Acta Biochim Biophys Sin 2022, 54(2): 243–251
https://doi.org/10.3724/abbs.2021018
Advance Access Publication Date: 23 December 2021
Original Article

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Aberrant expression of the extracellular matrix component Biglycan regulated by Hedgehog signalling promotes colorectal cancer cell proliferation

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Received 2 June 2021 Accepted 14 October 2021

Abstract

Hedgehog (Hh) signalling plays essential roles in regulating embryonic development and contributes to tumour initiation, growth and progression in multiple cancers. The detailed mechanism by which Hh signalling participates in tumour growth warrants thorough study, although several downstream target genes have been identified. Herein, a set of novel targets of Hh signalling was identified in multiple types of tumour cells via RNA-Seq analysis. Among these targets, the expression regulation and oncogenic function of the extracellular matrix component biglycan (BGN) were investigated. Further investigation verified that Hh signalling activates the expression of BGN via the transcription factor Gli2, which directly binds to the promoter region of BGN. Functional assays revealed that BGN facilitates tumour cell growth and proliferation in colorectal cancer (CRC) cells, and xenograft assays confirmed that BGN also promotes tumour growth *in vivo*. Moreover, analysis of clinical CRC samples showed that both the protein and mRNA levels of BGN are increased in CRC tissues compared to those in adjacent tissues, and higher expression of BGN is correlated with poorer prognosis of CRC patients, further confirming the function of BGN in CRC. Taken together, aberrantly activated Hh signalling increases the expression of BGN, possibly regulates the extracellular matrix, and thereby promotes tumour growth in CRC.

Key words RNA-Seq, Hedgehog signalling, biglycan, colorectal cancer

Introduction

Hedgehog (Hh) signalling plays essential roles in regulating embryonic development and cell differentiation and proliferation; therefore, its dysregulation leads to multiple developmental abnormalities [1]. Activation of Hh signalling is initiated by the binding of a ligand to the membrane receptor Ptch, which releases the inhibition of Smo. Smo is then transported to the cell membrane and enters the primary cilium, where the full-length form of the Gli protein is activated and assumes its activated form. After being transported to the nucleus, activated Gli (GliA) recruits transcription cofactors, such as p300/CBP, and initiates the expressions of downstream target genes (Gli1, Ptch1, c-Myc, Bcl-2, etc.) [2].

Aberrant activation of Hh signalling is ubiquitous in tumours, and somatic mutations in Ptch1, Smo and SuFu have been reported to be among the leading causes of basal cell carcinoma and medulloblastoma; however, in colorectal cancer (CRC), it is widely accepted that Hh signalling facilitates tumour maintenance and growth but does not contribute to tumour initiation [3]. Although many target genes in the downstream of Hh signalling have been shown to promote cell proliferation and tumour growth, the detailed mechanism by which Hh signalling initiates or facilitates tumour formation remains unclear. More importantly, whether different tumours with activated Hh signalling share a cluster of functional genes or similar mechanisms requires further study.

Extracellular matrix (ECM) is composed of a lattice-like network of collagen, glycosaminoglycans, proteoglycans, and glycoproteins,

and provides both biochemical and biomechanical contexts within which cancer cells exist [4]. Biglycan (BGN) is a member of the class I family of leucine-rich proteoglycans (SLRPs) that share a 42-kDa core protein. BGN is ubiquitously expressed as a structural component of the ECM, and has been revealed as a key signalling molecule that participates in multiple tumour-associated signalling pathways [5], including the nuclear factor kappa-light-chain-enhancer of activated B cell (NF-κB) pathway [6], the transforming growth factor-β(TGF-β) and bone morphogenetic protein (BMP) pathways [7,8], and the Wnt pathway [9,10]. It has been well established that BGN facilitates angiogenesis by increasing the expression of vascular endothlial growth factor (VEGF) [11] and attenuates apoptosis through the NF-κB signalling in tumour cells [12]. Furthermore, BGN expression has been shown to be upregulated in multiple tumours [5], including melanoma [13], urothelial carcinoma [14] and colorectal cancer [15]. Interestingly, it has been reported that urothelial carcinoma increases BGN levels in the serum [14], which makes BGN a potential diagnostic marker. However, the mechanisms by which BGN expression is upregulated in these tumours remain unknown.

Herein, we found that *BGN* is a novel target gene of Hh signalling, suggesting that aberrantly activated Hh signalling promotes the expression of BGN and facilitates tumour growth and progression through these BGN-regulated cell signalling pathways.

Materials and Methods

Patients and clinical tissue samples

Eight patients diagnosed with primary CRC who underwent surgical resection at the First Affiliated Hospital of Nanchang University were included in the current study. All patients were diagnosed based on the histopathological criteria. All relevant ethical regulations were followed. Clinical samples were collected after an informed written consent was obtained from the participants in accordance with the Ethics Committee requirements at the participating institutes and the World Medical Association's Declaration of Helsinki. All experiments with human tissue samples were approved by the Ethics Committee of the First Affiliated Hospital of Nanchang University (No. 2020-32).

Cell culture and transfection

HT29 was purchased from the American Type Culture Collection (ATCC; Manassas, USA), and HCT-116, H4, and Hep3B cells were purchased from Cell Bank of the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). The H4 cells was cultured in Dulbecco's modified Eagle's medium (DMEM; C11995500BT; Gibco, Grand Island, USA), the Hep3B cells were cultured in Minimum Essential Medium (MEM; C11095500BT; Gibco), and the HT-29 and HCT-116 cells were cultured in McCoy's 5A Medium (16600082; Gibco), all supplemented with 10% FBS (10091148; Gibco) and 1% penicillin/streptomycin (15140-122; Gibco) at 37°C in a humidified 5% CO_2 atmosphere. The cells were grown on coverslips in 35-mm diameter culture dishes until they reached approximately 70%-80% confluence. Then, the cells were transfected with the indicated plasmids utilizing Lipofectamine 3000 (Invitrogen, Carlsbad, USA) according to the manufacturer's instructions.

