



EGR1 promotes stemness and predicts a poor outcome of uterine cervical cancer by inducing SOX9 expression

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Abstract

Background Early growth response-1 (EGR1) is a transcription factor involved in the progression of several cancer types. However, the expression and clinical significance of EGR1 in uterine cervical cancer (CC) have not been elucidated.

Objective To investigate the expression, clinical significance and prognostic value of EGR1 in CC.

Methods The expression of EGR1 was detected in 13 CCs and paired adjacent tissues with qRT-PCR and in 144 CC tissues with immunohistochemistry (IHC). The IHC scores were used to divide the patients into subsets with low and high EGR1 expression. The correlations between the EGR1 expression and clinicopathological factors were analyzed with the chi-square test, and the prognostic significance of EGR1 expression was evaluated with univariate and multivariate analyses. The functions of EGR1 in the proliferation, invasion and stemness of CC cells were investigated, and the molecular mechanism was assessed by in vitro experiments.

Results High expression of EGR1 was significantly associated with low survival rates of CC. EGR1 is an independent prognostic biomarker of CC, and its high expression predicted a poor outcome. EGR1 facilitated stemness and thus promoted proliferation and invasion of CC cells. SOX9 played an essential role in the EGR1-induced progression of CC cells.

Conclusions EGR1 is an independent prognostic biomarker of CC. High EGR1 expression promoted proliferation, invasion and stemness by increasing SOX9 expression in CC cells. Our results suggested that the EGR1-SOX9 axis may be a potential drug target and that blocking the EGR1-SOX9 axis may be a possible approach to treating CC.

Keywords EGR1 · Prognosis · Uterine cervical cancer · SOX9 · Stemness

Introduction

Uterine cervical cancer (CC) is the third most common malignancy in women worldwide. Globally, there are approximately 527,600 newly diagnosed CC cases and approximately 265,700 CC-related deaths every year (Torre

et al. 2015). CC is a particular threat to women in developing countries. In China, the incidence and mortality of CC are the highest among all gynecologic cancers (Chen et al. 2016).

The morbidity and mortality of CC have significantly decreased in recent decades due to improvements in CC prevention, such as HPV vaccines, and advances in individual treatment, but approximately 40% of patients still suffer recurrence (Gao et al. 2018). Targeted therapies, such as bevacizumab, have been used to treat CC, resulting in a significant improvement in the overall survival (OS) rate. However, the 5-year OS rate of locally advanced CC in FIGO (Federation International of Gynecology and Obstetrics) stage (IIB-IVA) is only approximately 40%–50% (Marquina et al. 2018). The current biomarkers and targeted drugs cannot fulfill the unmet need of CC treatment to establish new therapeutic approaches and to improve patient OS time.

Early growth response-1 (EGR1, also known as NEF1A and Krox-24) is a transcription factor implicated in several essential physiological processes, including cell

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development, metabolism, cell growth and proliferation. EGR1 has a highly conserved DNA-binding domain consisting of three zinc fingers that interact with the GC-rich consensus sequence (Li et al. 2019). EGR1 expression is induced by many extracellular signaling molecules, such as hormones, neurotransmitters, growth and differentiation factors (Chen et al. 2017), and it participates in many pathological processes, such as cardiovascular diseases and cancers. EGR1 has been identified to be involved in the tumorigenesis and progression of many cancers, including breast, prostate, colon, and esophageal cancers (Parra et al. 2009; Shan et al. 2014; Yang and Abdulkadir, 2003; Yang et al. 2006), but the role of EGR1 in tumor progression has not received a complete consensus. It is generally accepted that EGR1 can function as a tumor suppressor or an oncoprotein depending on the type of tumor (Hasen and Gammie 2006). The function and expression of EGR1 in CC have not been well studied, so in this study, we investigated the clinical significance of EGR1 in CC.

SRY-related high-mobility group box 9 (SOX9) is a member of the family of sex-determining region Y (SRY)-related high-mobility-group box transcription factors (Leung et al. 2016). SOX9 is an indispensable factor that regulates multiple developmental pathways related to stemness, differentiation, and progenitor development (Jana et al. 2020). SOX9 has been proven to drive tumorigenesis as an oncoprotein by participating in tumor initiation, proliferation, migration, chemoresistance, and stem cell maintenance, thereby regulating tumorigenesis (Dy et al. 2012; Finzsch et al. 2008; Wang et al. 2008; Yagi et al. 2005). However, the oncogenic role of SOX9 in CC is still controversial. A previous study showed that SOX9 was a potential tumor suppressor in CC, functioning by transactivating p21WAF1/CIP1 (Wang et al. 2015), but other studies found that SOX9 was an oncoprotein and showed that inhibition of SOX9 could suppress CC progression (Feng et al. 2018; Liu et al. 2019). However, the clinical functions of SOX9 in CC and the correlation of SOX9 with EGR1 have not been fully elucidated.

In our study, we investigated the clinical significance of EGR1 by detecting its expression, evaluating its correlation with clinicopathological variables, and analyzing its prognostic value. Moreover, we assessed the role of EGR1 and SOX9 in CC progression with *in vitro* experiments and explored the underlying molecular mechanisms.

Materials and methods

Ethics and patients

From 2012 to 2015, a total of 209 patients were diagnosed with CC in three medical centers, including Women & Children's Health Care Hospital of Linyi, Hospital of Shandong

Medical College and the First Affiliated Hospital of Shandong First Medical University. The validation cohort was selected from among those patients, consisting of 128 patients, according to these criteria: (1) no preoperative treatment, (2) follow-up of more than 3 months, (3) standard postoperative treatment, and (4) radical surgery and available tissues for IHC. The average age of the validation cohort was 42.0 years old. The basic information of the patients with CC is provided in Supplemental Table 1. A follow-up was performed every 3 months after the surgery. The average follow-up time of these patients was 62.5 months. The TNM stage was based on the 8th AJCC/UICC staging system. All specimens were obtained with signed informed consent from the patients. The whole study was approved by the Ethics Committees of the Women and Children's Health Care Hospital of Linyi, the Hospital of Shandong Medical College and the First Affiliated Hospital of Shandong First Medical University.

