Original Article

BCL6-SPECC1L: A Novel Fusion Gene in Nasopharyngeal Carcinoma

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Abstract

Background: Nasopharyngeal carcinomas (NPCs) are malignant tumors originating from the lining epithelium of the nasopharynx. Fusion genes have been confirmed to play important roles in the occurrence and development of various malignant tumors, but the role of fusion genes in NPC is poorly understood. We aimed to explore new fusion genes that promote the occurrence and development of NPC. **Methods:** RNA-seq was used to search for interchromosomal translocations in 18 NPC tissues. Polymerase chain reaction (PCR) and Sanger sequencing were applied to verify the presence of BCL6-SPECC1L (BS); quantitative PCR (qPCR) and Western blotting were used to measure the expression level of BCL-6 in NPC cells; MTT and *in vivo* tumorigenesis assays were applied to evaluate the cell proliferation ability; immunofluorescence assays were used to determine the cellular localization of BCL6 and BS; and a luciferase reporter assay was performed to evaluate the ability of BCL6 and BS to inhibit transcription. **Results:** BS was present in 5.34% (11/206) of primary NPC biopsies and 2.13% (1/47) of head and neck cancer biopsies. The expression of BCL6 was downregulated in NPC, and silencing of endogenous BCL6 promoted NPC cell proliferation *in vitro*. Overexpression of BCL6 but not BS inhibited the growth of NPC cells *in vivo* and *in vitro*. Mechanistically, BCL6 localized in the nucleus can inhibit the G1/S transition to suppress the growth of NPC cells. However, after the fusion of BCL6 and SPECC1L, the product cannot localize to the nucleus, and the transcriptional inhibitory function of BCL6 is abolished, eventually abolishing its tumor suppressor effect and leading to the development of NPC. **Conclusion:** BS is a novel fusion gene in NPC that may play an important role in the occurrence and development of this cancer. The clinical significance of the BS fusion gene needs further elucidation.

Keywords

fusion gene, BCL6, SPECC1L, nasopharyngeal carcinoma, RNA-seq

Abbreviations

PCR, polymerase chain reaction; BS, BCL6-SPECC1L; qPCR, quantitative PCR; HNC, head and neck cancer; DLCL, diffuse large-cell lymphoma; Abs, antibodies; HRP, horseradish peroxidase; KSFM, keratinocyte serum-free medium; DMEM, Dulbecco's modified Eagle's medium; WB, Western blotting; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; SPF,

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Introduction

Nasopharyngeal carcinoma (NPC) is a malignant tumor originating from the epithelium of the nasopharynx, with the highest incidence in southeastern China, Southeast Asia, and North Africa.[1] Although great progress has been made in the treatment of NPC as the radiotherapy techniques have improved, approximately 10% of patients experience relapse after treatment. Therefore, it is very important to elucidate the molecular mechanism of NPC and to identify early diagnostic markers and therapeutic targets for this cancer.

In 2011, Weinberg proposed that genomic instability and mutation are hallmarks of cancer that can lead to chromosomal rearrangement and gene fusion.[2,3] Over the past decades, many fusion genes have been identified in leukemia and lymphoma, and it has recently been found that fusion genes are widely present in solid tumors and can serve as diagnostic and therapeutic markers. For example, the TMPRSS2-ERG fusion gene was identified in 33% of prostate cancer specimens[4]; FGFR3-TACC3 was observed in 3.1% of glioblastoma specimens[5]; EML4-ALK was detected in 6.7% of NSCLC specimens[6]; and NAB2-STAT6 was found in 51% of solitary fibrous tumor specimens.[7] However, reports on fusion genes in NPC are limited. In 2013, the fusion genes UBR5-ZNF423 and RARS-MAD1L1 were identified in the NPC cell line C666 by Chung GT et al and were found to greatly promote the malignant behaviors of NPC cells *in vivo* and *in vitro*.[8,9] In 2014, researchers identified the recurrent FGFR3-TACC3 fusion gene in NPC tissues through transcriptome sequencing and polymerase chain reaction (PCR). FGFR3-TACC3 promotes the proliferation of NPC cells by continuously activating fibroblast growth factor kinase, and this effect can be significantly inhibited by the fibroblast growth factor inhibitor PD173074.[10] Therefore, fusion gene may be highly important in the occurrence and development of NPC, a possibility worthy of further exploration.