Hh target gene screening

Lentiviruses carrying GFP (Lv-GFP) or Gli2A (Lv-Gli2A) were purchased from GeneChem Co., Ltd. (Shanghai, China), and HT-29, H4 and Hep3B cells were infected with these lentiviruses. After 72 h of infection, GFP-positive cells were then sorted with a FACSAria SORP Cell Sorter (BD Biosciences, Franklin Lakes, USA). Total RNA was extracted from HT29, H4, and Hep3B cells stably overexpressing GLI2A using Trizol reagent (Invitrogen), and the gene expression profiles were determined via next-generation sequencing (NGS) with Illumina NovaSeq by Novogene Co. Ltd. (Beijing, China), and genes with expression changes over 2 folds were considered differentially-expressed genes (DEGs). Genes with expression changes in over 2 folds in all three cell lines were considered to be commonly regulated genes in the downstream of Hh signalling.

Plasmid construct and RNAi

Plasmid construction was conducted as previously reported [16]. Specifically, the coding sequence of BGN was amplified with the indicated primers, digested with the restriction enzymes *Bam*HI and *Xho*I, and inserted into the empty vector pLVX (Clontech, Mountain View, USA). The *BGN* promoter was amplified from HCT116 genomic DNA via PCR with primers, cut with the restriction enzymes *Nhe*I and *Eco*RV, and inserted into the empty vector pGL4.2 (Promega, Madison, USA). The primers used in molecular cloning are listed in Table 1. *BGN* and *Gli2* were knocked down via short harpin RNA (shRNA) targeting its coding sequence. The sequences are shBGN#1: 5′-GCCATTCATGATGAACGATGA-3′, shBGN#2: 5′-GCCACAACCAGATCAGGATGA-3′ and shGli2: 5′-TCCTGAACATGATGACCTA-3′. The negative control is shCon: 5′-CCTAAGGTTAAGTCGCCCTCG-3′.

Dual-luciferase assay and chromosome immunoprecipitation (ChIP)

Dual-luciferase reporter assay was performed as previously described [16]. Specifically, HCT116 cells were grown to 70% confluence in 24-well plates and transfected in triplicate with 0.2 μg of pGL4.2 or the BGN promoter luciferase reporter and 0.2 μg of the Gli2 expression plasmid or empty vector along with 0.05 μg of pRL-TK for normalization. After 48 h of incubation, the luciferase activity was determined with an illuminometer using a dual-luciferase assay kit (Promega, Madison, USA) according to the manufacturer's instructions. The activity of the pGL4.2-BGN promoter luciferase reporter was normalized to that of the pRL-TK Rluc reporter and compared between HCT116 cells transfected with the Gli2 expression plasmid or empty vector.

ChIP was conducted as described previously [17]. Briefly, HCT-

Table 1. Sequence of primers used in plasmid construction

	Primer name	Sequence (5′→3′)
Cds	BGN-*Bam*HI-pLVX-f	GGATCCGGATCCATGTGGCCCCTGTGGCGC
	BGN-*XhoI*-pLVX-r	CTCGAGCTCGAGCTTTTTGTAGTTGCC
Promoter	BGN-prmt-*NheI*-4.2-f	GCTAGCGCTAGCGTCAGGCTTCGCTGCCTGTCCCGCT
	BGN-prmt-*Eco*RV-4.2-r	GATATCGATATCCCCATTCTGAGTCCCAGTCC

116 cells stably expressing Gli2A were grown to 90% confluence and cross-linked with 1% (v/v) formaldehyde in PBS, followed by ChIP as described previously. The fragments were mixed with anti-Gli2 antibody (sc-271786; Santa Cruz Biotechnology, Santa Cruz, USA) and protein A-agarose beads (11134515001; Roche, Palo Alto, USA) to enrich DNA fragments bound to Gli2 via immunoprecipitation. Normal mouse immunoglobulin (IgG; sc-2025; Santa Cruz Biotechnology) was used as a control for the ChIP assay. The ChIP PCR primer sequences are listed in Table 2.

Western blot analysis and real-time qPCR

Western blot analysis and real-time qPCR were conducted as previously described [16]. Specifically, an antibody recognizing Gli1 was purchased from Cell Signalling Technology (cat: L42B10; Beverly, USA), an antibody recognizing Gli2 was purchased from Santa Cruz (cat: sc-271786), an antibody recognizing BGN was purchased from Abcam (cat: ab58562; Cambridge, UK), and an antibody recognizing GAPDH was purchased from Millipore (cat: MAB374; Billerica, USA). Total protein was extracted from the cells or tissues using lysis buffer (0.5% Lubrol-PX, 50 mM KCl, 2 mM $CaCl_2$, 20% glycerol, 50 mM Tris-HCl, pH 7.4, containing 1% protease inhibitor cocktail), and the relative levels of the indicated proteins were analysed by western blot analysis using the indicated antibodies. For qPCR, total RNA was extracted from the cells or tissues using Trizol reagent, and 1 μg of total RNA was used for reverse transcription. An ABI StepOne Plus detection system (Applied Biosystems, Foster City, USA) was used to perform real-time qPCR. The sequences of the primers used to detect the target gene are shown in Table 3.

Colony formation assay

HCT116 cells were seeded into a 6-well plate at 500 to 2000 cells/well, and cultured in DMEM supplemented with 10% FBS for approximately 2 weeks. Then, after the cell colonies were fixed with 4% paraformaldehyde and stained with crystal violet, the colonies were photographed with Epson Perfection V800 (Epson, USA) and quantified using ImageJ software (National Institutes of Health, Bethesda, USA).