Cell culture and agents

The human CC cell line HeLa was purchased from the Cell Bank of Chinese Sciences Academy (Shanghai, China) and cultured in DMEM supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. Antibodies against

Table 1 Correlation between EGR1 and other factors

Characters	EGR1		P*
	Low	High	
Age			
< 50	67	16	0.045
≥ 50	29	16	
Tumor size			
< 4 cm	50	20	0.412
≥ 4 cm	46	12	
HPV			
Absent	17	9	0.213
Present	79	23	
Histological grade			
I + II	30	5	0.111
III	66	27	
T stage			
I + II	82	21	0.021
III + IV	14	11	
Lymph node invasion			
No	83	21	0.017
Yes	13	11	
TNM			
I + II	82	21	0.017
III + IV	14	11	

*Chi square test

the following proteins were used: EGR1 (Cell Signaling Technology, MA, USA, Cat# 44D5, RRID:AB_2097035), SOX9 (Abcam Cat# ab59265, RRID:AB_945590), Nanog (Cell Signaling Technology, 8822), Oct-4 (Cell Signaling Technology, 2750), and GAPDH (Cell Signaling Technology, 5174).

RNA extraction and qPCR

The mRNA levels of EGR1 in fresh CC tissues and corresponding tumor adjacent tissues were evaluated by qRT-PCR. Fresh tissues were obtained from 13 consecutive CC patients during surgery with prior consent. Total RNA of the CC tissues and adjacent tissues was extracted with TRIzol according to the manufacturer's instructions. Reverse transcription and real-time PCR were accomplished with a ReverTra Ace qPCR RT kit (TOYOBO, Japan). mRNA reverse transcription was conducted with a ReverTra Ace qPCR RT kit and SYBR Green Master Mix (Roche, USA) using the Light Cycler Roche 480 PCR. The GAPDH level was set as a baseline, and the $2^{-\Delta\Delta C_t}$ method was used to evaluate the mRNA level of EGR1. The primers of GAPDH and EGR1 were as follows:

EGR1: Forward: 5'-CTTCAACCCTCAGGCGGACA-3', Reverse: 5'-GGAAAAGCGGCCAGTATAGGT-3';

GAPDH: Forward: 5'-GAGTCAACGGATTGGTCGT-3', Reverse: 5'-GACAAGCTTCCCGTTCTCAG-3'.

Transfection

Knockdown and overexpression were achieved by transfection with shRNA, siRNA or overexpression plasmids by using Lipofectamine 2000 (Thermo Fisher) according to the manufacturer's guidelines. The target sequences of shEGR1-1 and shEGR1-2 were 5'-TATCCCATGGGCAATAAAG-3' and 5'-ACATTCTGGAGAACCGAAG-3'. The target sequence of siSOX9 was 5'-CAGCGAACGCACATCAAGACGGAGCAGCT-3'. The open reading frames of EGR1 and SOX9 were purchased from Sinobiological (Beijing, China) and transferred into the pFLAG-CMV vector (Sinobiological, China).

Immunohistochemistry and evaluation

The expression levels of EGR1 and SOX9 were estimated with IHC. The detailed methods applied were as described in our previous study (Zhang et al. 2016). Briefly, tissues were deparaffinized and rehydrated with graded ethanol and xylene. Optimal antigen retrieval was realized by boiling in citrate buffer (pH = 6.0). Endogenous hydrogen peroxidase was blocked by incubating with hydrogen peroxide

for 10 min. After that, 5% fetal bovine serum was used to block nonspecific antigen binding. Primary (1:100 for both EGR1 and SOX9) and secondary antibodies were incubated with the tissues in sequential order, and final visualization of antigen was realized by applying a DAB kit (Solarbio, Beijing, China).

The IHC results were semiquantified by IHC scores by two pathologists who were unaware of the patient's clinical information. The IHC scores were the products of two-aspect multiplication of the score for positive cell percentage and the score for staining intensity. The score for positive cell percentage was from 0 to 3: 0 (0–5%), 1 (5–25%), 2 (26–50%) and 3 (51–100%). The staining intensity was scored from 0 to 3 as follows: score 0 for negative staining; 1 for weak staining (pale yellow); 2 for moderate staining (dark yellow); and score 3 for strong staining (brown). The patients were divided into subsets based on the cutoff of the IHC scores.

CCK-8 Assay

A Cell Counting Kit 8 (CCK-8) assay was used to evaluate the proliferation of HeLa cells. After successful knockdown or overexpression of EGR1 and/or SOX9, HeLa cells were seeded into a 96 plate well, cultured for 6 h for adhesion, and then incubated for the indicated time (0–3 days). Ten microliters of CCK-8 solution was added to the cells after incubation, and the optical density at 450 nm was detected. The OD₄₅₀ of the control cells was set as the baseline, and the proliferation index was calculated by the ratio of the OD of the other cells to the baseline.

Transwell assay

A Transwell assay was performed to assess tumor cell invasion with 8 μ m Matrigel-coated Transwells (BD Biosciences, USA). HeLa cells were first transfected with shEGR1 or pFLG-CMV-EGR1 for EGR1 knockdown or overexpression 48 h before the assay. After that, the cells were placed in the top chamber at a concentration of 10^5 cells per well and then incubated for 12 h. Finally, the cells in the lower chamber were fixed with formalin and stained with 0.05% crystal violet for observation. Cell numbers in five visual fields were counted, and the average number was defined as the invaded cell number.

