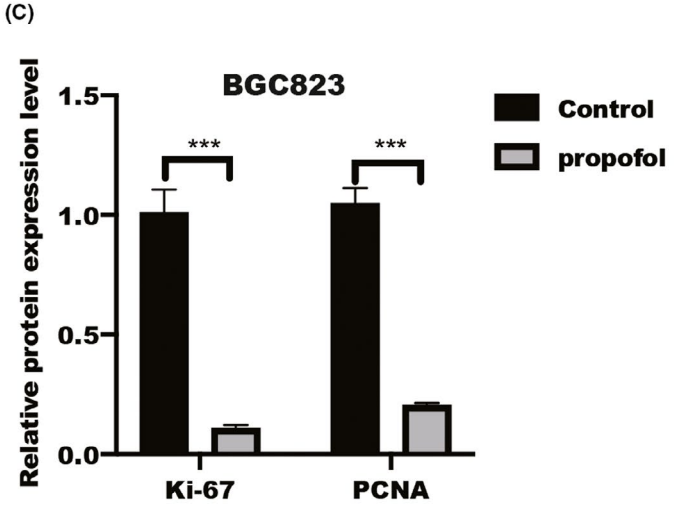
GES-1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BGC823
Relative protein expression level
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0.5
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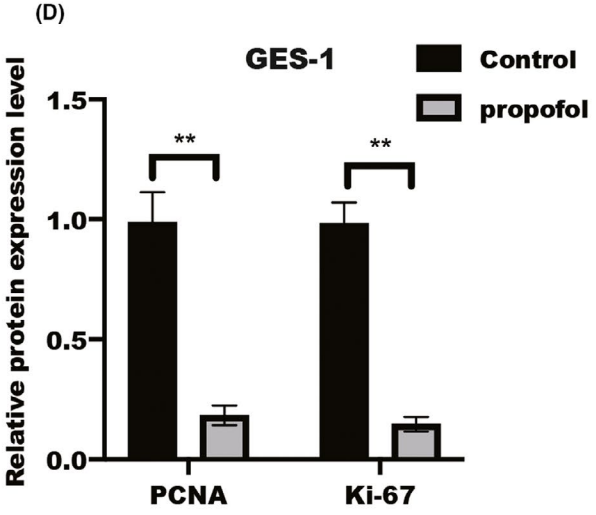
Control
propofol
Ki-67
PCNA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GES-1
Relative protein expression level
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Control
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PCNA
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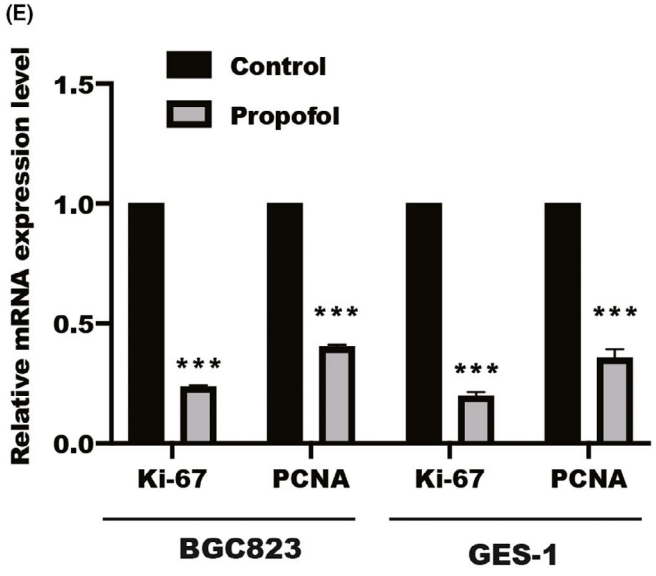
(E)
Relative mRNA expression level
1.5
1.0
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Control
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Ki-67
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Ki-67
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BGC823
GES-1