Table 2. Sequences of primers used in ChIP analysis

Primer name	Sequence (5′→3′)
BGN-ChIP-1-f	TGCCGAAAGACAAGCCATCT
BGN-ChIP-1-r	TGGCACCTTGACCAAACTGT
BGN-ChIP-2-f	ACAGTTTGGTCAAGGTGCCA
BGN-ChIP-2-r	CTGAGGGTCTTCAACCCCTG
BGN-ChIP-3-f	CGTACTAAGGACCTGGGCTC
BGN-ChIP-3-r	GGTCCCCTCGGACATGAGA
BGN-ChIP-4-f	GCAGGCTCAGGACCAAATTC
BGN-ChIP-4-r	CAGCAAAGGAACGGACACAC
BGN-ChIP-5-f	TTTGCCAATGGCCCTGTTTC
BGN-ChIP-5-r	AGTCTTTCAGCCCATGCCAA
BGN-ChIP-6-f	TTAGTTTCCCAGGCACACCC
BGN-ChIP-6-r	CTTCCCCTCCACACCCATTC

Table 3. Sequences of primers used in qPCR

Primer name		Sequence (5′→3′)
BGN	BGN-rt-f	GAGACCCTGAATGAACTCCACC
	BGN-rt-r	CTCCCGTTCTCGATCATCCTG
Gli2	Gli2-rt-f	CCACCACCTCACCCAGTCCA
	Gli2-rt-r	CAAAGCCTGCTGTAGCCACCC
Ptch1	Ptch1-rt-f	GGGTGGCACAGTCAAGAACA
	Ptch1-rt-r	GGTCGTGGTGGTGAAGGAA

Immunohistochemistry (IHC)

The excised clinical samples were fixed in a 10% neutral buffered formalin solution, dehydrated and embedded into paraffin wax blocks. Embedded-tissues from human CRC samples or mouse xenograft samples were cut into 3-μm-thick sections, mounted onto slides, and processed for histopathological evaluation. All samples were stained with hematoxylin and eosin (H&E) and immunohistochemistry procedures were performed as described previously [17]. Briefly, the tissues were de-paraffinized, rehydrated, and treated with 3% hydrogen peroxide to block endogenous peroxidase activity. Then, the tissues were treated with EDTA (pH 9.0) and heated in a microwave for 45 min. Following a standard antigen retrieval protocol, the slides were incubated with the appropriate primary antibody BGN (ab58562; Abcam), or Ki-67 (9449S; Cell Signalling Technology, Beverly, USA) overnight at 4°C in a humidified chamber. Subsequently, the slides were rinsed with PBS and incubated for 30 min at 37°C with appropriate biotinylated immunoglobulins (PV-6000; Zhongshan Biotechnology, China). Then a Polink-2 HRP DAB detection kit (ZLI-9018; Zhongshan Biotechnology) was used to visualize the immunoreactivity. A negative control was set up in each case with normal IgG. IHC images were captured using a FSX100 microscope equipped with a digital camera system (Olympus, Tokyo, Japan).

Subcutaneous xenograft assay

For *in vivo* experiments, 2×10^7 HT-29 cells stably expressing BGN or control cells were digested by trypsin, resuspended in sterile PBS (200 μL) and then injected subcutaneously into the flanks of 5-week-old female BALB/c-nu athymic nude mice (SLAC Laboratory Animal CO. Ltd, Shanghai, China). Subcutaneous tumour formation was observed from 6 days post-injection. Tumour sizes were measured every 2 days using Vernier callipers. Tumour volume was calculated with the formula: Tumour volume = (length × width^2)/2. At 18 days after injection, tumours were harvested for immunohistochemistry and western blot analysis. Protocols for animal experiments were approved by the Ethical Committee of the First Affiliated Hospital of Nanchang University and conformed to the guidelines of the National Institutes of Health on the Ethical Use of Animals. All surgeries were performed under sodium pentobarbital anaesthesia, with minimized suffering.

Statistical analysis

Data are presented as the mean ± SD of at least three independent experiments. Pearson's correlation test was used to analyse the correlation of the expression level of BGN and Gli1 or Gli2 in CRC samples, and $P < 0.05$ was considered statistically significant. Paired or unpaired two-tailed Student's *t*-test was used to assess the statistical significance of the differences between two different groups of quantitative data. $P < 0.05$ was considerd statistically significant. ANOVA was used to analyse multiple level comparisons, and $P < 0.01$ was considered statistically significant.

Results

BGN expression is positively regulated by Hh signalling

To screen the common target genes of Hh signalling, HT-29 CRC cells, H4 glioma cells, and Hep3B hepatocellular carcinoma cells constitutively expressing activated Gli2 (Gli2A) were generated, and the gene expression profiles of these cells were determined via RNA-Seq. Among the DEGs, 211 genes responded to Gli2A in all three cell lines (Figure 1A and Supplementary Table S1) and were then defined as common target genes of Hh signalling. As shown in Figure 1B, BGN, whose expression was dramatically upregulated by 3–20-folds in response to Gli2A, attracted our interest. Moreover, in the CRC cohort of TCGA dataset, the expression of BGN showed a correlation with that of both Gli1 and Gli2 (Figure 1C,D and Supplementary Table S2), further indicating that the expression of BGN may be regulated by Gli transcription factors in the downstream of Hh signalling.

Gli2 elevates BGN transcription

Although it is well established that BGN plays an essential role in tumorigenesis, the potential mechanism underlying the regulation of its expression remains poorly understood [5]. The findings described above showed that BGN is a potential target gene of Hh signalling; thus, we further investigated whether Hh signalling regulates BGN expression.