BCL6 is a transcriptional suppressor reported by most studies to function as an oncogene in several cancer types, such as lymphoma and breast cancer.[11] Many mutations in BCL6 occur in diffuse large-cell lymphoma, all of which are activating mutations.[12] BCL6 was found to activate a malignant transformation process by forming a transcriptional repression complex with BCOR,[13] SMRT,[14] and NCOR[15] to inhibit various tumor suppressor genes, such as TP53[16] and TNFRs. In B-cell lymphoma, various fusion gene forms of BCL6, such as fusions with the chr14q32 and chr22q11 loci, were also found. The fusion forms mainly altered the promoter of BCL6,[17] thus constitutively activating BCL6 expression.

However, a few studies have reported that BCL6 could be used as a tumor suppressor gene. For example, in medulloblastoma, BCL6 was found to inhibit the expression of the Gli1 and Gli2 transcription factors in the SHH pathway at the transcriptional level by recruiting the transcription inhibitor BCOR and deacetylase SIRT1 and forming a complex, thus inhibiting the occurrence and development of neuroblastoma.[18]

In this study, we found that BCL6 functions as a tumor suppressor gene rather than an oncogene in NPC and discovered a novel fusion gene, BS, which causes loss of the DNA-binding domain in BCL6, impairing its tumor suppressor function and thus leading to the nasopharyngeal epithelial carcinogenesis. Our research confirmed that BS may play an important role in the occurrence and development of NPC.

Materials and Methods

Reagents

The reagents used were as follows: mouse monoclonal antibodies (Abs) against FLAG (F1804, Sigma-Aldrich, 1:2000 dilution) and GAPDH (KC-5G4, Kangcheng Biotech, 1:3000 dilution), horseradish peroxidase (HRP)-conjugated goat anti-mouse/rabbit secondary Abs (Fisher Scientific, 1:3000 dilution), an Alexa Fluor 594-conjugated goat anti-mouse Ab (Molecular Probes, 1:2000 dilution), and 4′-6-diamidino-2-phenylindole (DAPI; Molecular Probes).

Cell Culture

NPEC-Bmi1 cells (N2 and N5) and NP69 cells, immortalized nasopharyngeal epithelial cells induced by Bmi-1 and SV40T, respectively, were grown in keratinocyte serum-free medium (KSFM; Invitrogen). All human NPC cell lines (HONE1, CNE1, CNE2, and HNE1) maintained in our laboratory were cultured in RPMI 1640 medium (Gibco) supplemented with 5% fetal bovine serum (FBS; Gibco). HEK-293FT cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Life Technologies) supplemented with 10% FBS. Cells were grown in a humidified 5% CO2 incubator at 37 °C and passaged using standard cell culture techniques.

RNA-seq

A total of 18 NPC samples were obtained from patients, and all related procedures were performed with the approval of the Internal Review and Ethics Board of our hospital (approval number: B2022-412-01). After extracting RNA from 18 NPC tissues, a library was constructed and sequenced by Igenomics Ltd. The raw sequencing data reported in this paper have been deposited in the Genome Sequence Archive of the BIG Data Center at the Beijing Institute of Genomics, Chinese Academy of Science, under accession number HRA000087 (accessible at http://bigd.big.ac.cn/gsa-human). A human genome (UCSC

hg19) was used as the reference genome. Fusion genes were identified using TopHat-Fusion with default parameters.

Identification of the Fusion Gene

Genomic DNA from tumor tissue was extracted with an E.Z.N.A.® Tissue DNA Kit (Omega, D3396-01) according to the manufacturer's instructions, and RNA was extracted with TRIzol and reverse transcribed into cDNA. Then, the 2 extraction products were subjected to PCR followed by agarose gel electrophoresis. Finally, the target band was excised and sent for Sanger sequencing. The primers used for PCR were as follows: BS (genomic level) forward primer, 5′-CAGCGA GAGCCACTCACCACTCTAC-3′; BS (genomic level) reverse primer, 5-GATTTAAAATTGGCTCTACCCCAC C-3′; BS (RNA level) forward primer, 5′-ATGGCCTCGCC GGCTGACAGCTGTA-3′; and BS (RNA level) reverse primer, 5′-TCAGGAACTCATGATGGAAGAGGTG-3′.

