Original Article

ISL1 and AQP5 complement each other to enhance gastric cancer cell stemness by regulating CD44 expression

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Background: Gastric cancer, a prevalent and life-threatening malignancy, is believed to involve cancer stem cells (CSCs) as a contributing factor to tumor progression. Insulin gene enhancer binding protein-1 (ISL1) is a transcription factor, and it has not been elucidated how ISL1 regulates gastric carcinogenesis. The aim of this paper is to investigate the role of ISL1 in gastric cancer development.

Methods: In this study, we investigated the effects of ISL1 on the stem-like properties of human gastric cancer cells by applying transcriptional, flow, and immunofluorescence techniques.

Results: In human gastric cancer samples, there is an observed elevation in ISL1 expression, which correlates with the expression of stem cell markers, notably LGR5. Functionally, ISL1 fosters the self-renewal, cell proliferation, migration, and the clonogenic potential of gastric cancer cells *in vitro*. Furthermore, it enhances the ability of these cells to form tumors and metastasize in vivo. Additionally, ISL1 collaborates with AQP5, collectively intensifying the tumorigenicity of gastric cancer cells. Mechanistically, transcriptomic analysis of cells overexpressing ISL1 unveils a notable activation of the forkhead box O (FOXO) pathway. This activation leads to increased nuclear expression of forkhead box O3 (FOXO3), subsequently resulting in elevated expression of the stemness-associated gene CD44 in gastric cancer cells.

Conclusions: These findings shed light on the role of ISL1 in promoting the stem-like characteristics of gastric cancer cells and emphasize the connection between ISL1 and AQP5 as a novel therapeutic target for individuals with gastric cancer.

Keywords: Insulin gene enhancer binding protein-1 (ISL1); AQP5; gastric cancer; CD44; stemness

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Introduction

Gastric cancer (GC), ranking as the fifth most common cancer globally, presents a persistent challenge with its high mortality rate. Perioperative or adjuvant chemotherapy has demonstrated promise in augmenting survival rates for patients with stage IB or higher cancer. However, in advanced stages of GC, chemotherapy, typically initiated with a combination of platinum-based agents and fluoropyrimidines, yields a median survival period of less than 1 year for patients (1). Targeted therapies approved for GC treatment include trastuzumab [for human epidermal growth factor receptor-2 (HER2)-positive patients in the first line], ramucirumab (a second-line anti-angiogenic therapy), and nivolumab or pembrolizumab [third-line anti-

programmed death-1 (PD-1) therapies] (2). The principal contributors to mortality in GC patients are cancer cell metastasis, recurrence, and chemotherapy resistance. Cancer stem cells (CSCs) have emerged as pivotal contributors to these challenges, underscoring the significance of targeting gastric cancer stem cells (GCSCs) as an effective approach in GC treatment (3). In the context of GCSCs, both genes and non-coding RNAs function as critical regulatory factors. Numerous experimental studies have identified specific drugs capable of targeting GCSCs by modulating these genes or non-coding RNAs, offering promising avenues for clinical GC treatment (4,5). Nevertheless, the precise marker genes and regulatory mechanisms governing GCSCs remain elusive.

Insulin gene enhancer binding protein-1 (ISL1) is a transcription factor characterized by two LIM domains (A zinc finger-binding, cysteine-enriched motif containing two repeating zinc fingers in tandem) and a homeodomain (HD). It has been demonstrated to play regulatory roles in various signaling pathways and biological processes (6). ISL1 assumes a critical role in the development of Langhans' islets during embryonic development. In mice lacking ISL1, embryonic heart development and differentiation of motor neurons in the neural tube are impaired, underscoring ISL1's significance in cellular differentiation and development (7,8). In the context of tumor cells, ISL1 exerts influence over cell proliferation, migration, and other cellular processes, thereby contributing to cancer initiation and progression. Moreover, ISL1 functions as a novel regulator of cell cycle genes such as cyclin D1, cyclin B, and c-myc, rendering it a prognostic factor for cancers like GC, bladder cancer, and a biomarker for neuroblastoma. ISL1 has also been demonstrated to mediate glucose transporters like GLUT4 in glycolysis and GC tumorigenesis (9). Furthermore, ISL1 can predict adverse outcomes in GC patients and, in concert with SETD7, binds to the ZEB1 promoter to drive tumor progression (10). While prior research has indicated ISL1's involvement in GC development, its relationship with GCSCs remains unexplored.

Forkhead box O proteins (FOXOs) constitute an evolutionarily conserved family of transcription factors characterized by their highly conserved forkhead box domain, also known as the winged helix domain. FOXOs play substantial roles in various biological processes, including development, metabolism, and stem cell maintenance, by orchestrating the spatial and temporal expression of target genes (11). In recent years, mounting evidence has linked members of the FOXO family to a spectrum of diseases, including GC (12). FOXO3, in particular, has been implicated in promoting GC malignancy, cancer stemness, and chemotherapy resistance, primarily through its downstream target, IFITM3 (13). FOXO3 instigates cathepsin L expression, thereby fostering the migration and invasion of GC cells (14). This underscores the potential role of FOXO3 in GC progression, although the precise mechanisms governing these interactions remain to be elucidated. We present this article in accordance with the ARRIVE and MDAR reporting checklists (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-248/rc).

Highlight box

Key findings

- In this study, we found that insulin gene enhancer binding protein-1 (ISL1) was highly expressed in gastric cancer tissues and significantly promoted the stemness of gastric cancer cells, and ISL1 cooperated with AQP5 to enhance the tumorigenicity of gastric cancer cells. ISL1 promoted the nuclear translocation of forkhead box O3 (FOXO3), which led to an increase in the expression of stemness-associated gene CD44 in gastric cancer cells.

What is known and what is new?

- ISL1 is highly expressed in a variety of tumour tissues and has an important role in cell differentiation and development.
- ISL1significantly promoted the stemness of gastric cancer cells, and ISL1 cooperated with AQP5 to enhance the tumorigenicity of gastric cancer cells. ISL1 promoted the nuclear translocation of FOXO3, which led to an increase in the expression of stemness-associated gene CD44 in gastric cancer cells.

What is the implication, and what should change now?