The expression level of BGN was then validated by qPCR, and the results showed that Gli2A elevated the mRNA level of BGN in HT-29 cells (Figure 2A). Furthermore, activating Hh signalling by N-Shh treatment also increased both the mRNA (Figure 2B) and protein levels (Figure 2C) of BGN in HT-29 cells. In contrast, inhibition of the Gli transcription factor via treatment with GANT61 decreased the mRNA level of BGN in HCT-116 cells (Figure 2D). Moreover, depletion of Gli2 via RNAi led to a dramatic reduction in both the mRNA and protein expression levels of BGN (Figure 2E,F), indicating the regulatory function of Gli2 in BGN expression.

To further determine whether Gli2 activates the transcription of BGN directly, Gli binding sites (GBSs) in the regulatory region of BGN were predicted via Genomatix (https://www.genomatix.de/), and 6 potential BGSs were identified (Figure 3A,B). The promoter region of BGN (from −1557 to + 949) was then cloned into pGL4.2 to generate the pBGN-Luc reporter. A dual-luciferase reporter assay revealed that ectopic Gli2 expression markedly increased the luciferase activity induced by the BGN promoter in HCT-116 cells (Figure 3C), and this regulatory effect was dose-dependent (Figure 3D). Moreover, ChIP was conducted, and the results revealed that 3 GBSs (#1, #2 and #5) might mediate the binding of Gli2 to the BGN promoter (Figure 3E). These results further confirmed that *BGN* is a novel target gene of Hh signalling that is directly regulated by Gli2.

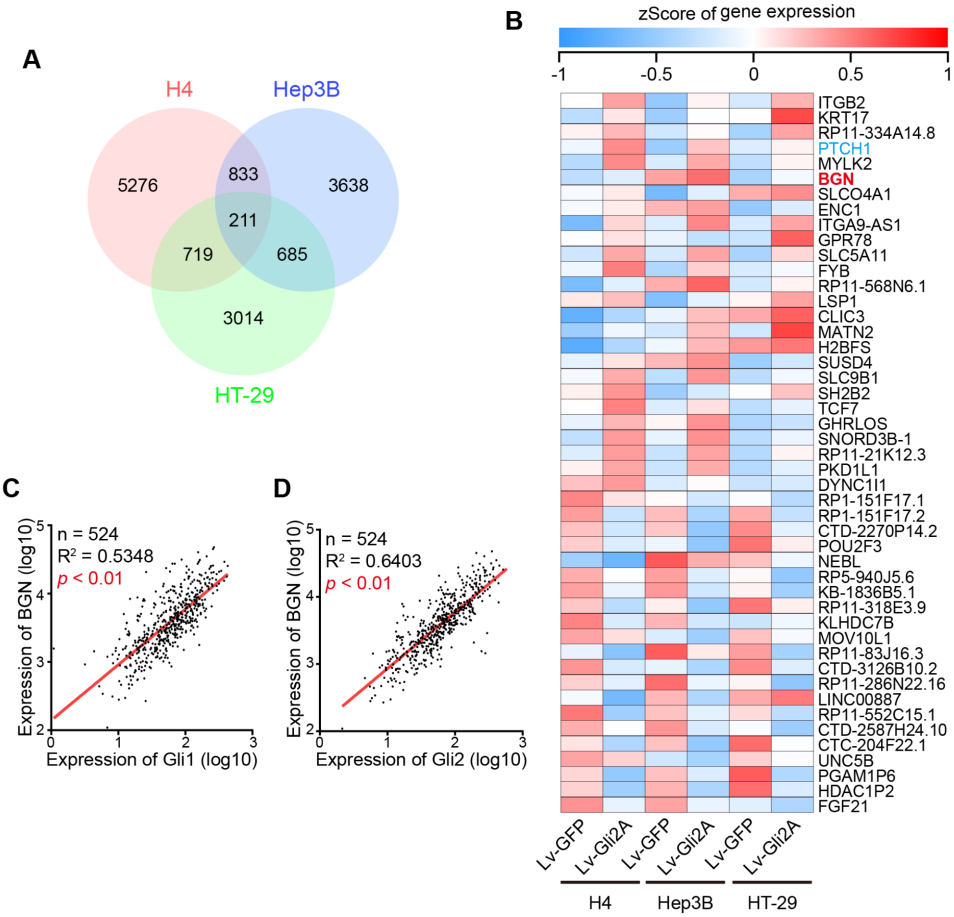

Figure 1. *BGN* is a novel target gene of Hh signalling (A) Venn diagrams showing the numbers of differentially-expressed genes (DEGs) in HT29, Hep3B and H4 cells ectopically expressing GLI2A. (B) Heatmap presenting the expression of commonly altered genes in response to Hh signalling. The Z score of expression is graded by colour. (C,D) Correlation between BGN and Gli1 (C) or Gli2 (D) mRNA expression in TCGA colon cancer cohort (n = 524).