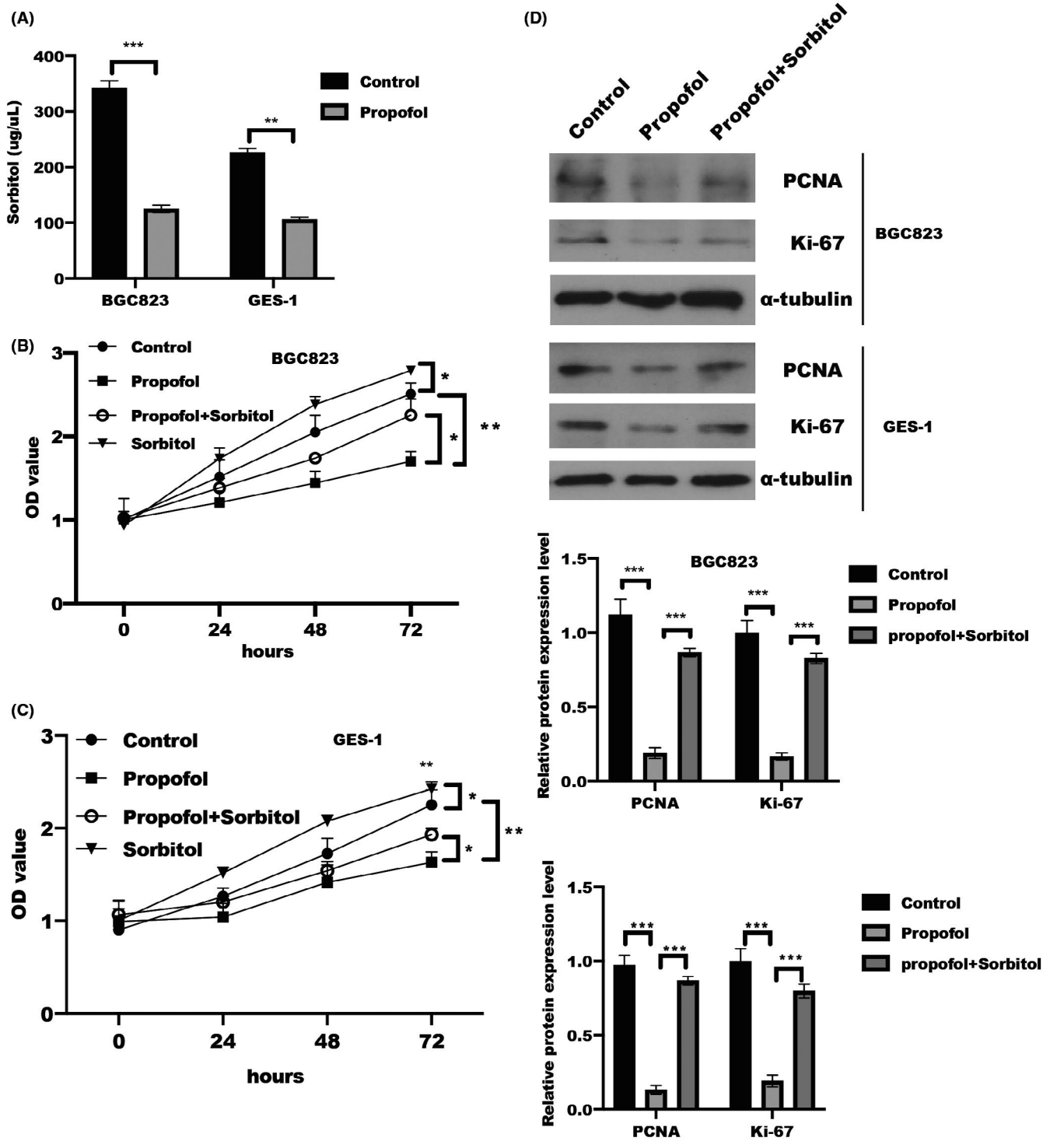


FIGURE 2 Propofol-induced cell proliferation suppression through sorbitol. (A) BGC823 and GES-1 cells were seeded at 12-well plate and treated with or without propofol stimulation for 72 h, the level of sorbitol was measured by sorbitol detection kit according to the instruction. Data represented the mean ± SD of three independent experiments and were analysed by one sample *t*-test for significance, $^{**}p < 0.01$, $^{***}p < 0.001$. (B) BGC823 and (C) GES-1 were reseeded in 96-well plates overnight to allow adhesion and treated with sorbitol, propofol and sorbitol combined with propofol for 48 h. The cell viability was detected by CCK-8 assay. Data represents the mean ± SD of three independent experiments and were analysed by one ANOVA for significance, $^{*}p < 0.05$, $^{**}p < 0.01$. (D) BGC823 and GES-1 cells were treated as A, the total protein was collected and subjected from SDS-PAGE to detect cell proliferation marker, PCNA and Ki-67. Data represented the mean ± SD of three independent experiments and were analysed by one ANOVA for significance, $^{***}p < 0.001$

stimulation for 48 h at mRNA level compared with the control group (Figure 3A). In line with this, the immunoblotting and quantified results showed that propofol treatment in BGC823 and GES-1 cells led to a significant downregulation of the protein level of AKR1B1 and AKR1B10 expression (Figure 3B).