- In the future, ISL1 may emerge as a promising candidate for cancer therapy, albeit research on its inhibitors remains relatively scarce. Comprehensive exploration of these aspects will establish a robust theoretical framework for clinical diagnostics and therapeutic interventions.

Methods

Human GC samples

Primary GC tissues and corresponding adjacent noncancerous tissues were obtained from GC patients who underwent surgery at the Affiliated Hospital of Jining Medical University (Table S1). We randomly obtained 12 tissue specimens from patients. All GC tissue samples were obtained with written consent from the patients. This study was conducted in accordance with the Declaration of

Helsinki (as revised in 2013). The study was approved by the Clinical Ethics Committee of the Affiliated Hospital of Jining Medical University (No. 2022B034).

Cell culture and transfection

HGC-27 (TCHu 22) and AGS (TCHu232) cells were obtained from the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China). HGC-27 cells were cultured in DMEM medium (Gibco, 11965092) containing 10% foetal bovine serum (Gibco, A5669401) and AGS cells were cultured in DF-12 medium (Gibco, 21331020) containing 10% foetal bovine serum. Cells were incubated in an incubator at a temperature of 37 ℃ with a carbon dioxide concentration of 5%.

Cells were transfected with pSLenti-ISL1 and and ISL1-specific shRNA according to the manufacturer's instructions. Stably transfected cell lines were obtained after 6 days of selection with 1.5 μg/mL puroMycin (Gibco, A1113803). Transfection of FOXO3 siRNA and negative control (NC) siRNA was performed using Lipofectamine 3000 reagent (Invitrogen, L3000150) according to the manufacturer's instructions.

Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was isolated from cells and tissues using RNA-easy reagent (Vazyme). mRNA was reverse transcribed to cDNA according to the manufacturer's (Vazyme, R323-01) protocol. Real-time quantitative RT-PCR (qRT-PCR) was performed using the QuantiTect SYBR Green PCR kit (Vazyme, Q711-03). Primer sequences are shown in Table S2.

Western immunoblotting

Total protein was extracted using cell lysate (Beyotime Biotechnology, P0013). Denatured proteins were separated on a 10% SDS polyacrylamide gel and transferred to a nitrocellulose membrane. The membranes were incubated overnight with primary antibodies against the selected proteins (CellSignalingTechnology, AQP5-59558, ISL1-12496, SOX2-23064, GAPDH-2118), and the secondary antibodies were incubated the next day according to the primary antibody properties (abcam, ab150077). Colours were developed using the Enhanced ECL Chemiluminescence Detection Kit (Vazyme, E422-01).

Flow cytometry

Cells were collected and stained using anti-AQP5 (abcam) antibody. Data were analysed using FlowJo software.

In vivo tumour xenograft model

Cell suspensions were collected and then injected subcutaneously into BALB/c nude mice (female, 6–8 weeks). These nude mice were aged 4–6 weeks and were obtained from the Shanghai Experimental Animal Centre, Chinese Academy of Sciences, the experimental units were allocated to groups of mice using a randomised approach, consistent rearing environment across groups (the temperature of the rearing room was controlled at 24 ℃ and 55% humidity, and the ammonia concentration in the air was controlled to be no more than 20 ppm with 15 air changes/hour). Tumour volume (V) was calculated using the following formula: $V=0.5\times a\times b^2$ (where a indicates the longer tumour diameter and b indicates the shorter tumour diameter). At the end of the experiment, Neck-breaking mice, the xenograft tumours of each group were removed. All experimental protocols were approved by the Animal Ethics Committee of the Affiliated Hospital of Jining Medical University (No. 2022B034, Ethics committee has approved the pre-study protocol), in compliance with institutional guidelines for the care and use of animals.

In vivo metastasis model

A total of 2×10^6 cells carrying the GFP vector were injected into the tail vein of nude mice. At the end of the experiment, lung metastasis was assessed by OV100 microscope (Olympus) based on GFP signals.

Statistical analysis

Prism statistical analysis software (GraphPad, San Diego, USA) was used for person analysis and Student's *t*-test to assess the statistical significance of differences between groups; $P<0.05$ was considered statistically significant.

Results

ISL1 is highly expressed in GC and correlates with the expression of stemness genes

We conducted an investigation into the expression of

ISL1 in GC patient tissues as compared to adjacent non-cancerous tissues. As depicted in *Figure 1A-1C*, there is a significant upregulation of ISL1 in GC tissues. Data sourced from The Cancer Genome Atlas (TCGA) reveals that ISL1 demonstrates its highest expression in adenocarcinoma, with a gradual increase in expression as GC progresses in pathological grading, as illustrated in *Figure 1D,1E*. Notably, *Figure 1F* demonstrates a correlation between higher ISL1 expression and lower differentiation levels in GC tissues, suggesting an association between ISL1 and the differentiation status of GC tissues. The data presented in *Figure 1G* highlight the potential of ISL1 as a reliable marker for distinguishing GC. Further analysis via Kaplan-Meier survival curves, as shown in *Figure 1H*, reveals that patients exhibiting higher ISL1 expression levels have poorer survival outcomes, emphasizing ISL1's utility as a prognostic marker for individuals with GC. To investigate the potential relationship between ISL1 and GC differentiation, we examined its correlation with stemness and differentiation markers. As depicted in *Figure 1I-1N*, ISL1 exhibits a positive correlation with stemness marker genes, including *LGR5*, *SOX2*, *CD44*, and *OCT4*, while displaying a negative correlation with differentiation markers CK18 and MUC1. Collectively, these findings provide compelling evidence that ISL1 may indeed play a role in the regulation of stemness in GC.

ISL1 regulates the malignant biological functions of GC cells

We established distinct cell lines with exogenous overexpression and knockdown of ISL1, and their validation is presented in Figure S1. The results revealed that the most significant knockdown efficiency was observed in the sh ISL1-1 cell line. Thus, the sh ISL1-1 cell line was chosen for subsequent cellular functional experiments. Cellular functional experiments conducted on AGS and HGC-27 cells unveiled that the exogenous overexpression of ISL1 promoted the proliferation, migration, and clonogenicity of GC cells. Conversely, ISL1 knockdown had an inhibitory effect on these cellular functions, as demonstrated in *Figure 2A-2L*. The collective findings from these experiments strongly support the notion that ISL1 can augment the malignant biological functions of GC cells.