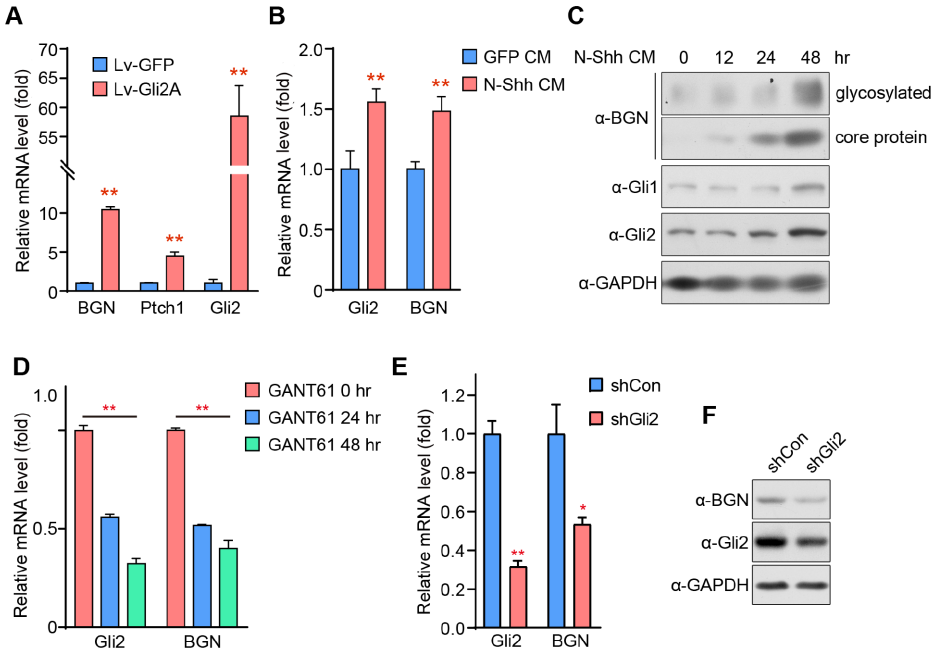

Figure 2. Hh/GLI2 activates BGN transcription (A) mRNA levels of BGN in HT-29 cells with or without stable Gli2A expression as determined by qPCR. (B,C) mRNA levels (B) and protein levels (C) of BGN in HT-29 cells treated with GFP- or N-Shh-conditioned medium (CM) as determined by qPCR or western blot analysis respectively. (D) mRNA levels of BGN in HCT-116 cells treated with or without the Gli inhibitor GANT61. (E,F) mRNA levels (E) and protein levels (F) of BGN in HCT-116 cells with stable Gli2 knockdown or control HCT-116 cells as determined by qPCR or western blot analysis respectively. $^{**}P < 0.01$.

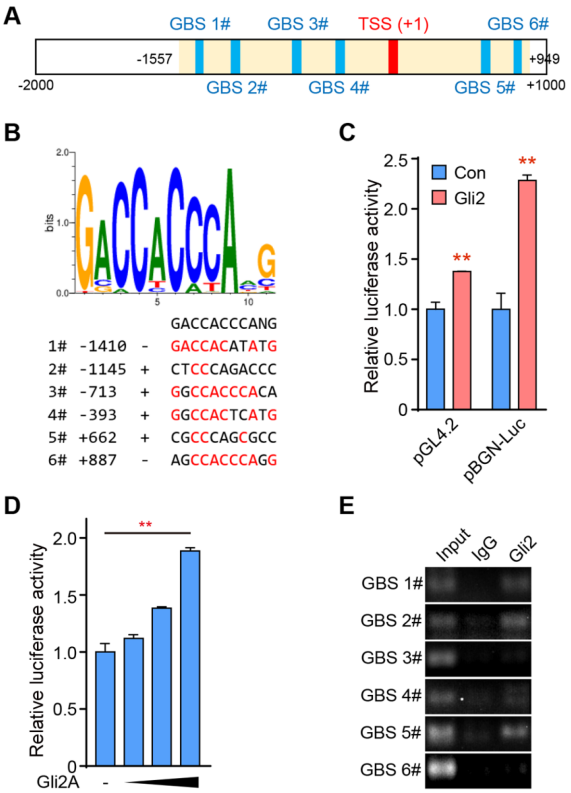

Figure 3. GLI2 direct binds to BGN promoter (A,B) Six potential Gli binding sites were identified in the promoter region of BGN. (C,D) Transcription activity of the BGN promoter region in HCT-116 cells ectopically expressing Gli2 (C) or Gli2A (D) was determined by dual-luciferase reporter assays. (E) Three potential Gli binding sites were validated via ChIP analysis. Data are shown as the mean ± SD ($n = 3$). $^{**}P < 0.01$.

BGN promotes colon cancer cell proliferation

BGN participates in regulating multiple tumour-associated pathways; therefore, we investigated its effect on CRC cell proliferation.

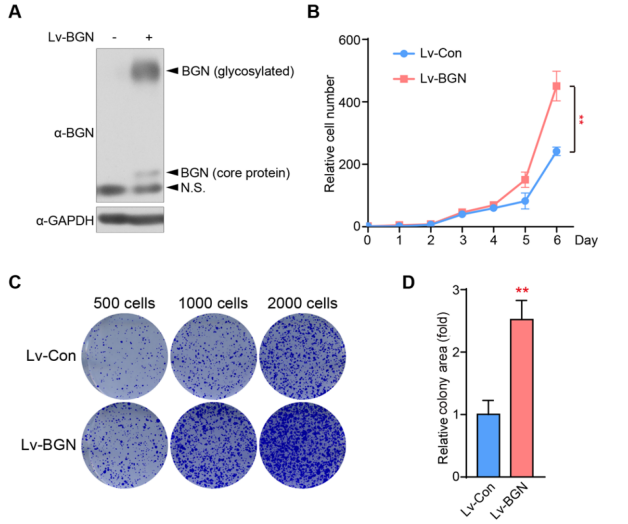

Figure 4. BGN promotes CRC cell proliferation (A) Validation of BGN expression in HCT116 cells stably expressing BGN determined by western blot analysis. N.S., nonspecific bands. (B) Growth curves of HCT-116 cells with or without ectopic BGN expression. (C) The proliferative ability of HCT-116 cells with or without stable BGN expression was assessed by colony formation assay. (D) Quantitative analysis of Figure 4C was performed using ImageJ software. $^{**}P < 0.01$.