Western blotting

Western blotting was applied to detect the amount of protein. In brief, the cells were lysed in RIPA lysis buffer (Beyotime) on ice for 20 min. The lysates were centrifuged at 10,000g

for 30 min, and the pellets were removed. The supernatant was detected with the BCA method to quantify the protein and then mixed with loading buffer. Ten micrograms of protein per sample were electrophoresed on SDS-PAGE and then transferred to a PVDF membrane (PALL Company, USA). The membrane was incubated in 5% fat-free milk to attenuate nonspecific binding and then with the primary antibody overnight. The corresponding HRP-labeled secondary antibody was incubated with the membrane for 1 h, and ECL (Thermo Fisher) was added to show the protein bands.

Statistical analysis

All statistical analyses were calculated with SPSS 22.0 statistical software (Chicago, IL, USA). The χ^2 method was applied to analyze the correlation between the level of EGR1 expression and the other clinicopathological variables. The Kaplan–Meier method was used to display the survival curves, and the log-rank test was used to analyze the differences among different subgroups. The Cox proportional hazard model was used to determine the independent prognostic factors in the multivariate analysis. In the *in vitro* experiments, statistical significance was analyzed with a *t* test or one-way ANOVA. A *P* value < 0.05 was considered statistically significant.

Results

EGR1 was upregulated in cervical cancers

In 13 CC tissues and their corresponding tumor-adjacent tissues, the mRNA level of EGR1 was detected with qRT-PCR (Fig. 1a). EGR1 expression was increased in all of the CCs compared with the adjacent tissues. Moreover, EGR1 expression in 128 CC tissues was detected by IHC. The EGR1 protein was mainly localized in the cell nucleus. Using the cutoff defined by the ROC curve, we divided these patients into subsets with low and high EGR1 expression (Fig. 1b), which accounted for 75.0% (96/128) and 25.0% (32/128), respectively.

The clinical significance of EGR1 in cervical cancer

The clinical significance of EGR1 in CC was evaluated by the chi-square method to estimate the correlations between EGR1 expression and other clinicopathological factors (Table 1). In our study cohort, high EGR1 expression was significantly associated with advanced T stage and lymph node invasion, indicating that EGR1 overexpression promoted the progression of CC.

The effects of EGR1 on CC prognosis were further estimated with univariate and multivariate analyses (Table 2). The survival curves of EGR1 and the other clinicopathological factors were calculated with Kaplan–Meier methods and analyzed with the log-rank test. In this study, EGR1 overexpression was markedly correlated with a poor prognosis of CC (Fig. 2a). Moreover, histological grade (*P* = 0.036), T stage (*P* < 0.001), lymph node invasion (*P* < 0.001) and TNM stage (*P* < 0.001) were all defined as prognostic factors, predicting a poor outcome of CC (Fig. 2b–e).

EGR1 promoted cell proliferation by upregulating SOX9

In the above clinical analysis, we observed that EGR1 was correlated with T stage and lymphatic invasion, indicating that EGR1 may be involved in tumor proliferation, infiltration and invasion, so we applied *in vitro* experiments to assess the effects of EGR1 on tumor progression. After silencing EGR1 with two independent shRNAs or overexpressing EGR1 by transfection of the pFLAG-CMV-EGR1 vector (Fig. 3a, b), we assessed the proliferation and invasion of the CC cell line HeLa. Both the proliferation and invasion of HeLa cells were attenuated after EGR1 knockdown and enhanced after EGR1 overexpression (Fig. 3c, d). Considering that a change in cell stemness can explain multiple processes of tumor progression, such as proliferation and invasion, we assessed the influence of EGR1 on cell stemness by detecting the relevant stemness biomarkers. The stemness biomarkers Nanog and OCT2 were substantially decreased after EGR1 silencing (Fig. 3e, f), supporting that CC stemness was regulated by EGR1.

EGR1 facilitated CC stemness by regulating SOX9 expression

Several studies have shown that EGR1 can influence tumor stemness in some special cancer types, such as breast cancer or glioma (Sakakini et al. 2016; Tang et al. 2019), but no definite molecular mechanism has been revealed. As a transcription factor, EGR1 mainly functions by regulating the transcription and expression of downstream target genes. Applying mRNA sequencing, a previous study showed that EGR1 and SOX9 mRNA had consistent changes (Berent et al. 2019), suggesting that EGR1 may regulate the expression of SOX9. Considering that SOX9 is a stemness modulator, we detected the regulation of SOX9 expression by EGR1. In HeLa cells, SOX9 expression was decreased after silencing EGR1 expression, supporting