ISL1 promotes the stemness of GC cells

Subsequently, we explored the influence of ISL1 on the stemness of GC cells. As expected, in AGS and HGC-27 cells, the exogenous overexpression of ISL1 substantially enhanced the formation of spheres (*Figure 3A*,*3B*). Conversely, ISL1 knockdown led to a significant reduction in sphere formation (*Figure 3C*,*3D*). To further substantiate the effect of ISL1 on the stemness of GC cells, we assessed the expression of stem cell markers. As depicted in *Figure 3E-3H*, ISL1 knockdown prominently inhibited the expression of stem cell markers, including including *CD44*, *AQP5*, *SOX2*, and *OCT4*, while ISL1 overexpression augmented the expression of *CD44*, *AQP5*, *SOX2*, and *OCT4* (*Figure 3I-3L*). Similarly, at the protein expression level, ISL1 overexpression heightened the protein expression of AQP5 and SOX2, whereas ISL1 knockdown significantly suppressed the protein expression of AQP5 and SOX2 (*Figure 3M*,*3N*).

To assess the impact of ISL1 on the tumorigenicity of GC cells, we established a xenograft model. Cells at varying concentrations (1×10^4–1×10^6) were subcutaneously injected into severely immunodeficient mice. As demonstrated in *Figure 4A-4E*, ISL1 overexpression amplified the tumorigenic potential of GC cells, resulting in increased tumor weight and volume. To evaluate the effect of ISL1 on the metastatic capacity of GC cells, we introduced GC cells overexpressing ISL1 and a control group of cells into severely immunodeficient mice. The results in *Figure 4F* illustrate that ISL1 overexpression promotes the metastasis of GC cells. In summary, these findings collectively suggest that ISL1 augments the stemness, tumorigenicity, and metastatic propensity of GC cells *in vivo*.

ISL1 and AQP5 synergistically promote the stemness of GC cells

Expanding upon our prior observation that ISL1 can enhance the expression of the stemness marker AQP5, we proceeded to investigate the interrelationship between ISL1 and AQP5. Flow cytometric analysis unveiled that the exogenous overexpression of ISL1 led to an increase in the proportion of AQP5+ cells, whereas conversely, ISL1 knockdown resulted in a reduction in the population of AQP5+ cells (*Figure 5A*,*5B*). Furthermore, the co-overexpression of AQP5 and ISL1 notably potentiated the formation of spheres by GC cells, while the overexpression of AQP5 alone exerted a comparatively modest effect (*Figure 5C*). Similar outcomes were observed *in vivo*, where the co-overexpression of AQP5 and ISL1 significantly heightened the tumorigenic potential of GC cells, surpassing