Activation of aldose reductase has been reported to catalyse glucose metabolise into sorbitol.[29] To confirm the effect of propofol on AR activity and sorbitol level, we analysed AR activity and assayed sorbitol concentration based on colorimetric approach. The result showed that AR activity was suppressed in BGC823 and GES-1 cells in response to propofol stimulation (Figure 3C), leading to a decreased sorbitol level in the supernatant (Figure 3D). Taken together, these findings suggested propofol suppressed the polyol pathway in BGC823 and GES-1 cells through AR.

2.4 | Propofol regulated AR expression through NRF2

In addition to NF-κB,[30] activator protein (AP1)[31,32] and nuclear factor of activated t-cells (NFAT5).[33] Many available reports have identified that NRF2 activity is critical for AR transactivity.[34-36] Next, we tried to explore whether propofol-mediated AR activity and expression could be attributed to downregulation of NRF2 signalling. We found that overexpression of NRF2 in BGC823 and GES-1 cells significantly reversed the inhibitory effect of propofol on AR expression, including AKR1B1 and AKR1B10 (Figure 4A-C). However, the exact mechanisms remained unclear.

Phosphorylation of NRF2 at Ser40 was increased in response to various stimulations, leading to Keap1-Nrf2 disassociation, which further caused NRF2 protein stabilisation and nuclear translocation, promoting targeted genes transcription and expression, including HO1 and NQO1.[37-39] As shown in Figure 4D, immunoblotting analysis showed that the phosphorylation of NRF2 at Ser40 is significantly inhibited in BGC823 and GES-1 cells with propofol stimulation compared with the control group. The subcellular fractionation extraction analysis indicated that the amount of NRF2 was increased significantly in cytosolic fraction upon propofol stimulation in BGC823 cells (Figure 4E). These findings suggested that propofol treatment led to a significant inhibition of NRF2 activity and nuclear translocation, leading to decrease AKR1B1 and AKR1B10 expression.

2.5 | NRF2 is essential for propofol-mediated AR transactivation

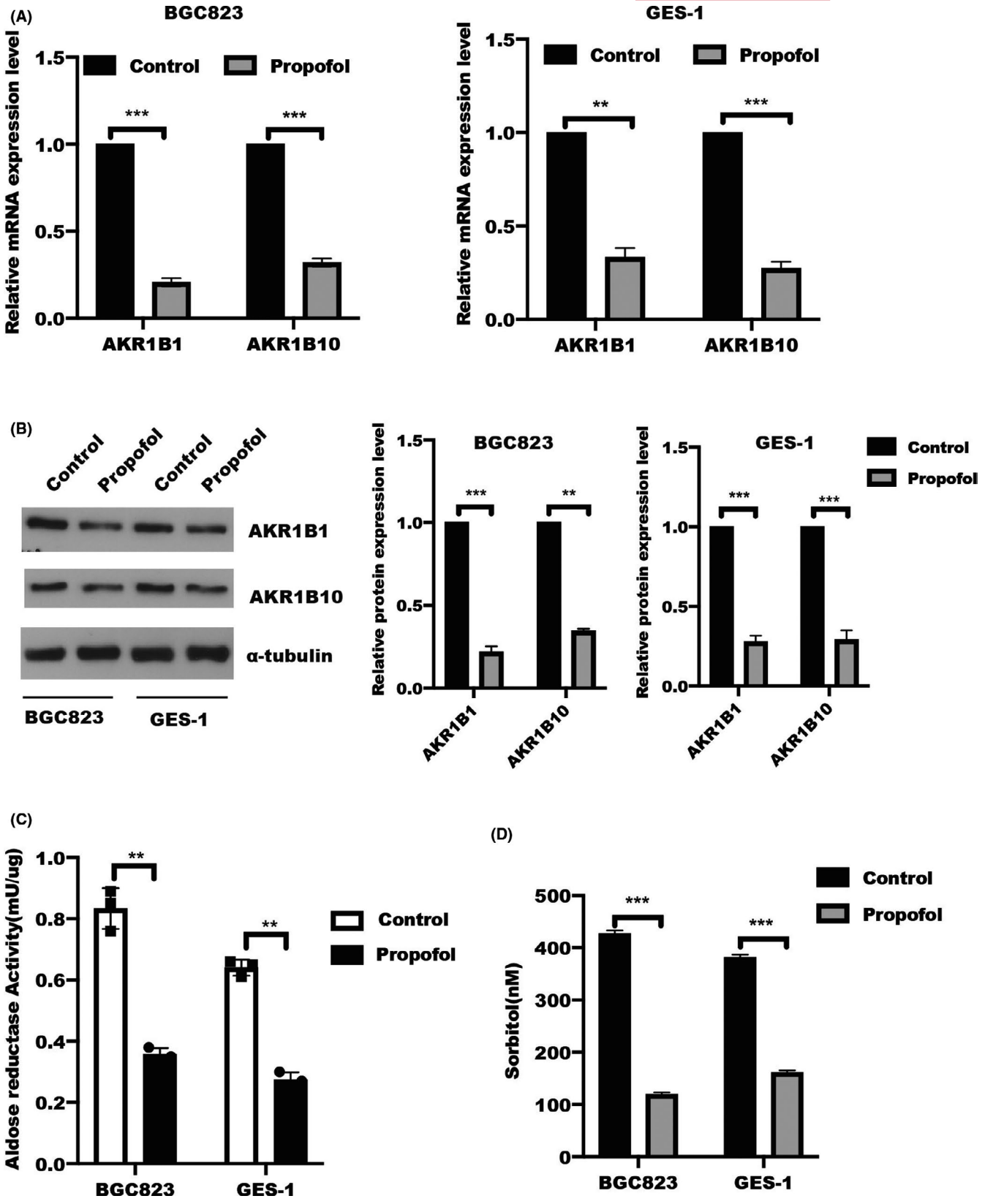
To further confirm the possibility that downregulation of AR transactivation caused by propofol-induced binding of NRF2 to the AR promoter, we utilised chromatin immunoprecipitation (ChIP). As shown in Figure 5A-B, the binding of NRF2 to the AKR1B1 and AKR1B10 promoter region were dramatically reduced in BGC823 cells in response to propofol stimulation. As a negative control, binding of NRF2 to the GAPDH was also detected (Figure 5C), also, no binding of IgG control was observed, indicating that the specific sensitive of IP and real-time PCR was conducted. Taken together, these results indicated that NRF2 is required for propofol-mediated transactivation of AR.