Reverse Transcription-PCR (RT-PCR)

BCL6 expression levels in multiple NPC cell lines were determined by RT-PCR, and N2, N5, and NP69 were set as normal control. After extracting RNA with TRIzol, the mRNA was reverse transcribed into cDNA with random primers, quantitative PCR (qPCR) was carried out with SYBR Green I to measure the BCL6 expression level. GAPDH mRNA expression was also measured as the internal reference. The primers used were as follows: BCL6 forward primer, 5′-GGAGTCGAGACATCTTGACTGA-3′; BCL6 reverse primer, 5′-ATGAGGACCGTTTTATGGGCT-3′.

Western Blotting (WB)

Cell lysates were prepared in RIPA lysis buffer (Biosharp, BL504A) with protease inhibitors (Roche, Indianapolis, IN, USA). Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to a polyvinylidene fluoride membrane. The membrane was blocked with 5% PBS-T (25 mM Tris, 0.8% NaCl, 2.68 mM KCl (pH 7.4), and 0.1% Tween 20) and subsequently incubated with an anti-Flag (Sigma-Aldrich, F1804, 1:2000 dilution), anti-BCL6 (Sigma-Aldrich, HPA004899, 1:500 dilution), anti-SPECC1L (SAB, 29423-1, 1:500 dilution), anti-P21 (CST, 2947S, 1:1000 dilution), and anti-p27 (CST, 3686T, 1:1000 dilution) Abs. The membrane was then incubated with an HRP-conjugated secondary Ab (Pierce, Thermo Fisher Scientific Inc., Rockford, IL, USA).

siRNA Transfection

First, we obtained 3 custom siRNAs targeting BCL6 from RiboBio. The sequences of the 2 single siRNA duplexes targeting BCL6 (RIBOBIO, China) were as follows: siBCL6-1, GTCGAGACATCTTGACTGA; siBCL6-2, GAAGAGGCA CGAAGTGATA.

siRNAs were transfected into cells using Lipofectamine RNAiMAX (Invitrogen). Twenty-four hours post-transfection, the interference efficiency was verified using qPCR and WB. Transfected cells were used for cell functional assays.

Plasmid Construction, Retroviral Infection, and Establishment of Stable Cell Lines

The coding sequence of BCL6 was amplified from C666-1 cellular cDNA, and the coding sequence of the fusion gene BS was amplified from fusion gene-positive tissue. The coding sequence was subcloned into the pMSCV vector to generate pMSCV-BCL6 and pMSCV-BS. Retrovirus was produced as described previously.[9] HNE1 and HONE1 cells were infected with retrovirus carrying BCL6, BS, or pMSCV (empty vector). HNE1 or HONE1 cells expressing BCL6, BS, or empty vector were selected with puromycin (Sigma-Aldrich, catalog no. 101-58-58-2) for 7 days after retroviral infection. After 10 days of selection, the stable cell lines were verified, and the resulting cells were cultured in fresh medium.

MTT Assay

To evaluate the cell proliferation ability, cells were seeded in 5 96-well plates. Beginning the next day, one plate was measured every day after 20 μL of MTT solution was added to each well. After 4 h, the culture medium was removed, and 150 μL of DMSO was added. Ten minutes later, the OD was measured at 490 nm. Finally, the growth curve was plotted with time on the horizontal axis and OD on the vertical axis.

In vivo Experiments

Female BALB/c nude mice (3-4 weeks old) were obtained from the Animal Center of Guangdong Province (Guangzhou, China) and housed under specific pathogen-free conditions. A total of 1×10^6 cells in 50 μL of PBS were injected into the thighs of nude mice. After tumors formed, the tumor size was measured every other day, and a tumor growth curve was drawn. All animal experiments were performed in accordance with a protocol approved by our institutional Animal Care and Use Committee (approval number: L102042015050A). The random animal allocation was performed by computer generation of random number.