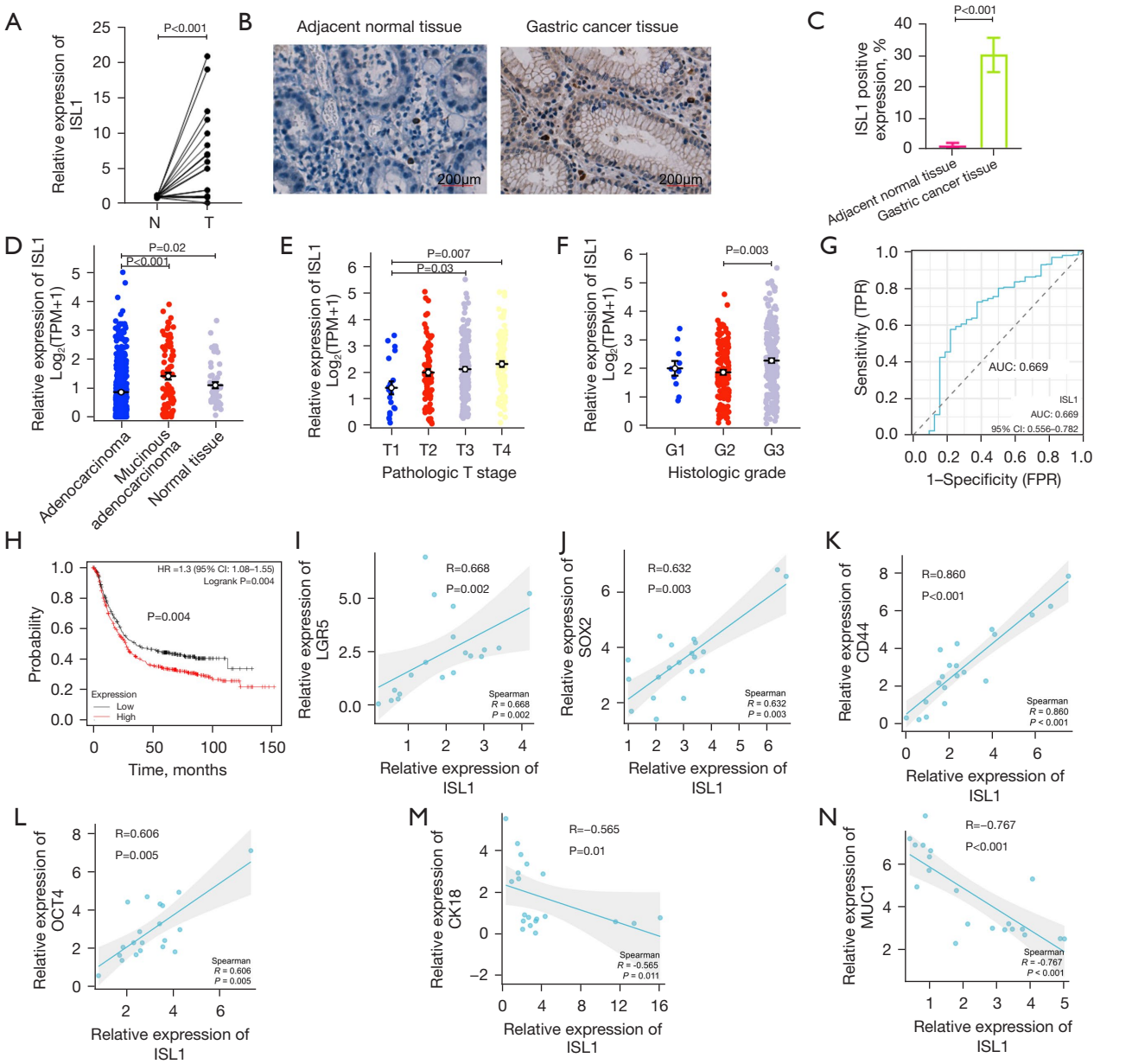

Figure 1 Expression of ISL1 in patients with gastric cancer. (A) Expression levels of ISL1 were measured in GCs and matched GMs (Cohort 1, n=12, log-rank test, two-sided). (B,C) Immunohistochemistry was used to detect ISL1 expression in tissues and the rate of ISL1 positivity per high magnification field of view was counted. (D-F) Expression of ISL1 in patients with different gastric cancer types in the TCGA database. (G) ROC curves were established to test the discriminative value of ISL1 for gastric cancer tissues. (H) Survival curves of OS between ISL1-high and -low patients with gastric cancer. (I) The correlation of ISL1 and LGR5 mRNA expression. (J) The correlation of ISL1 and SOX2 mRNA expression. (K) The correlation of ISL1 and CD44 mRNA expression (biological repeat 3 times). (L) The correlation of ISL1 and OCT4 mRNA expression. (M) The correlation of ISL1 and CK18 mRNA expression. (N) The correlation of ISL1 and MUC1 mRNA expression. ISL1, insulin gene enhancer binding protein-1; N, normal tissue; T, gastric cancer tissue; TPM, transcripts per million; TPR, true positive rate; FPR, false positive rate; AUC, area under the curve; HR, hazard ratio; GCs, gastric cancer tissues; GMs, gastric mucosal tissues; TCGA, The Cancer Genome Atlas; ROC, receiver operating characteristic; OS, overall survival; LGR5, leucine rich repeat containing G protein-coupled receptor 5; SOX2, SRY-box transcription factor 2; CD44, CD44 molecule; OCT4, POU class 5 homeobox 1; CK18, keratin 18; MUC1, mucin 1. Pathological types of gastric cancer: adenocarcinoma, mucinous adenocarcinoma.

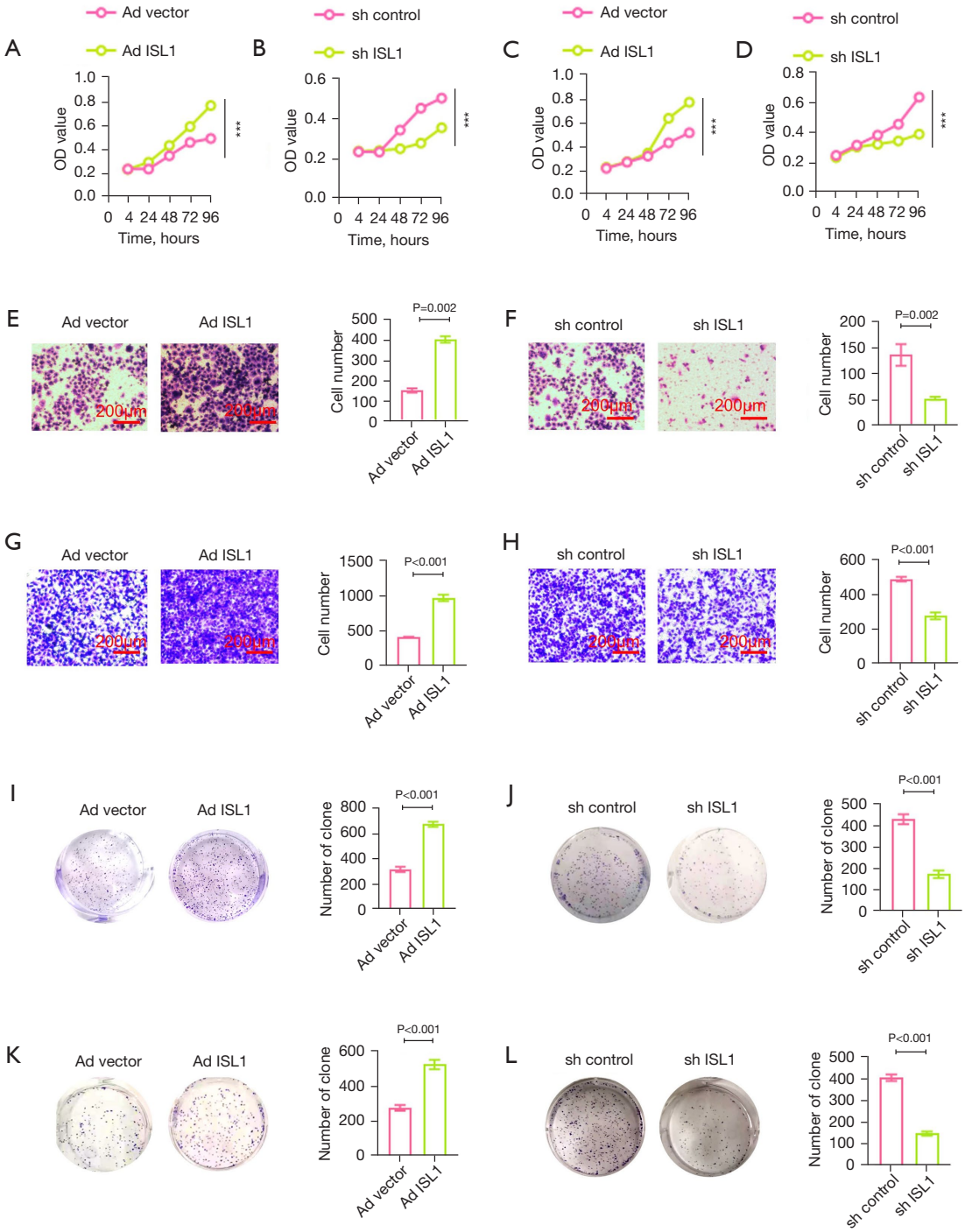

Figure 2 ISL1 promotes gastric cancer development *in vitro*. ISL1 was knocked down or overexpressed in the AGS or HGC-27 cell lines, and the cells were analyzed to measure cell proliferation (A-D, A and B are AGS cells, C and D are HGC-27 cells); cell migration: the cells were stained using crystal violet dye and then observed and photographed under a 10× lens (E-H, E and F are AGS cells, G and H are HGC-27 cells); colony formation: direct observation and photographing of cells after staining with crystal violet dye (I-L, I and J are AGS cells, K and L are HGC-27 cells) (biological repeat 3 times). ***, P<0.001. OD, optical density; Ad vector, empty vector control of exogenous overexpression of ISL1; Ad ISL1, exogenous overexpression of ISL1; sh control, empty vector control of knockdown of ISL1 expression; sh ISL1, knockdown of ISL1 expression; SOX2, SRY-box transcription factor 2; CD44, CD44 molecule; OCT4, POU class 5 homeobox 1; ISL1, insulin gene enhancer binding protein-1.