3 | DISCUSSION

Available studies have demonstrated the tumour-suppressing role and non-anaesthetic effect of propofol in human gastric cancer, including cell proliferation, apoptosis and invasion,[40-42] through various mechanisms. In the present study, as shown in Figure 6, we showed that propofol suppressed gastric cancer cell proliferation by inactivation of the polyol pathway through the NRF2 pathway. Sorbitol addition, the production of the polyol pathway, in BGC823 and GES-1 cells could reverse the inhibitory effect of propofol on cell proliferation. ChIP analysis showed that propofol treatment led to decrease the level of binding of the NRF2 to the promoter of the gene encoding AKR1B1 and AKR1B10, suppressing transcriptional activation of them, which further inhibited the conversation of glucose into sorbitol and cell proliferation in BGC823 and GES-1 cells, while ectopic expression of NRF2 in BGC823 cells reversed the inhibitory effect of propofol on AR expression, leading to increase sorbitol level. Mechanistically, propofol stimulation in BGC823 caused a significant reduction of NRF2 phosphorylation, resulting in attenuation of NRF2 nuclear translocation. In summary, these findings supplied a novel insight of the suppressive effect of propofol in gastric cancer cells.

As shown in the current study, treatment with propofol in BGC823 and GES-1 cells diminished gastric cancer cell proliferation, moreover, the data from RT-qPCR, western blotting, and sorbitol level detection demonstrated that both AKR1B1- and AKR1B10-mediated sorbitol production were regulated by propofol, implying the critical role of the polyol pathway in propofol-induced gastric cancer cells proliferation suppression, which has been reported to be pivotal for the doxorubicin and cisplatin resistant gastric cancer.[43-45] In addition to the above metabolism mentioned, whether other metabolic reprogramming, such as ketogenesis, is involved in the possible role of propofol in tumour cell biology remains to be addressed. Although the propofol-mediated polyol pathway has been shown to influence cell proliferation in this study, the changes of other biology function in gastric cancer cells could be further demonstrated in the future work, such as resistance, and tumour initiation.

The NRF2 pathway plays a critical role in the anti-oxidative effect during cellular senescence and apoptosis.[46-48] NRF2 phosphorylation and nuclear translocation was reduced in BGC823 and GES-1 cells caused by propofol stimulation in the current study, leading to decrease the binding of NRF2 to AR promoter. Interestingly, KEAP1 served as a cytoplasmic factor that interacted with the Neh2 domain and formed the KEAP1-NRF2 complex to sequester NRF2 in the cytoplasm and initiate degradation of NRF2 by ubiquitination and proteasomal degradation unless



there is a stress stimulus around.[49-51] However, how propofol regulated NRF2 activation requires future exploration. Moreover, our current work could not completely rule out the speculation that propofol directly regulated NRF2 and/or AR, including activity and expression, which could be address in our next work. In addition to the NRF2 pathway, whether NF-κB or AP1 signalling is involved in propofol-mediated AR expression.