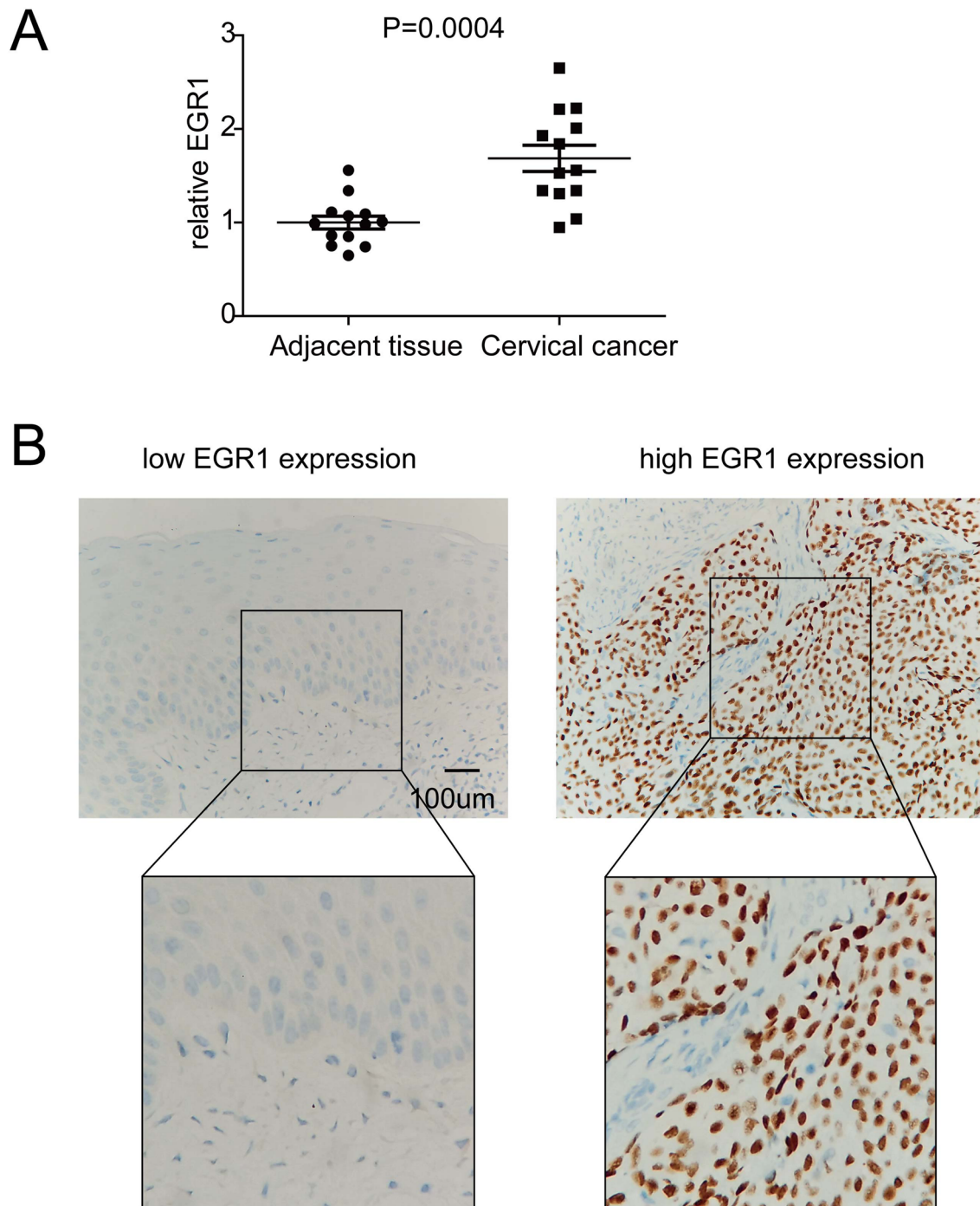


Fig. 1 The expression of EGR1 in CC tissues. **a** The mRNA levels of EGR1 in 13 pairs of CC tissues and their corresponding adjacent tissues were detected with qRT-PCR. The data were from three biological

independent experiments and were analyzed by paired *t* tests. **b** The expression and intracellular location of EGR1 was detected with IHC in 144 CC cases

the hypothesis that EGR1 regulated SOX9 expression. To detect the role of SOX9 in the EGR1-induced progression of CC, we performed a rescue assay by overexpressing SOX9 after knocking down EGR1. SOX9 overexpression

extensively rescued the effect of EGR1 knockdown, indicating that SOX9 was essential in EGR1-induced proliferation and invasion of CC (Fig. 4b, c). By detecting the expression of stemness biomarkers, we proved that

Table 2 The prognostic significance of EGR1

Characters	Univariate		Multivariate		
	5-year OS%	<i>P</i> *	HR	95%	<i>P</i> #
Age					
< 50	71.4	0.001	1		
≥ 50	51.9		6.91	3.28–14.56	0.001
Tumor size					
< 4 cm	68.6	0.769	1		
≥ 4 cm	70.4		1.07	0.54–2.13	0.847
HPV					
Absent	65.4	0.375	1		
Present	70.4		0.64	0.30–1.39	0.262
Histological grade					
I+II	82.9	0.036	1		
III	64.3		1.48	0.93–2.37	0.100
T stage					
I+II	76.7	<0.001	1		
III+IV	36.1		1.34	1.05–3.32	0.003
Lymph node invasion					
No	76.9	<0.001	1		
Yes	35.4		2.52	1.42–8.72	0.003
TNM					
I+II	76.7	<0.001	–	–	–
III+IV	38.1		–	–	
EGR1					
Low	81.1	<0.001	1		
High	40.6		2.44	1.21–4.94	0.013

*Log-rank test

#Cox-regression model

SOX9 overexpression increased CC stemness, which was impaired by EGR1 knockdown (Fig. 4d). To further validate our findings, we overexpressed EGR1 but silenced SOX9 (Fig. 4e). Consequently, SOX9 knockdown almost totally wiped out the EGR1-induced stemness, suggesting that EGR1 facilitated CC stemness by inducing SOX9 expression. Taken together, EGR1 promoted CC proliferation and invasion by inducing CC stemness, which required the participation of SOX9.

SOX9 expression was associated with EGR1 and the OS rate

The expression of SOX9 in CC tissues was detected with IHC. The percentages of low and high SOX9 expression accounted for 64.34% and 35.66%, respectively. The SOX9 protein was mainly localized in the nucleus (Fig. 5a). We

compared the IHC scores of SOX9 in the patients with low or high EGR1 expression and found that high EGR1 was significantly associated with upregulated SOX9 expression in CC patients (Fig. 5b). In the 13 pairs of frozen CC tissues, we analyzed the correlation between SOX9 and EGR1 (Fig. 5c). SOX9 expression was positively associated with EGR1 expression in CC tissues ($P < 0.0001$, $r^2 = 0.7809$). Moreover, we estimated the prognostic significance of SOX9 and demonstrated that high SOX9 expression was a predictive biomarker of poor outcomes of CC ($P = 0.001$). The 5-year OS rates of patients with low or high SOX9 expression were 81.6% and 47.8%, respectively (Fig. 5d).