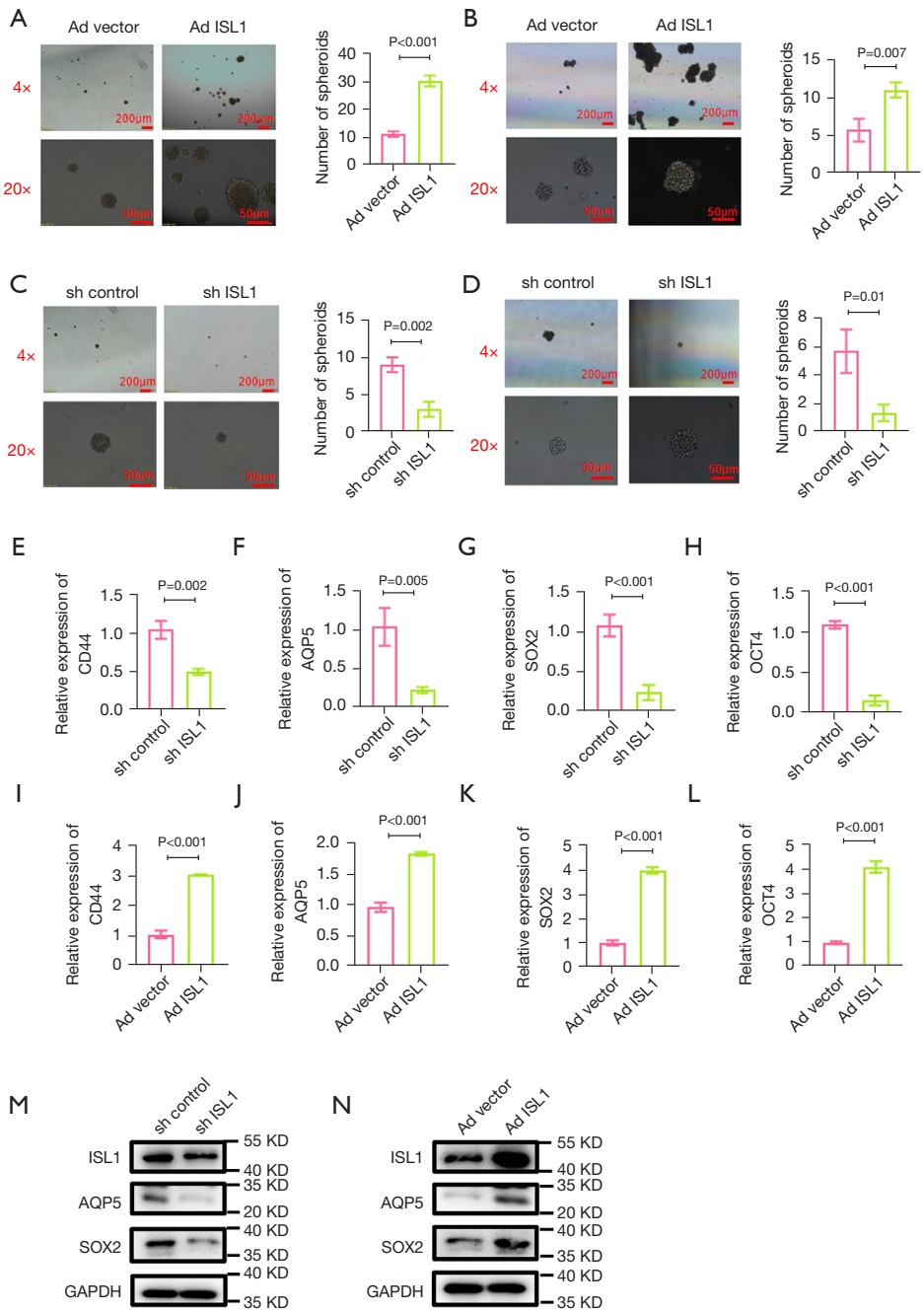

Figure 3 ISL1 promotes the stemness of GC cells *in vitro*. Representative images of exogenous ISL1 overexpressing (A,B) or knockdown ISL1 (C,D) cells cultured in serum-free medium for 10 days (biological repeat 3 times), observed and photographed using a microscope at 4× and 20× respectively. And statistical analysis was performed on the number of spheroids (diameter >50 μm). Real-time qRT-PCR was used to detect the expression of CD44 (E), AQP5 (F), SOX2 (G) and OCT4 (H) in ISL1 knockdown cells and negative control cells. qRT-PCR was used to detect the expression of CD44 (I), AQP5 (J), SOX2 (K) and OCT4 (L) in ISL1 overexpressing cells and adenovirus control cells (biological repeat 3 times). (M,N) The protein expression of ISL1, AQP5, SOX2 and GAPDH in ISL1 knockdown or ISL1 overexpressing cells was assayed using Western blotting. Ad vector, empty vector control of exogenous overexpression of ISL1; Ad ISL1, exogenous overexpression of ISL1; sh control, empty vector control of knockdown of ISL1 expression; sh ISL1, knockdown of ISL1 expression, SOX2, SRY-box transcription factor 2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; CD44, CD44 molecule; OCT4, POU class 5 homeobox 1; AQP5, aquaporin 5; ISL1, insulin gene enhancer binding protein-1; GC, gastric cancer; qRT-PCR, quantitative reverse transcription-polymerase chain reaction.