Taken together, we have demonstrated that propofol might serve as a switch to inhibit NRF2 nucleus shuttle and activation through diminished ser40 phosphorylation of NRF2, leading to inhibit AR

FIGURE 3 Propofol regulated AR-mediated polyol pathway. (A) BGC823 (left panel) and GES-1 (right panel) cells were treated with or without propofol for 72 h and collected for total RNA using TRIZOL reagents, RT and Real-time PCR were conducted to detect the AKR1B1 and AKR1B10 mRNA expression level, Data represented the mean ± SD of three independent experiments and were analysed by one sample *t*-test for significance vs. the control group, $^{***}p < 0.001$, $^{**}p < 0.01$. (B) After 72 h of propofol stimulation, the total protein was collected from BGC823 and GES-1 cells, respectively, to analyse AKR1B1 and AKR1B10 expression level by immunoblotting, α-tubulin served as the internal control. The intensity of bands was quantified, and statistical analysis by *t*-test. $n = 3$; $^{***}p < 0.001$, $^{**}p < 0.01$. (C-D) At 72 h post-treatment, culture medium from BGC823 and GES-1 cells were harvested and measured for AR activity and sorbitol production (see Materials and Methods). Data represent the mean ± SD of three independent experiments and analysed by *t*-test. $n = 3$; $^{***}p < 0.001$, $^{**}p < 0.01$

expression and cell proliferation. These findings extended the function of propofol and supplied the novel insights into the function of propofol on in gastric cancer cell proliferation.

4 | MATERIALS AND METHODS

4.1 | Chemical reagents and antibodies

Propofol (YZ-1572503) and D-sorbitol (S8090) were obtained from Solarbio life science. Sorbitol level assay kits (MAK010) were purchased from Sigma. The SimpleChIP Enzymatic Chromatin IP Kit (Magnetic Beads) (9003) was from Cell Signaling Technology. Aldose reductase activity kit (Colorimetric) (ab273276), Antibodies targeting AKR1B1, AKR1B10, phosphorylated NRF2 at Ser40 and NRF2 were purchased from Abcam Company; antibodies targeting α-tubulin and β-actin and all unconjugated secondary antibodies were from Santa Cruz Biotechnology; PCNA (Abclonal, A0264), Histone 3 (Abclonal, A2348) and Ki-67 (Abclonal, A16919) were obtained from Abclonal, and fetal bovine serum (FBS), Alexa-488- and 594-conjugated secondary antibodies were from Molecular Probes (Invitrogen). NRF2 plasmid was from Public Protein/Plasmid Library. The trizol was purchased from Invitrogen and All-in-One First-Strand cDNA Synthesis Kit and All-in-One qPCR Mix were obtained from GeneCopoeia. Subcellular fractionation was conducted using NE-PER Nuclear and Cytoplasmic Extraction Reagents (Thermo Fisher) following the manufacturer's recommendations. All ultrapure reagents were from Promega.

4.2 | Cell Culture, transfection, and treatment

BGC823 and GES-1 cells were purchased from American Type Culture Collection (ATCC) and cultured according to the manufacturer's recommendations. For treatment, cells were treated with propofol at concentation of 300 μmol/L for 72 h, while plasmids were transfected into cells with lipofectamine3000 (L-3000) following the manufacturer's instructions.

4.3 | RNA extraction and real-time PCR

As described in the study of Xu et al.,[52] the whole RNA from each group was extracted with Trizol reagents according to the instruction. Reverse transcription and quantitative PCR (qPCR) were performed using the All-in-One First-Strand cDNA Synthesis Kit and All-in-One qPCR Mix (GeneCopoeia) according to the manufacturer's protocol. The primer used in this study is as followed: AKR1B1[53] forward, 5′-TATTCACTGGCCGACTGGCTTTA-3′ and reverse, 5′-GAACCACATTGCCCGACTCA-3′; AKR1B10[54] forward, 5′-CCCAAAGATGATAAAGGTAATGCC-3′, and reverse, 5′-TCAGTCCAGGTTTGTTCAAGAGC-3′; UBC (internal control) forward, 5′-ATTTGGGTCGCGGTTCTTG-3′ and reverse, 5′-TGCCTTGACATTCTCGATGGT-3′.