Discussion

EGR-1 is a transcription factor that mediates several critical processes, such as proliferation, apoptosis and migration, in response to many factors, including hormones, neurotransmitters, hypoxia, fluid shear stress and vascular injury (Pagel and Deindl 2012). EGR1 is a well-known mediator of PI3K/MAPK signaling and is activated by the MAPK cascade. More than 70% of tumors have alterations in PI3K/MAPK (Burki 2017), and aberrant activation of PI3K/MAPK can also induce tumorigenesis and progression in CC (Hu et al. 2020; Zhou et al. 2020). However, the expression and functions of EGR1 in cancer have not been found to be consistent. EGR1 can function as a tumor suppressor or a tumor promoter in a time- and tissue-specific way. EGR1 expression is downregulated in several cancer types, such as breast cancer, but upregulated in other cancer types, such as prostate cancer (Huang et al. 1997; Li et al. 2019). Within the same cancer type, such as breast cancer, both the tumor-suppressing and tumor-promoting functions of EGR1 have supporting evidence (Crawford et al. 2019; Tang et al. 2019; Yang et al. 2016). In CC, the function of EGR1 is still controversial. EGR1 was reported to be upregulated in CC cells (Simpson et al. 1997) but was also reported to be involved in the inhibition of the proliferation and invasion of CC cell lines (Ma et al. 2019). For the first time, in this study, we identified the clinical significance of EGR1 in CC and showed that high expression of EGR1 was predictive of a poor outcome. This is a supplement to CC biomarker studies and suggests that EGR1 detection could help stratify CC patients by their molecular patterns.

EGR1 participates in many physiological processes, such as proliferation, invasion or migration. However, studies on the correlations between EGR1 and cell stemness are relatively rare. Several studies have shown that EGR1 can influence tumor stemness in breast cancer under EGF

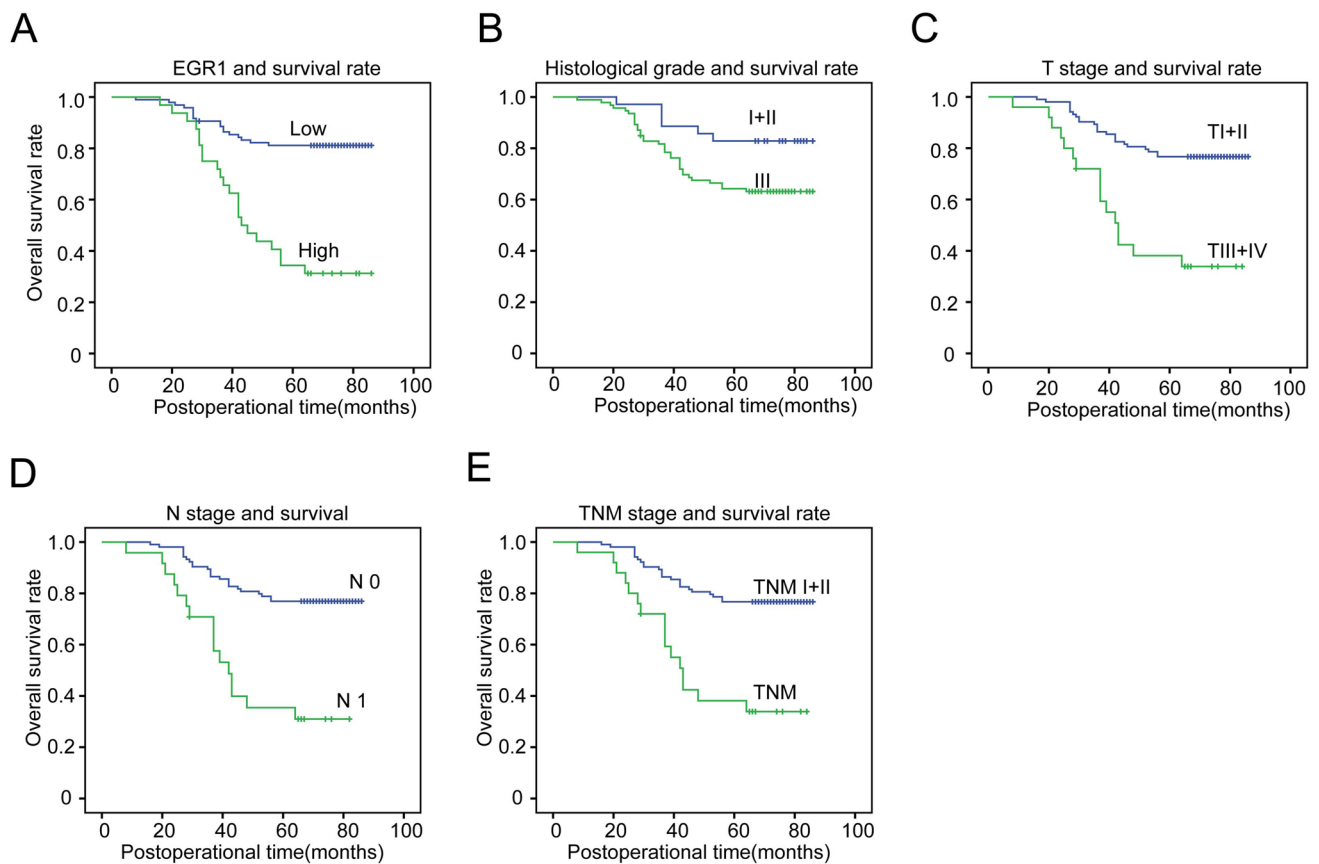


Fig. 2 The survival curves of EGR1 expression and clinicopathological factors. The total cohort was classified according to **a** EGR1 expression, **b** histological grade, **c** T stage, **d** N stage and **e** TNM stage. Data were analyzed with the log-rank test

stimulation and in glioma under PDGF stimulation (Sakakini et al. 2016; Tang et al. 2019). This is the first report on the influence of EGR1 on CC stemness, revealing the underlying mechanism of how EGR1 promotes CC progression and correlates with the T stage and lymphatic invasion of CC. Moreover, our study unveiled a new molecular mechanism of EGR1-induced tumor progression, which was dependent on inducing SOX9 expression. This result may not be specific to CC and may apply to all types of tumors. The EGR1-SOX9 axis may be a common feature of most cancer types, and further experiments are worth performing to validate this hypothesis.