Immunofluorescence

First, coverslips were placed in a 24-well plate, and a certain number of cells (HONE1-BCL6 or HONE1-BS) were seeded in each well. After the cells grew to a suitable density, the medium was discarded, and the cells were washed with PBS and fixed with precooled 4% paraformaldehyde for 15 min. After lysis with 0.5% Triton X-100 for 10 min and blocking with 5% BSA for 25 min, the cells were incubated with an anti-FLAG primary Ab (F1804, Sigma-Aldrich, 1:200 dilution)

overnight and were then washed with 0.1% PBST 3 times for 5 min each. Then, the cells were protected from light, incubated with a fluorescent secondary Ab for half an hour, and counterstained with DAPI for 5 min. An antifade mounting medium was used to preserve fluorescence, and confocal fluorescence microscopy was used for imaging.

Luciferase Reporter Assay

5X BCL6 reporter plasmid containing the BCL6 binding site was constructed according to a previous study.[19] 5 μg of BCL6 reporter plasmid plus 5 μg(+) or 10 μg(++) of BCL6, BS, or BCL6[1–513] plasmid were transfected into 293 cells. About 48 h later, luciferase activity was detected using a luciferase assay kit (Luc-Pair Duo-Luciferase HS Assay Kit, iGenobio, LF004) according to the manufacturer's instructions.

Statistical Analysis

For the functional assays, data from all experiments that were repeated 3 times are presented as the means ± standard deviations (SDs) as determined by Student's t-test (unpaired, 2-tailed). The sample size was chosen based on the need for statistical power. Differences with $P < .01$ (**) and $P < .05$ (*), as determined by GraphPad Prism 5 (La Jolla, CA, USA), were considered statistically significant.

Results

Discovery and Verification of the BS Fusion Gene in NPC

Transcriptome sequencing was performed on 18 NPC tissues to identify novel fusion genes in NPC, and the characteristics of the corresponding 18 patients are summarized in Supplemental Table 1. After bioinformatics analysis, a new fusion gene, BS, was identified in NPC tissue 271. The fusion occurred at exon 6 of BCL6 and exon 2 of SPECC1L (Figure 1A). Based on the sequencing results, we designed upstream primers in exon 6 of BCL6 and downstream primers in exon 2 of SPECC1L, performed qPCR on cDNA reverse transcribed from the sample, and found specific bands (Figure 1B, left). The fusion gene was confirmed using Sanger sequencing (Figure 1C), and the fusion site was consistent with that indicated by transcriptome sequencing. Thus, the accuracy of the high-throughput sequencing results was verified by Sanger sequencing in the sample.

According to current research, gene fusion may occur at the genomic level due to chromosomal translocation. However, fusion transcripts generated by cis-splicing at the RNA level may be present only at the mRNA level (eg, SLC45A3-ELK4 fusion occurs only at the mRNA level).[19] To identify whether the fusion gene was present at the genomic level, a specific primer was used, and PCR of genomic DNA from the sample revealed specific bands (Figure 1B, right). The results of Sanger sequencing are shown in Figure 1D.

The fusion gene BS was also further identified by qPCR in more samples of NPC, as well as in esophageal squamous cell carcinoma (ESCC) and head and neck cancer (HNC). Finally, we found that this fusion gene was present in 5.34% (11/206) of primary NPC biopsies, 2.13% (1/47) of HNC biopsies, and 0.70% (1/143) of ESCC biopsies. The Sanger sequencing results for these fusion events are shown in Supplemental figure 1. Therefore, this fusion gene may be a multivariant, multicancer, and multilevel fusion gene.

Through ORF analysis of the fusion gene, we found that SPECC1L fusion occurred at exon 2 in the 5′UTR of the gene and predicted that the fusion gene can be translated into 21 new amino acid sequences and then produce terminators. Therefore, this sequence is a new protein sequence. The predicted structural variation map is shown in Figure 1E, and the position of the new 21 amino acid sequence is denoted by "?" in the figure. By comparison with the human genome in NCBI, these 21 amino acids were identified as specific amino acid sequences, and there were no homologous sequences.

BCL6 Functions as a Tumor Suppressor Gene in NPC

BCL6 has a total length of 706 amino acids, and a protein containing amino acids 1-513 is expressed after fusion. The fusion gene is speculated to be a truncation of the BCL6 gene body that lacks the complete DNA-binding region—that is, zinc finger domain—of BCL6, thus without the transcription factor function of BCL6. Therefore, we hypothesized that the effect of BS on the development of NPC does not depend on the transcription factor function of BCL6. Furthermore, we hypothesized that BCL6 plays a tumor suppressor role in NPC and that the fusion gene abolishes this tumor suppressor role, thus promoting the occurrence and development of NPC.