4.4 | Subcellular fractionation and western blotting

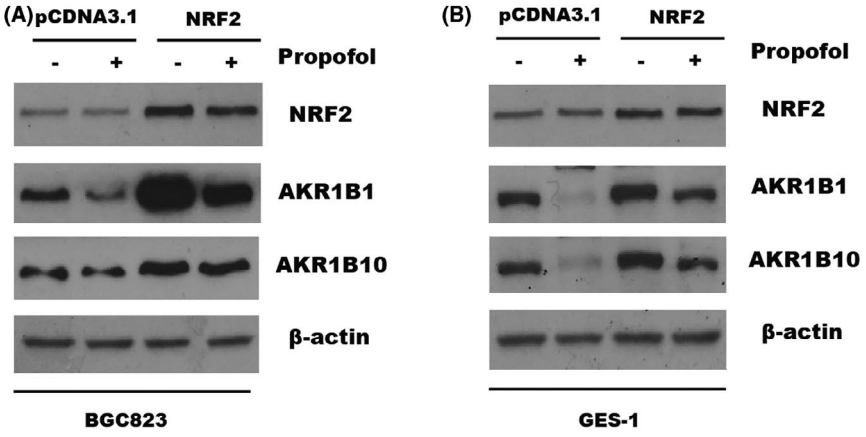
Western blotting was performed as previously described.[55] Briefly, the protein was subjected to SDS-PAGE and transferred into nitrocellulose transfer membrane to further incubate with 5% (w/v) milk in PBS/0.05% (v/v) Tween-20 (PBST) for 1 h; the antibodies were added into incubate with the indicated proteins overnight at 4°C, subsequently followed by incubation with a horseradish peroxidase secondary antibody (Jackson ImmunoResearch) for 1 h at room temperature. Proteins were detected using an enhanced chemiluminescence (Perkin Elmer).

4.5 | Immunofluorescence

Cells were digested and reseeded at a density of 0.5×10^5 into coverslips in 6-well plates overnight, allowing to attach. Briefly, fixed and permeabilised cells were incubated with 2% BSA in PBS/0.05% Triton X-100 for 30 min. The slips were incubated with the primary antibody overnight at 4°C, followed by incubation with Alexa-488- or Alexa 594-conjugated secondary antibodies for 1 h at room temperature. The coverslips were mounted onto glass slides with Prolong Gold Antifade reagent (after staining the nuclei with DAPI), and stained cells were imaged under a laser scanning confocal fluorescent microscope.

4.6 | Aldose reductase activity and sorbitol level detection

BGC823 and GES-1 cells were stimulated with or without propofol for 72 h. The conditional medium was directly assayed using the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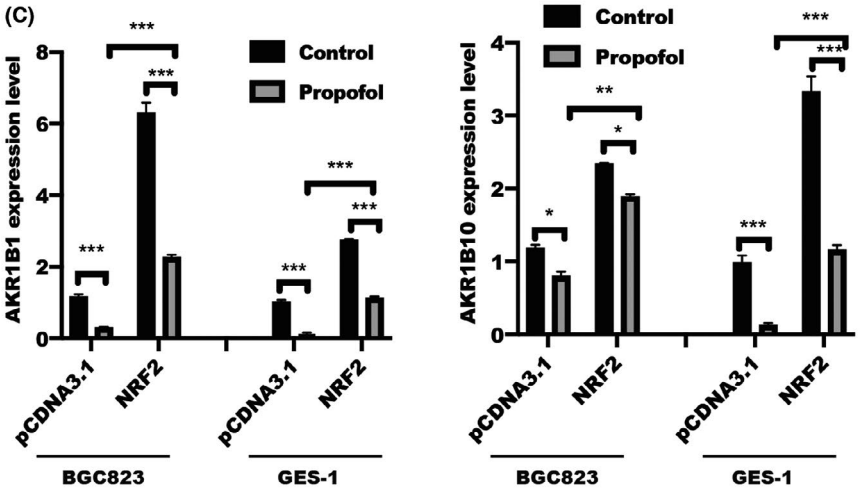
(C)
AKR1B1 expression level
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Propofol
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AKR1B10 expression level
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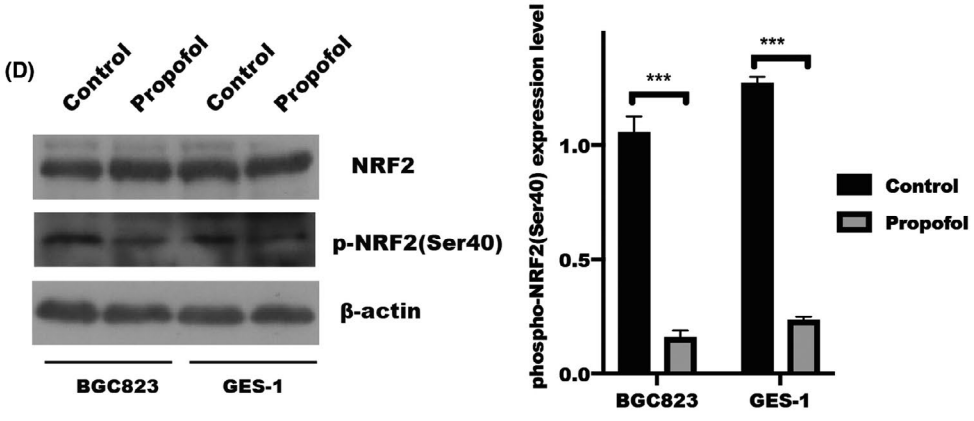
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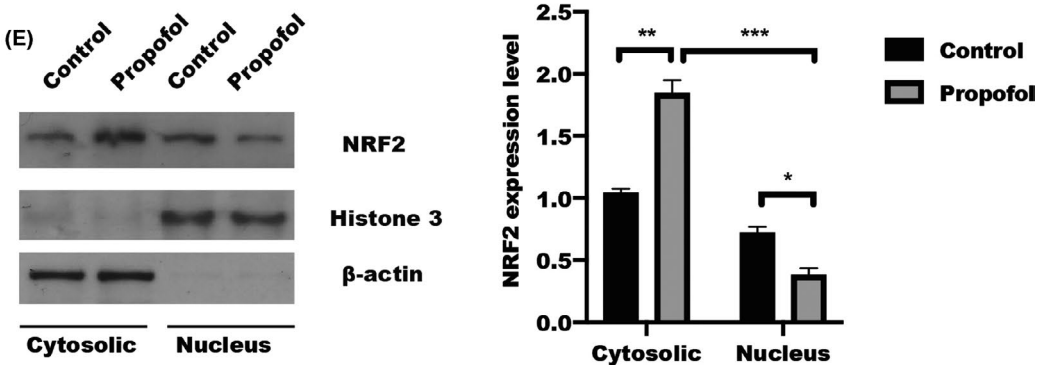
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NRF2 expression level
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Cytosolic
Nucleus