EGR1 is involved in numerous signaling pathways by interacting with other proteins or regulating the transcription of downstream effectors. EGR1 has been demonstrated to interact with CEBPB, CREB-binding protein, EP300,

NAB1, P53, PSMA3, and many other proteins (Liu et al. 2001; Silverman et al. 1998; Zhang et al. 2003). Several studies reported that SOX9 and EGR1 had simultaneous expression changes by applying mRNA sequencing (Bert et al. 2019; Falconi et al. 2016), indicating that they may have regulatory effects on each other, but there is no direct evidence showing that EGR1 can directly induce the expression of SOX9. This is the first report showing that EGR1 regulates CC proliferation and invasion by regulating SOX9 expression, which provides more detail on the oncogenic mechanism of EGR1 because most studies consider EGR1 and SOX9 as parallel downstream effectors of Wnt signaling.

Here, we demonstrated that EGR1 can induce the progression of CC and is correlated with poor outcomes of CC, but several issues are still not clear. For example, what

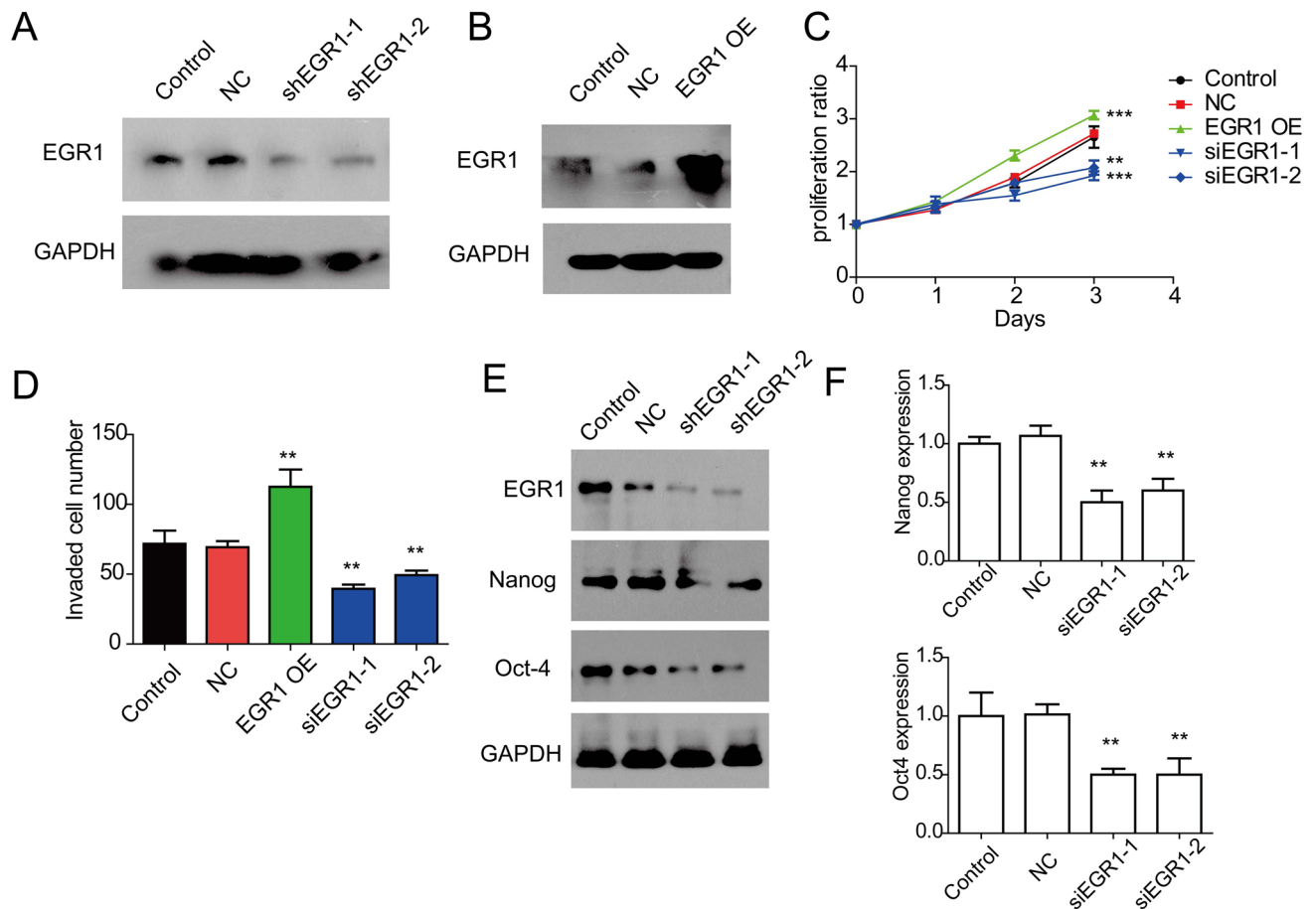


Fig. 3 EGR1 promoted CC cell proliferation, invasion and stemness. **a, b** Successful EGR1 knockdown and overexpression were verified by Western blotting of protein extracted from the HeLa cells. **c** EGR1 knockdown significantly decreased HeLa cell proliferation, while EGR1 overexpression extensively facilitated proliferation. Two different shRNAs of EGR1, or pFLAG-CMV-EGR1, were transfected into HeLa cells 48 h before the CCK-8 assay ($n=8$). $**P<0.01$, $***P<0.001$, with two-way ANOVA. **d** HeLa cell invasion was impaired by EGR1 knockdown and promoted by EGR1 overpres-

sion. Two different shRNAs of EGR1, or pFLAG-CMV-EGR1, were transfected into HeLa cells 48 h before the Transwell assay ($n=6$). $**P<0.01$ compared with the control group, analyzed with one-way ANOVA. **e** EGR1 knockdown decreased the stemness of HeLa cells. HeLa cells were transfected with shEGR1 48 h before the detection of stemness biomarkers Nanog and Oct-4. **f** The quantification of the WB bands in (E) ($n=3$). $**P<0.01$ compared with the control group and analyzed with one-way ANOVA. All experiments were performed in biological triplicates

causes EGR1 over-expression? Which stimulator activates EGR1? How does EGR1 regulate SOX9 expression? All of these issues need further research.