To test this hypothesis, we first used qPCR and WB to detect BCL6 expression in NPC cell lines and found that BCL6 was significantly downregulated in NPC cell lines (Figure 2A and B). Bioinformatics analysis of the NPC microarray data in the NCBI GEO database also revealed that BCL6 expression was downregulated in NPC samples compared with normal control samples (Figure 2C). These results suggested that BCL6 might play a tumor suppressor role in NPC.

Experiments with siRNA-mediated interference with BCL6 expression were carried out to further verify this conclusion. First, 2 siRNAs with knockdown effects were identified by WB (Figure 2D, left; Figure 2E, left), and the MTT assay showed that knockdown of BCL6 promoted the proliferation of the NPC cell lines HNE1 and HONE1 (Figure 2D and E). In contrast, overexpression of BCL6 in HNE1 and HONE1 cells significantly inhibited their proliferation (Figure 3A and B). Then, we carried out *in vivo* experiments in nude mice with the HONE1 cell line overexpressing BCL6. Overexpression of BCL6 significantly inhibited the tumorigenic ability of NPC cells in vivo; for example, reducing tumor proliferation (Figure 2F) and tumor weight (Figure 2G).

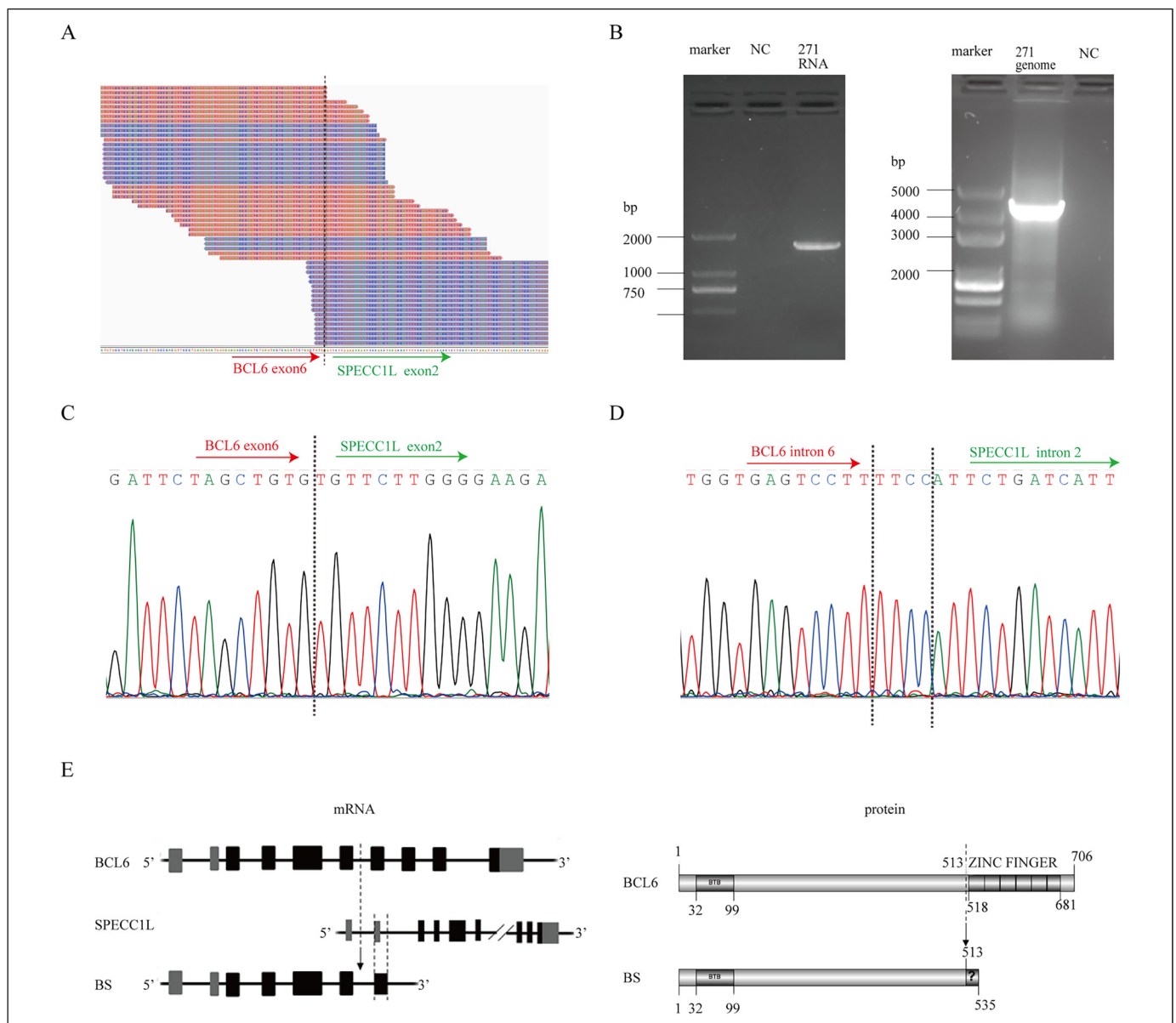In summary, we demonstrated that BCL6 might play a tumor suppressor role in NPC.