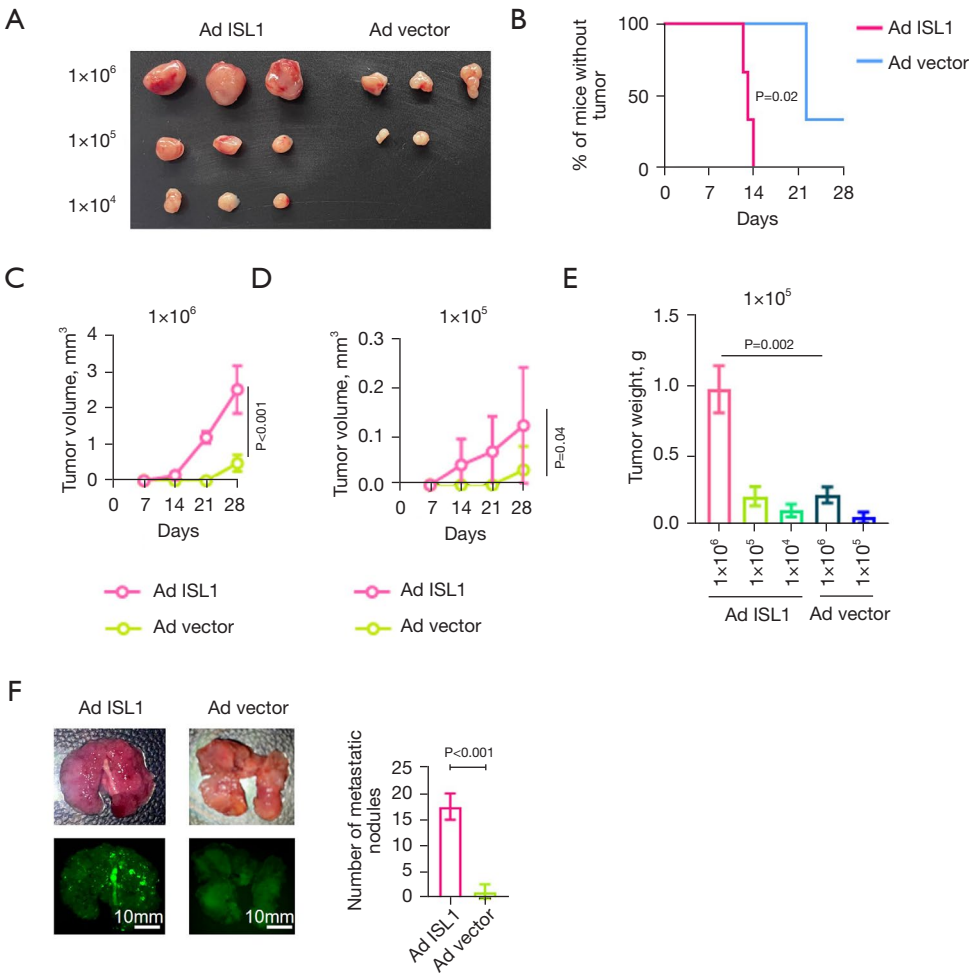

Figure 4 ISL1 promotes the tumorigenicity and metastatic capacity of GC cells *in vitro*. ISL1 was overexpressed in AGS cells. (A) These cells were diluted and subcutaneously injected into severely immunodeficient mice. Tumors were examined over a 28-day period (n=3 for each group, biological repeat 3 times). The tumor occurrence time (B), volume (C,D) and tumor weight (E) were monitored in the indicated groups and at the indicated time points. (F) ISL1 overexpressing and control cells (GFP labeled) were injected into the tail vein of mice, the number of metastatic nodules in the lungs of mice was observed (using the stereomicroscope) after 28 days, and the number of metastatic nodules in the lungs of mice was counted (biological repeat 3 times). Ad vector, empty vector control of exogenous overexpression of ISL1; Ad ISL1, exogenous overexpression of ISL1; ISL1, insulin gene enhancer binding protein-1; GC, gastric cancer; GFP, green fluorescent protein.

the effect of AQP5 overexpression in isolation (*Figure 5D*). These findings collectively establish that AQP5 and ISL1 synergistically promote tumorigenesis in GC cells.

ISL1 promotes CD44 expression by regulating FOXO3 nuclear translocation

To elucidate the molecular mechanism underlying ISL1's regulation of GC cell stemness, we conducted transcriptome sequencing on cells overexpressing ISL1 and control cells. The results unveiled a significant enrichment of differentially expressed genes in the FOXO signaling pathway, with the most pronounced distinction observed in the expression of *FOXO3* (*Figure 6A,6B*). Subsequent investigations further demonstrated that ISL1 overexpression facilitates the nuclear translocation of *FOXO3* (*Figure 6C*). Of particular importance, we observed that ISL1 overexpression leads to a substantial