The role of SOX9 in tumor stemness has been revealed gradually in recent years. SOX9 was demonstrated to be

related to stemness in many cancer types, such as breast cancer, osteosarcoma, ovarian cancer, gastric cancer, and esophageal squamous cell carcinoma (Chen et al. 2020; Siu et al. 2019; Wang et al. 2019). Although there is no available small-molecule inhibitor of SOX9, recent studies have indicated that targeting SOX9 may be a potential

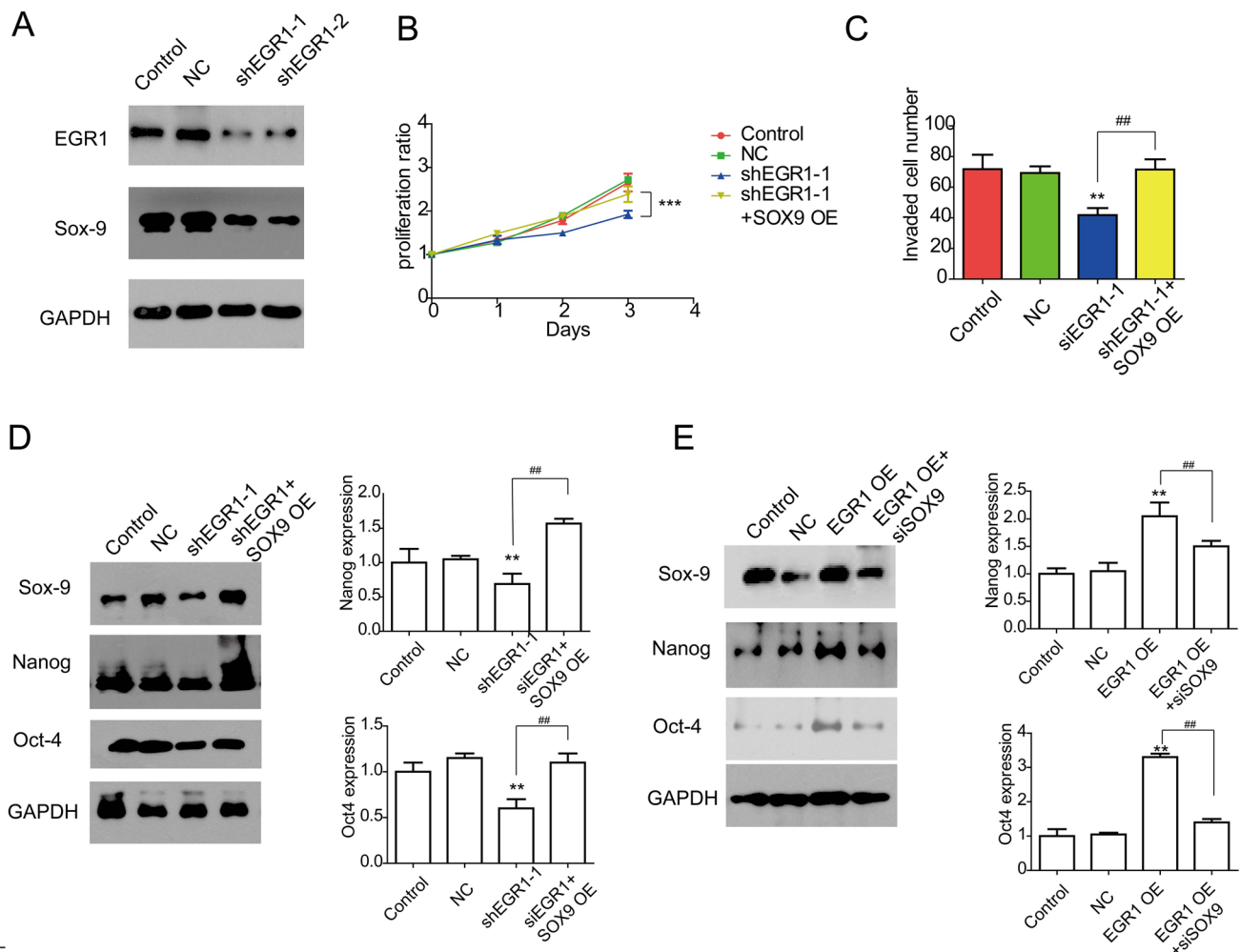


Fig. 4 SOX9 was required for EGR1-induced proliferation, invasion and stemness. **a** SOX9 expression was decreased by EGR1 in HeLa cells. **b, c** SOX9 overexpression rescued the downregulation of proliferation (**b**) ($n=8$) and invasion (**c**) ($n=6$) in HeLa cells, which was caused by EGR1 knockdown. CCK-8 and Transwell assays were performed 48 h after transfection with shEGR1 and/or pFLAG-CMV-SOX9 plasmids. OE represents overexpression. **d** Left panel: EGR1