Figure 1. Discovery of the BS fusion gene in NPC. (A) Detection of the BS fusion gene using transcriptome sequencing. The reads are aligned across the junctions of the predicted fusion transcripts. (B) The existence of BS at the transcriptional level (left) and genomic level (right) in NPC tissue 271 was confirmed by RT-PCR. (C) Identification of the fusion breakpoint at the transcriptional level using Sanger sequencing. (D) Identification of the fusion breakpoint at the genomic level using Sanger sequencing. The black bar indicates the 4 bp (TTCC) microhomologous region in the junction. (E) RNA structure (left) and protein structure (right) of the predicted fusion. All experiments were repeated 3 times and representative results are shown.

Effect of the Fusion Gene on the Anticancer Effect of BCL6 and the Underlying Mechanism

To explore the influence in the tumor suppressor role of BCL6 after its fusion, we overexpressed BCL6 and its fusion gene in the NPC cell lines HONE1 and HNE1 and found that wild-type BCL6 inhibited the proliferation of these cells, but BS had a significantly weaker ability to inhibit proliferation (Figure 3A and B).

The SPECC1L gene encodes a coiled-coil domain-containing protein that may play a critical role in actin cytoskeleton reorganization during facial morphogenesis. Next, we interfered with the expression of SPECC1L via siRNA transfection in the nasopharyngeal cancer cell lines HNE1 and HONE1 and found that cell proliferation was not affected, ruling out the possibility that the fusion gene promotes the proliferation of NPC cells by affecting SPECC1L (Figure 3C and D).