HCT-116 cells were infected with lentivirus carrying *BGN* to generate stable cell lines expressing BGN (Figure 4A). The numbers of cells were counted every day to generate a cell growth curve, and the results showed that BGN expression increased the proliferation of HCT-116 cells (Figure 4B). Consistently, a colony formation assay also confirmed that the expression of BGN led to a dramatic increase in tumour cell growth (Figure 4C,D).

To further investigate the function of BGN in CRC cells, HCT-116 cells with *BGN* knockdown were constructed (Figure 5A,B). Both the growth curve and colony formation assay indicated the suppressive effect of BGN depletion on CRC cell proliferation (Figure 5C–E).

Moreover, a xenograft model was established to reveal how BGN affects tumour growth *in vivo* by employing HT-29 cells stably expressing BGN (Figure 6A). The results revealed that BGN expression led to rapid tumour growth, with a dramatic increase in both tumour size (Figure 6A,B) and tumour weight (Figure 6C). The protein expression in these tumours was determined via both western blot analysis (Figure 6D) and IHC (Figure 6E), and the results showed that Ki67-positive cells were increased in BGN-expressing tumours, indicating that BGN can promote cell proliferation and tumour growth *in vivo*. Collectively, these results demonstrated that BGN plays key roles in the proliferation of colon cancer cells.

BGN expression is abnormally elevated in colon cancer tissues

The expression profile of BGN in the CRC cohort of TCGA dataset indicated that BGN is highly expressed in CRC tissues (Figure 7A), and high expression of BGN is correlated with the poor prognosis of

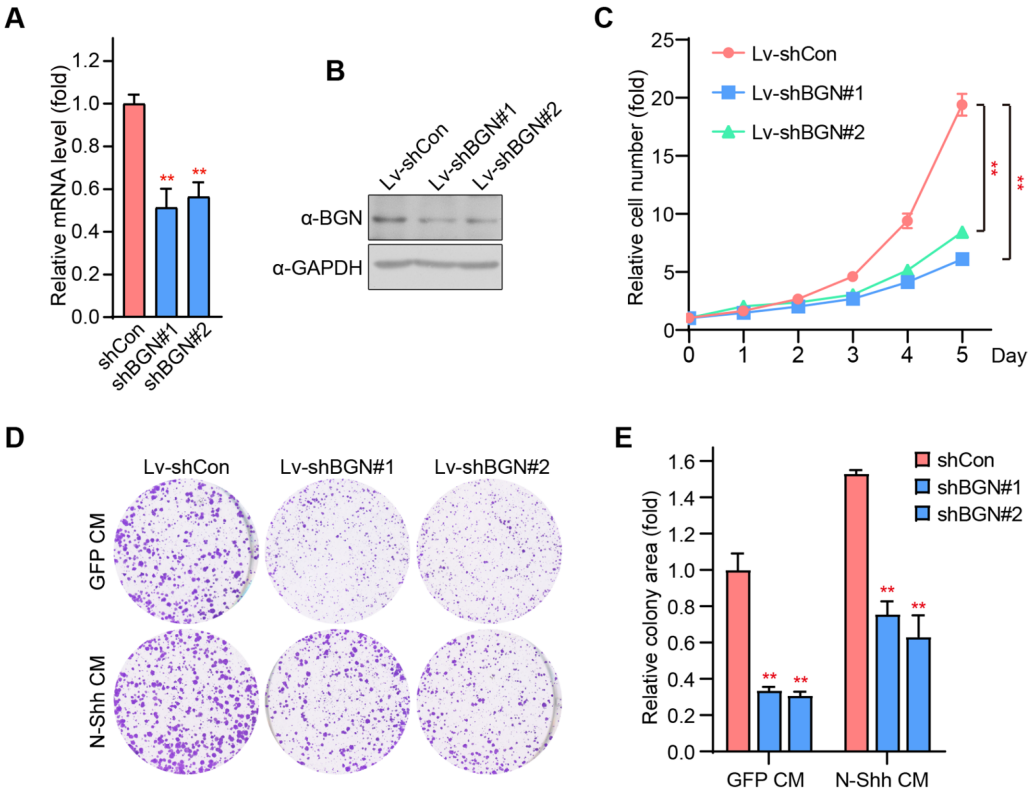

Figure 5. Depletion of BGN attenuates CRC cell proliferation (A,B) Validation of BGN expression in HCT116 cells with stable BGN knockdown by qPCR (A) or western blot analysis (B). (C) Growth curves of HCT-116 cells with or without BGN knockdown. (D) The proliferative ability of HCT-116 cells with BGN knockdown or control cells and treated with GFP or N-Shh was assessed by colony formation assay. (E) Quantitative analysis of Figure 5D was performed using ImageJ software. $^{**}P < 0.01$.