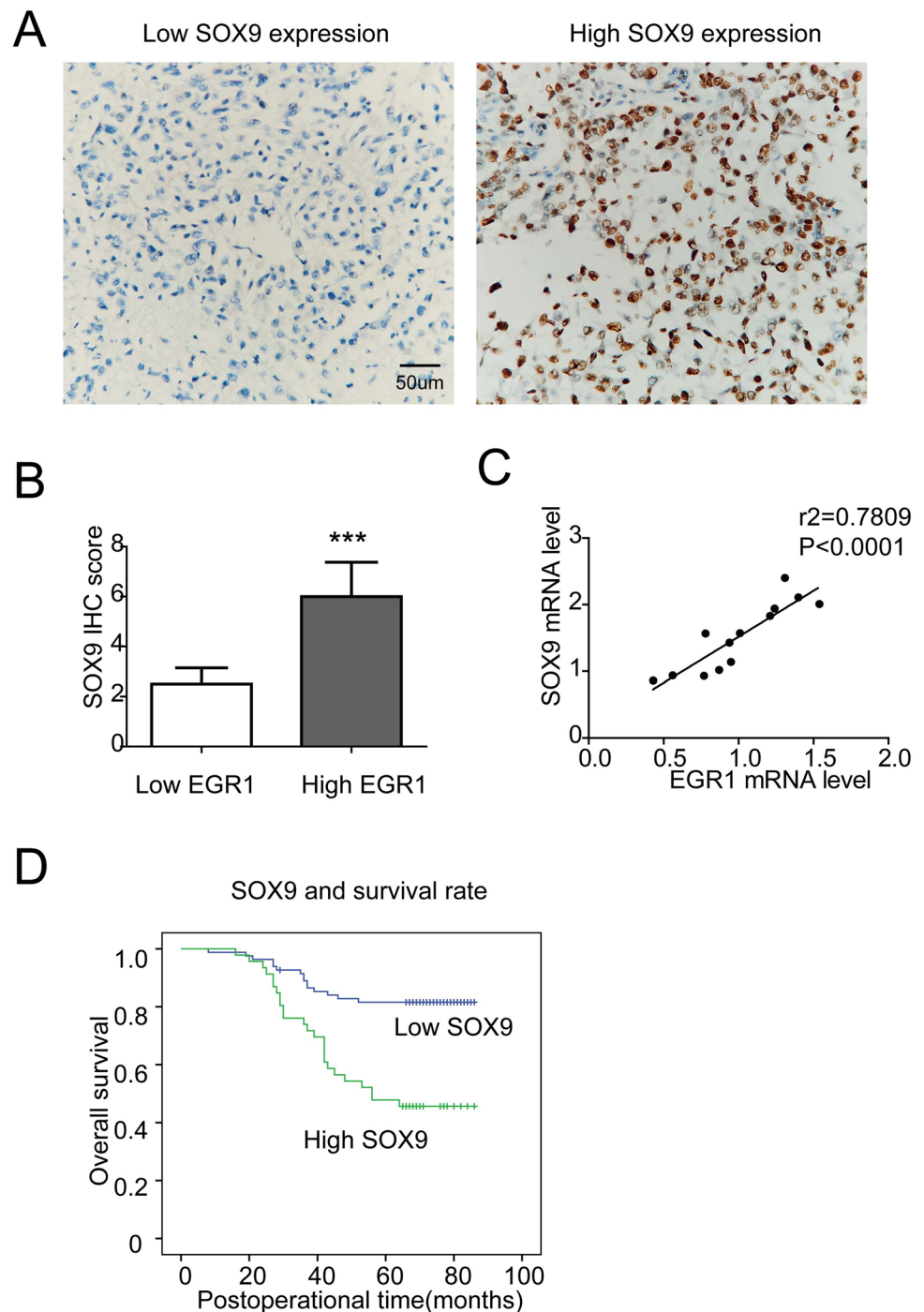
knockdown attenuated HeLa cell stemness, while SOX9 overexpression rescued that tendency. Right panel: the quantification of Nanog and Oct-4 in the left panel ($n=3$). **e** Left panel: EGR1 overexpression facilitated the stemness of HeLa cells, and SOX9 knockdown impaired EGR1-induced stemness. Right panel: the quantification of Nanog and Oct-4 in the left panel ($n=3$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, analyzed with one-way ANOVA or two-way ANOVA

approach to inhibit tumor cell pluripotency and progression. For example, melatonin exhibited tumor-suppressive ability in osteosarcoma stem cells by inhibiting SOX9-mediated signaling (Qu et al. 2018). Our study indicated that EGR1 and SOX9 are potential drug targets of CC and suggested that targeting the EGR1-SOX9 axis may be a possible approach to treat CC.

In conclusion, we demonstrated that EGR1 was an independent prognostic biomarker of CC, and its

overexpression was associated with a poor outcome. EGR1 promoted proliferation, invasion and stemness by elevating SOX9 expression in CC cells. Our results suggested that the EGR1-SOX9 axis may be a potential drug target and that blocking the EGR1-SOX9 axis may be a possible approach to treat CC.

Fig. 5 SOX9 expression was correlated with EGR1 expression in CC tissues. **a** SOX9 expression was investigated by IHC in 144 CC cases, which was then used to divide these patients into low and high SOX9 expression groups. **b** High EGR1 expression was significantly associated with SOX9 expression in CC tissues. IHC scores of SOX9 were substantially higher in patients with high IHC scores of EGR1. **c** In the 13 frozen CC tissue pairs, EGR1 expression was significantly associated with SOX9 expression. qPCR data were from 3 independent biological experiments and analyzed with the Spearman correlation test. **d** High SOX9 expression was significantly associated with lower survival rates of CC among the 144 CC cases. The survival curves were plotted by the Kaplan–Meier method, and the significant differences were analyzed by the log-rank test



Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s13258-021-01064-5>.

Author contributions HL and JZ performed all of the experiments and collected the specimens. MY designed the study, wrote the paper and collected the specimens.

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Compliance with ethical standards

Conflict of interest Author Haixia Li, author Juanhong Zhao, and author Miao Yuan declare that they have no conflicts of interest.

Ethical approval All procedures performed in studies involving human participants were conducted in accordance with the ethical standards of the Women and Children’s Health Care Hospital of Linyi, Hospital of Shandong Medical College, the First Affiliated Hospital of Shandong

First Medical University and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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