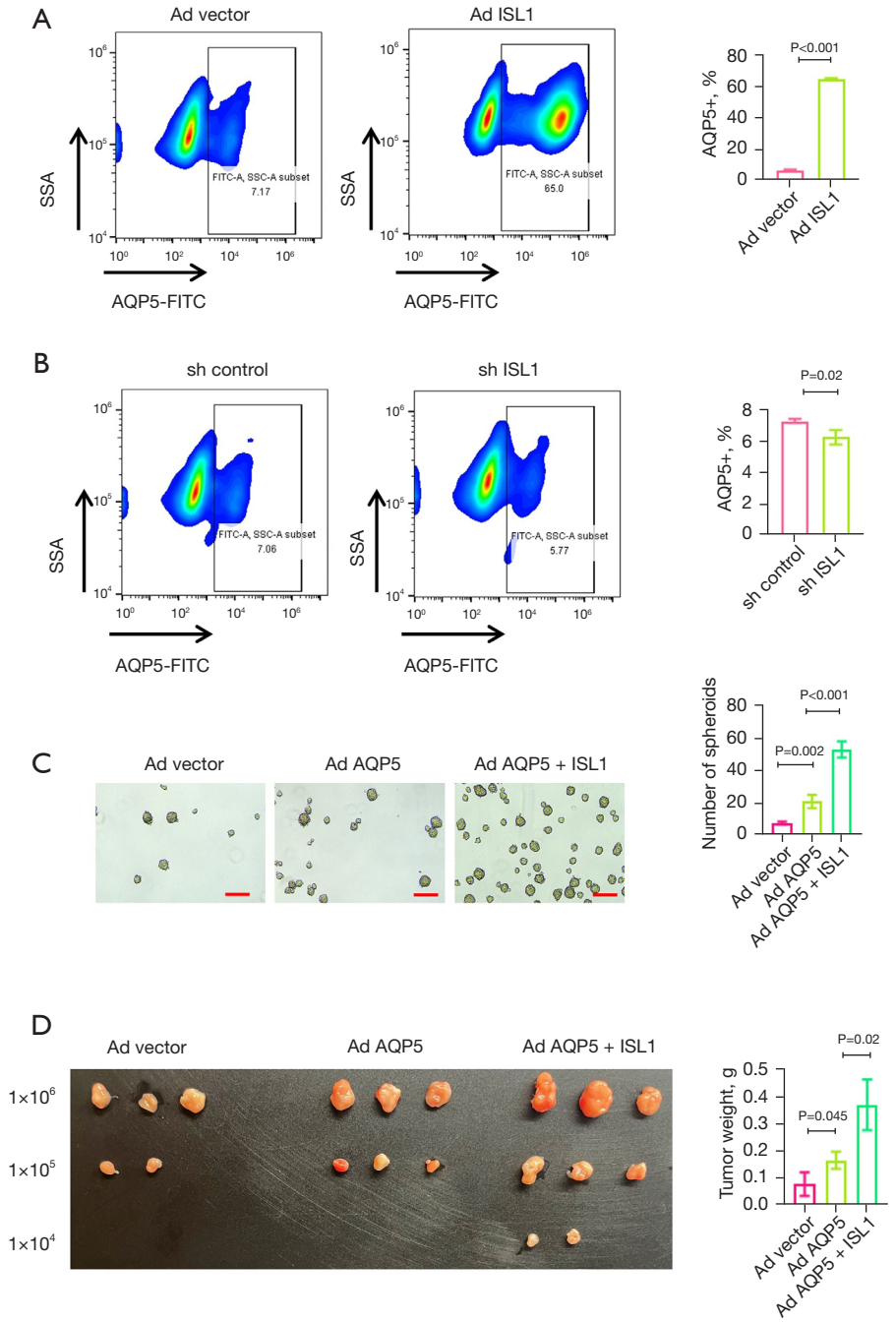

Figure 5 Co-overexpression of ISL1 and AQP5 significantly enhances the malignant biological functions of gastric cancer cells. Representative flow cytometry results and statistical analysis of AQP5 positive cells in ISL1-overexpressing (A) or ISL1-knockdown (B) AGS cells (biological repeat 3 times). (C) AQP5 and ISL1 were overexpressed in AGS cells, and the cells were analyzed to assess sphere formation in serum-free culture, observed and photographed using a microscope at 10× (biological repeat 3 times). (D) Cells were diluted and subcutaneously injected into severely immunodeficient mice. Tumors were examined over a 28-day period (n=3 for each group, biological repeat 3 times). The tumor weight was monitored in the indicated groups. Ad vector, empty vector control; Ad AQP5, exogenous overexpression of AQP5; Ad AQP5 + ISL1, exogenous overexpression of AQP5 + exogenous overexpression of ISL1; AQP5, aquaporin 5; ISL1, insulin gene enhancer binding protein-1.