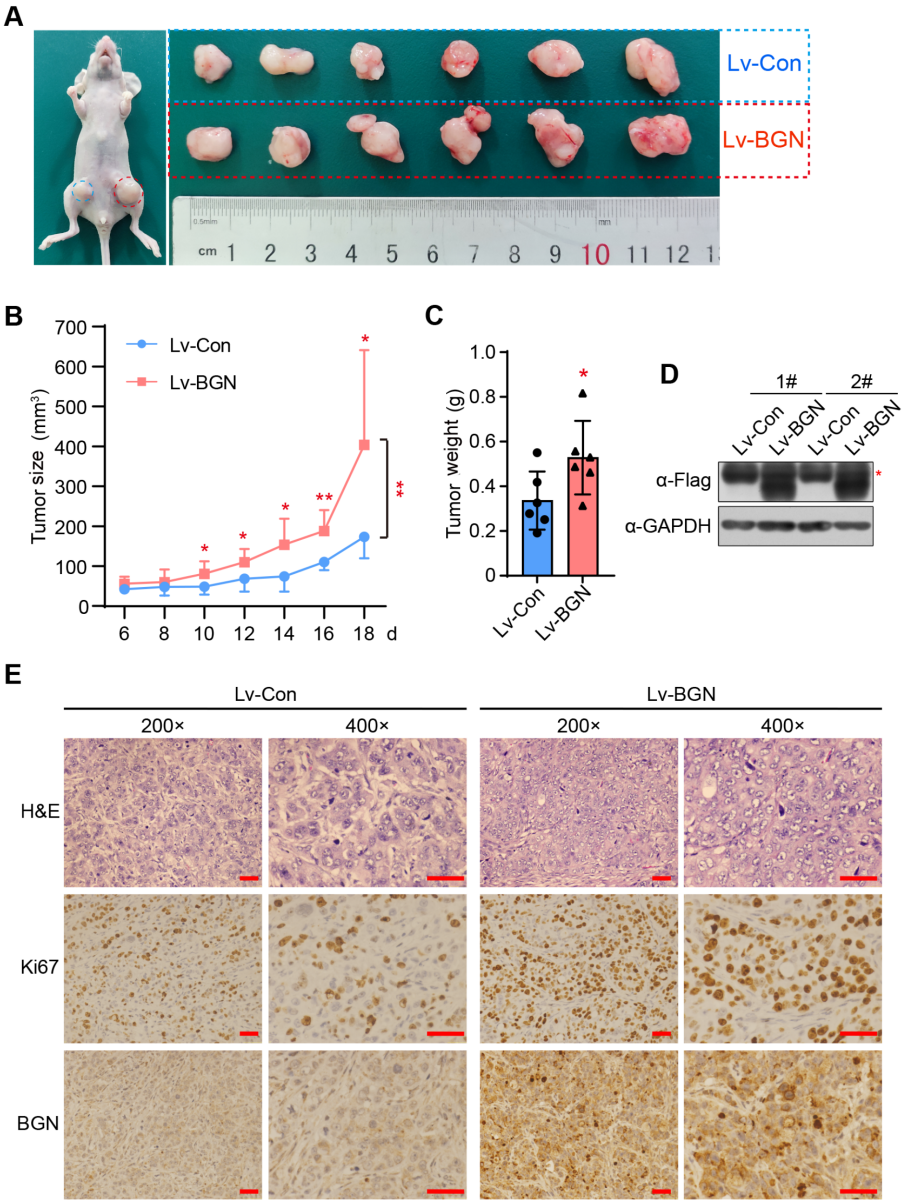

Figure 6. BGN increases tumour growth *in vivo* (A) HT-29 cells (2×10^7 cells) expressing BGN or control cells were subcutaneously injected into nude mice on each side of the inguinal region ($n = 6$ in each group). (B) Tumour sizes on either side were monitored every other day. (C) Tumour weight was measured at the end of experiment. Data are presented as the mean ± SD ($n = 6$). *$P < 0.05$, **$P < 0.01$. (D,E) Protein levels of BGN or Ki67 in randomly selected tumours were determined via western blot analysis (D) and IHC (E). Scale bar: 40 μm.

CRC patients (Figure 7B). Therefore, we further verified its expression in CRC. The mRNA levels of BGN in 8 pairs of randomly chosen CRC samples together with their adjacent tissues were determined by qPCR, and the results confirmed that the mRNA level of BGN was elevated in tumour samples compared with that in adjacent samples (Figure 7C). The protein level of BGN was also investigated, and as shown in Figure 7, both the IHC (Figure 7D) and western blot analysis (Figure 7E) results revealed that the protein levels of BGN were upregulated in most CRC tissues. Taken together, these results demonstrated that BGN could be a prognostic biomarker of human colon cancer.

Discussion

Aberrant activation of Hh signalling has been observed in many tumours, including CRC [18], hepatocellular carcinoma [19], and glioma [20], indicating that Hh signalling may play an important role in the progression of these tumours. Although many studies have identified a series of downstream target genes of Hh signalling, few studies have examined the common target genes of Hh signalling in different tumours. In our study, ectopic expression of Gli2A in HT-29 CRC cells, Hep3B hepatocellular carcinoma cells and H4 glioma cells was found to activate genes downstream of Hh signalling, and 211 DEGs were identified as common target genes of Hh signalling in all three tumour cell lines. As these genes respond to Hh signalling regardless of the cell type, they should play essential roles in mediating the downstream cellular function of Hh signalling.