FIGURE 4 Propofol is responsible for NRF2 phosphorylation and nuclear translocation. (A) BGC823 and (B) GES-1 cells were transfected with pCDNA3.1 or pCDNA-NRF2 plasmid as indicated for 24 h, the cells were serum starved for 18 h and stimulated with or without propofol for additional 24 h, the total proteins were collected and detected AKR1B1 and AKR1B10 expression at protein level by western blotting. The quantified results of AKR1B1 and AKR1B10 in BGC823 and GES-1 cells were represented the mean ± SD of three independent experiments. The two-way analysis of variance (ANOVA) and Dunnett's multiple comparison test were used to analyse statistical significance, $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$, $n = 3$ (C). (D) BGC823 and GES-1 cells were incubated with serum-free medium for 24 h and stimulated with or without propofol for 1 h. The whole cell lysates were collected and detected against NRF2 and phosphorylation of NRF2(ser40) by western blotting. β-actin served as internal control, the band intensity of p-NRF2(Ser40) was represented the means ± SD of three independent experiments and were analysed by *t*-test, $^{***}p < 0.001$. (E) After serum starved for 24 h, BGC823 cells were treated with or without propofol for 1 h. The levels of nuclear and cytosolic NRF2 were determined by immunoblotting. β-actin and Histone 3 were used as internal controls for the cytosolic and nuclear fractions, respectively, $n = 3$. the protein level of NRF2 was quantified. Statistical analysis was performed with two ANOVA test. $n = 3$; $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$, $n = 3$