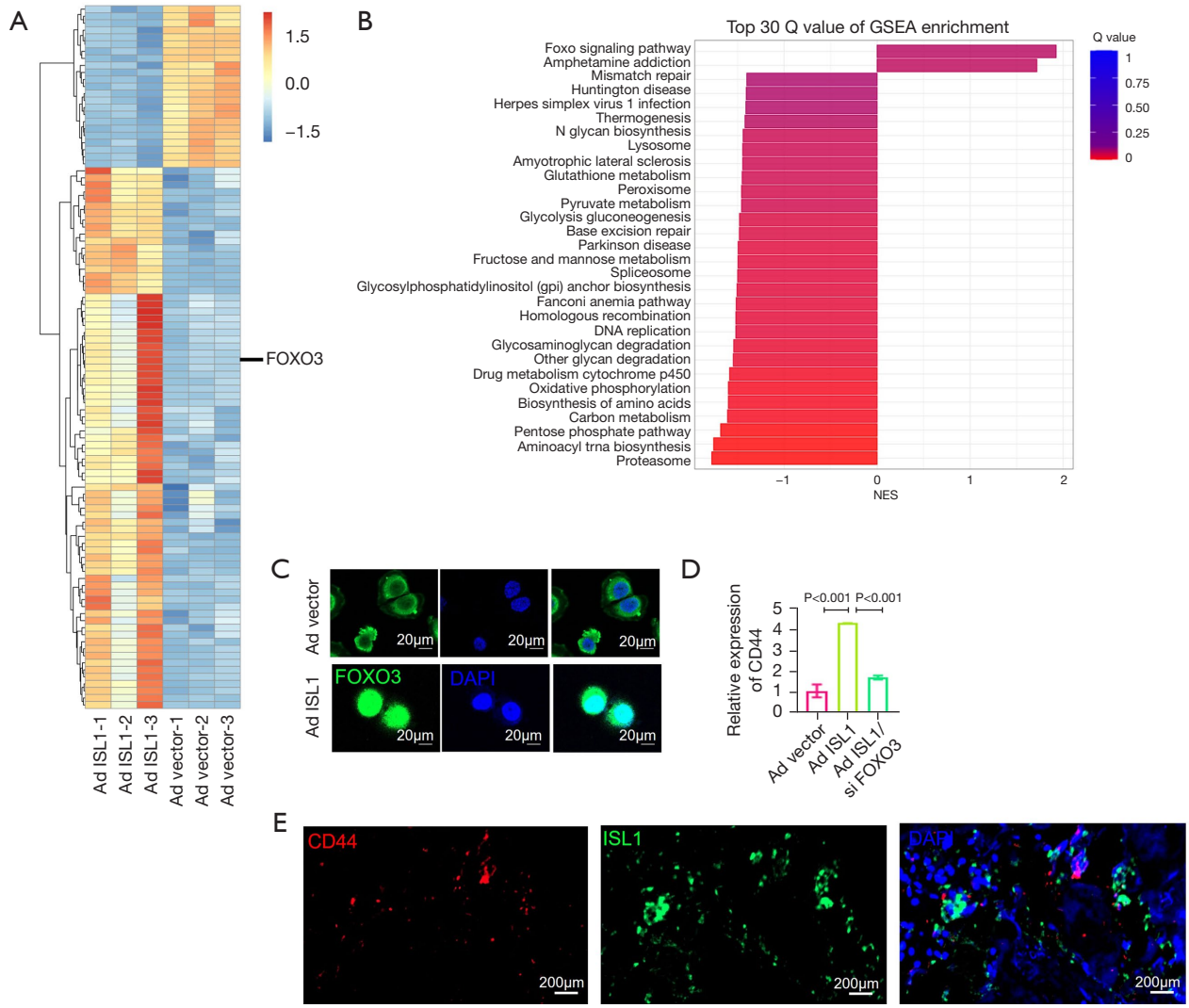

Figure 6 ISL1 promotes CD44 expression through FOXO3. (A) Heat map analyze the expression of different genes (biological repeat 3 times). (B) Differential genes were subjected to gene set enrichment analysis, and the enriched pathways in the top 30 of the Q value were displayed. (C) Immunofluorescence of FOXO3 in ISL1 overexpressing cells or control cells. (D) Real-time qRT-PCR was used to detect the expression of CD44 in different cells (biological repeat 3 times). (E) Expression of CD44 and ISL1 in gastric cancer tissues detected by multi-colour immunofluorescence, CD44 is labelled in red and ISL1 in green. FOXO3, forkhead box O3; AQP5, aquaporin 5; CD44, CD44 molecule; GSEA, gene set enrichment analysis; Ad vector, empty vector control; Ad AQP5, exogenous overexpression of AQP5; siFOXO3, small interfering RNA targeting FOXO3; ISL1, insulin gene enhancer binding protein-1; qRT-PCR, quantitative reverse transcription-polymerase chain reaction.

upregulation in the expression of the stemness-related gene *CD44*, whereas FOXO3 knockdown notably diminishes *CD44* expression (*Figure 6D*). In addition, we detected co-localisation of CD44 and ISL1 in GC tissues, further demonstrating the interconnection between ISL1 and CD44 (*Figure 6E*). These findings collectively suggest that ISL1 promotes the nuclear translocation of FOXO3, thereby enhancing CD44 expression and modulating the stemness of GC cells.

Discussion

The stemness of GC cells encompasses traits such as invasiveness, heterogeneity, and drug resistance, rendering

it for being imperative for gaining insight into the molecular mechanisms governing this phenomenon for the development of novel therapeutic strategies (15). Our study demonstrates that the overexpression of ISL1 enhances self-renewal and fosters tumorigenesis in GC cells.

ISL1 has established roles in the development and progression of diverse tumors, encompassing pheochromocytoma, gastrointestinal, pancreatic, lung, and cholangiocarcinoma (16). Our investigation reveals a heightened expression of ISL1 in GC, particularly in advanced-stage cases when compared to low-grade counterparts. Based on this, we divided GC samples into high and low ISL1 expression groups, and subsequently plotted survival curves. These Kaplan-Meier curves unveiled a correlation between elevated ISL1 mRNA expression and an adverse prognosis in GC. Therefore, ISL1 emerges as a pivotal transcription factor governing the progression of GC and serving as an independent prognostic indicator. Subsequent research is warranted to delineate ISL1's role in driving GC progression. Notably, findings by Guo *et al.* underscore ISL1's prognostic value in GC, elucidating its role in tumor progression through the binding to the ZEB1 promoter in conjunction with SETD7 (10). Our study similarly demonstrates ISL1's capacity to enhance the proliferation, migration, and clonogenicity of GC cells.

ISL1 assumes a critical role in neuronal (17), cardiac (18), and sensory development (19), directly or indirectly modulating numerous genes pivotal for proliferation and differentiation, many of which are implicated in cancer pathogenesis (20). Our investigation reveals that ISL1 expression correlates with stemness genes like SOX2 and CD44, crucial players in the proliferation and differentiation of GC cells. Furthermore, we establish ISL1's crucial role in the stemness capacity of GC cells, demonstrating its capacity to promote tumorigenicity and metastatic potential *in vivo*. These findings collectively suggest that ISL1 propels the progression of GC by regulating the stemness of GC cells.

Tumor stem cells self-renewal and differentiation capabilities significantly contribute to the overall tumor mass. Our prior research has identified AQP5 as a marker gene for GCSCs, with AQP5+ cell subpopulations being a critical tumor-initiating component (21). The current study underscores ISL1's significant role in expanding AQP5+ cells, implying its potential role in driving GC development.

This study unveils a previously undisclosed mechanism by which ISL1 governs the stemness of GC cells. FOXO3, an essential transcription factor implicated in various human cancers, regulates cancer progression by modulating the expression of multiple genes (22). Our investigation establishes that the heightened nuclear expression of FOXO3 activates the transcription of CD44. Prior literature has emphasized FOXO3's indispensability for CD44 expression and the attributes of CSCs (23). CD44 serves as a marker for CSCs in numerous tumors, encompassing breast cancer, pancreatic cancer, colorectal cancer, and liver cancer, with the upregulation of CD44 being sufficient to trigger CSC properties (24).

Conclusions

Our study has unveiled hitherto unrecognized biological roles of ISL1 in facilitating the progression of GC. Furthermore, we have elucidated the mechanisms through which ISL1 governs the deleterious biological characteristics of GC cells. In the future, ISL1 may emerge as a promising candidate for cancer therapy, albeit research on its inhibitors remains relatively scarce. Comprehensive exploration of these aspects will establish a robust theoretical framework for clinical diagnostics and therapeutic interventions.

Acknowledgments

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Footnote

Reporting Checklist: The authors have completed the ARRIVE and MDAR reporting checklists. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-248/rc

Data Sharing Statement: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-248/dss

Peer Review File: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-248/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-248/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study, which involved human participants and animal experiments, was approved by the Ethics Committee of the Affiliated Hospital of Jining Medical University (No. 2022B034). Research involving human participants was conducted in accordance with the Declaration of Helsinki (as revised in 2013), and informed consent was obtained from all patients. The animal experiments complied with institutional guidelines for the care and use of animals.

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