BGN, one of these common target genes, has been reported to

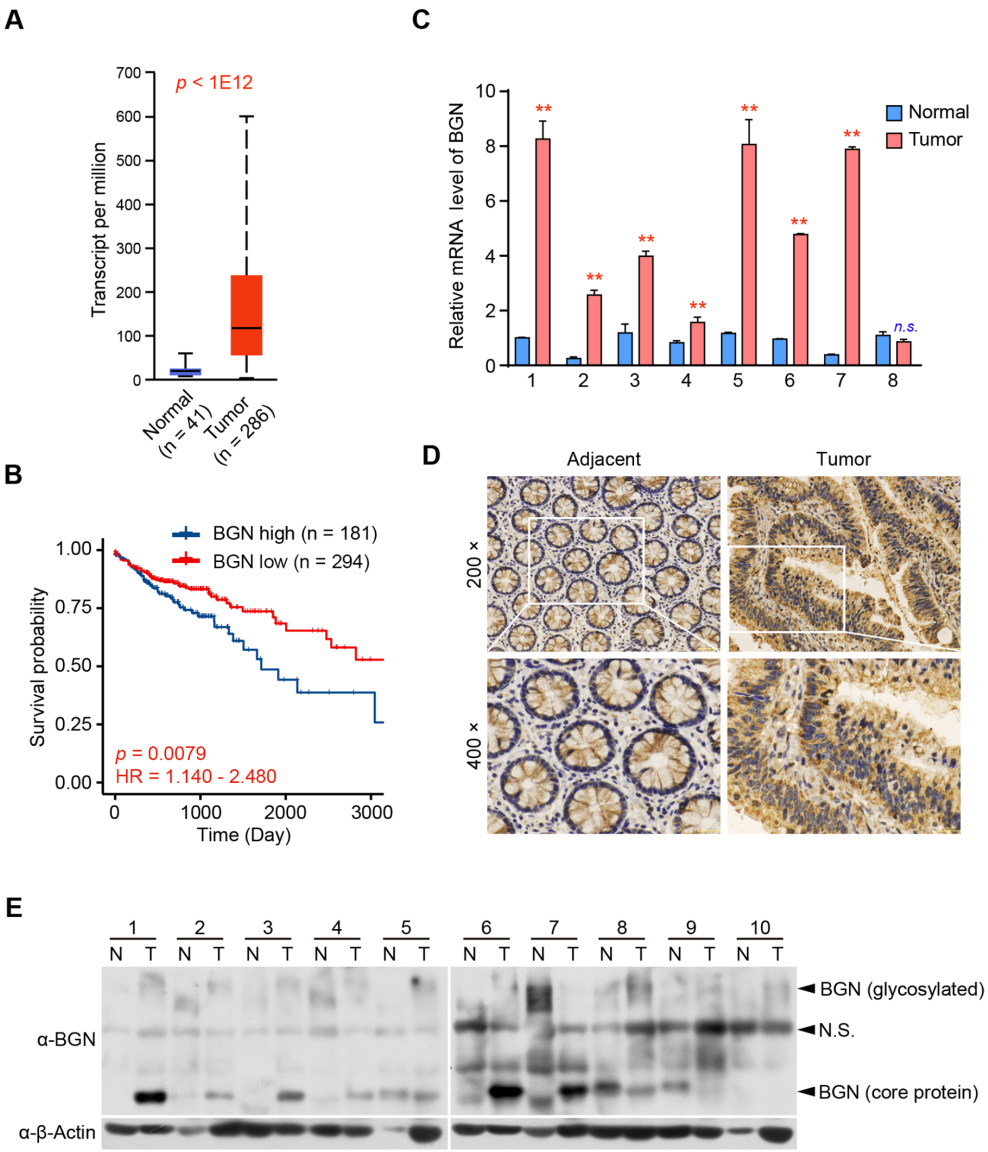

Figure 7. BGN is highly expressed in CRC tissues (A) BGN expression in the CRC cohort from TCGA dataset. (B) Higher expression of BGN was correlated with poorer prognosis of CRC patients. (C) Relative mRNA levels of BGN in 8 pairs of CRC tissues and adjacent tissues were determined by qPCR. (D) Protein levels of BGN in paired CRC tissues and adjacent tissues were determined by IHC. (E) Protein levels of BGN in paired CRC tissues and adjacent tissues (n = 10) were determined by western blot analysis. N.S., nonspecific bands. The data are shown as the mean ± SD (n = 3). $^{**}P < 0.01$; *n.s.*, no significance.

exhibit elevated expression levels in multiple types of tumours [5], although it remains unclear which transcription factor regulates the abnormal accumulation of BGN. In 2004, Heegaard *et al* [21] reported that TGF-β signalling might induce BGN expression via SP1; however, TGF-β/BMP signalling is usually silenced in CRC due to its function as a tumour suppressor [21]. Herein, we further confirmed the elevated expression of BGN in CRC and provided a potential explanation for why aberrantly activated Hh signalling induces the transcription of BGN. Further study revealed that Gli2 directly increases the transcriptional activity of the promoter region of BGN and increases the expression of BGN. Interestingly, we previously reported that the expression of matrix metalloproteinase-7 (MMP-7), another ECM component, is also regulated by Hh signalling [22], which, together with the current findings about the regulation of BGN expression, indicates the essential role of Hh signalling in regulating the tumour microenvironment by increasing the expressions of ECM-regulating proteins.

It has been revealed that BGN facilitates chemoresistance and tumour invasion in CRC cells [12]. Our results suggested that BGN could also increase cell proliferation and tumour growth in CRC. BGN binds to both Wnt ligands and receptors and restrains Wnt3a in cell layers to facilitate Wnt signalling [10]. Indeed, our preliminary results also confirmed that BGN can increase Wnt signalling in CRC cells (data not shown). Considering the oncogenic role of Wnt signalling in CRC, we hypothesized that BGN might promote cell proliferation and tumour growth in CRC by enhancing Wnt signalling, and more evidence should be collected in future studies. In addition, crosstalk between Hh signalling and Wnt signalling is a known but important phenomenon in embryonic development and oncogenesis [23]. Herein, we described a novel signalling commu-

nication axis in which Hh signalling might facilitate Wnt signalling by increasing the expression of BGN, and we will determine the function of this signalling axis during development in future studies.

In summary, we identified *BGN* as a novel target gene of Hh signalling that facilitates cell proliferation and tumour growth in CRC.

Supplementary Data

Supplementary data is available at *Acta Biochimica et Biophysica Sinica* online.

Funding

This work was supported in part by the grants from the National Natural Science Foundation of China (No. 31900559 to M.C., No. 31671476 to S.L., No. 82060517 to L.X., No. 81860491 to Z.Z., and No. 82160507 to H.W.) and the Natural Science Foundation of Jiangxi Province (No. 20181BAB214009 to M.C., No. 20192BAB205061 to Z. Z., and No. 20202BABL206101 to X.C.).

Conflict of Interest

The authors declare that they have no conflict of interest.

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