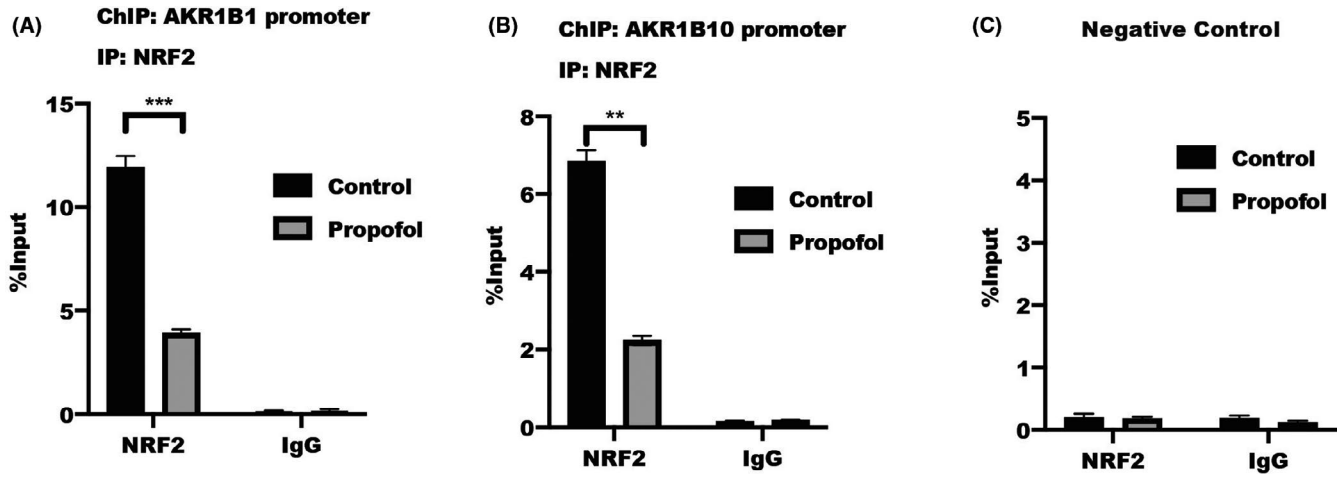

FIGURE 5 Propofol is crucial for binding of NRF2 to the AR promoter. BGC823 cells were serum starved and treated as indicated. Whole cell lysates were immunoprecipitated with an anti-NRF2 antibody, co-precipitating chromosome fragments binding to NRF2 were amplified and quantified by real-time PCR with ChIP primers. Results are presented as a ratio of the immunoprecipitated product to the input product. (A) Real-time PCR of the NRF2-enriched AKR1B1 promoter region. (B) Real-time PCR of the NRF2-enriched AKR1B10 promoter region. (C) Real-time PCR of a nonspecific region corresponding to the GAPDH gene enriched by NRF2 (negative control). Data represent the mean ± SD of three independent experiments. $^{***}p < 0.001$, $^{**}p < 0.01$

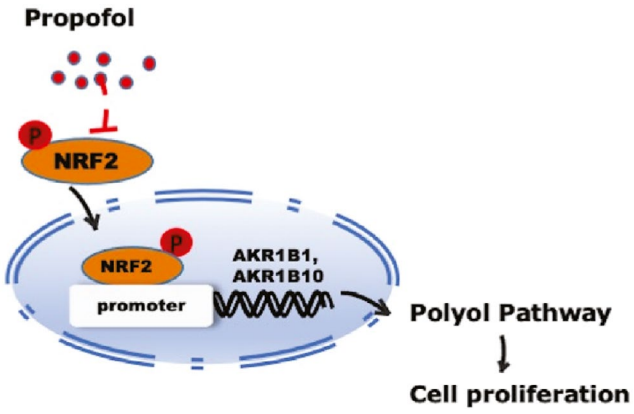

FIGURE 6 The schematic illustration for propofol regulated cell proliferation. Propofol treatment led to inhibit NRF2 phosphorylation and nuclear translocation, subsequently decreased AKR1B1 and AKR1B10 transactivation, which further reduced sorbitol level and cell proliferation

aldose reductase activity kit and sorbitol level detection kit following the manufacturer's protocol, respectively.

4.7 | CCK-8 assay

BGC823 and GES-1 cells were measured using a Cell Counting Kit-8 (CCK-8) (Dojindo). Briefly, cells were reseeded into 96-well plate at a concentration of 5×10^4 cells per well followed by propofol stimulation for a time course. Each 100 μL medium contained 10 μL CCK-8 were added into incubated for 1 h at 37°C, and absorbance were measured at 450 nm. The assays were performed in triplicate.

4.8 | Chromatin immunoprecipitation assay

Chromatin immunoprecipitation was conducted as described in Zhang et al. study.[55] Briefly, ChIP was performed using an anti-NRF2

antibody or rabbit IgG (Millipore) to pulldown chromatin extracts equivalent to 2 × 10 cells. ChIP samples were quantified by qPCR (SYBR Green Master Mix; Applied Biosystems) and the ChIP qPCR data were normalised using the percent input method. The ChIP primer used in the study for AKR1B1 and AKR1B10 were purchased from Thermo Fisher.

4.9 | Statistical analysis

Data analysis was performed using the GraphPad Prism V software. A difference in comparison with a p less than 0.05 was considered statistically significant. Statistical differences among groups were determined by Student's t-test, one-way analysis of variance (ANOVA) was used to determine the significance for mRNA and intensity quantified.

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CONFLICT OF INTEREST

The authors declared that there are no conflict of interests.

DATA AVAILABILITY STATEMENT

The datasets during the current study are available from the corresponding author on reasonable request.

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