As mentioned above, the DNA-binding domain of BCL6 was lost after fusion. By immunofluorescence experiments, we found that wild-type BCL6 was localized mostly in the nucleus, while after fusion, BS was localized mostly in the cytoplasm (Figure 3E). The BS fusion could no longer be localized
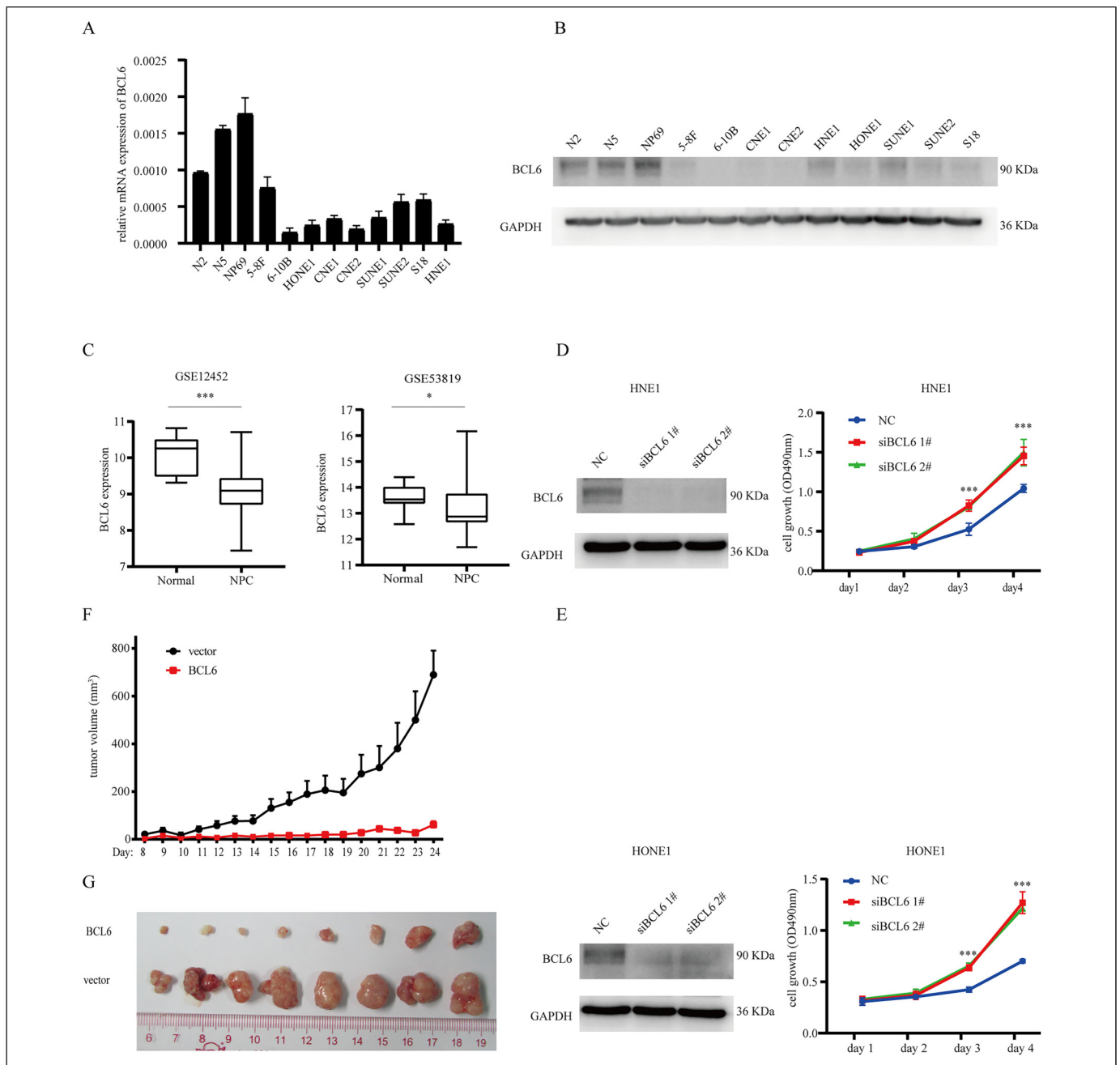
Figure 2. BCL6 is a tumor suppressor gene in NPC. The expression level of BCL6 in various NPC cell lines was measured by qPCR (2A) and WB (2B). (C) In 2 GEO datasets, BCL6 was downregulated in NPC. MTT assays of HNE1 cells (D) and HONE1 cells (E) seeded 24 h after transfection with siRNA and then cultured for the indicated period; the siRNA interference efficiency is shown on the left. $n = 6$ wells per group. * refers to differences between NC and siBCL6-1 or siBCL6-2 (* $P < .05$; ** $P < .01$; and *** $P < .001$ (two-way analysis of variance (ANOVA) followed by the Bonferroni post-hoc test)). Growth curve (F) and images (G) of tumors from nude mice inoculated with HONE1 cells stably expressing BCL6 or pMSCV (vector). All *in vitro* experiments were repeated 3 times and representative results are shown.

in the nucleus, indicating that it did not participate in transcriptional inhibition.

To further confirm that the transcriptional repression ability of the fusion gene is affected, we constructed a 5X BCL6 reporter plasmid containing the BCL6 binding site according to a previous study[19] and carried out a luciferase reporter assay. The BS fusion, like BCL6[1-513], which lacks the DNA-binding domain of BCL6, could not inhibit the transcription of pGL3-5X BCL6, whereas wild-type BCL6 could (Figure 3F). In conclusion, the nuclear entry ability and transcriptional inhibition function of BCL6 decreased after fusion. Since BCL6 acts as a transcriptional repressor and a tumor suppressor in NPC, the generation of the fusion gene ultimately leads to the development of NPC.
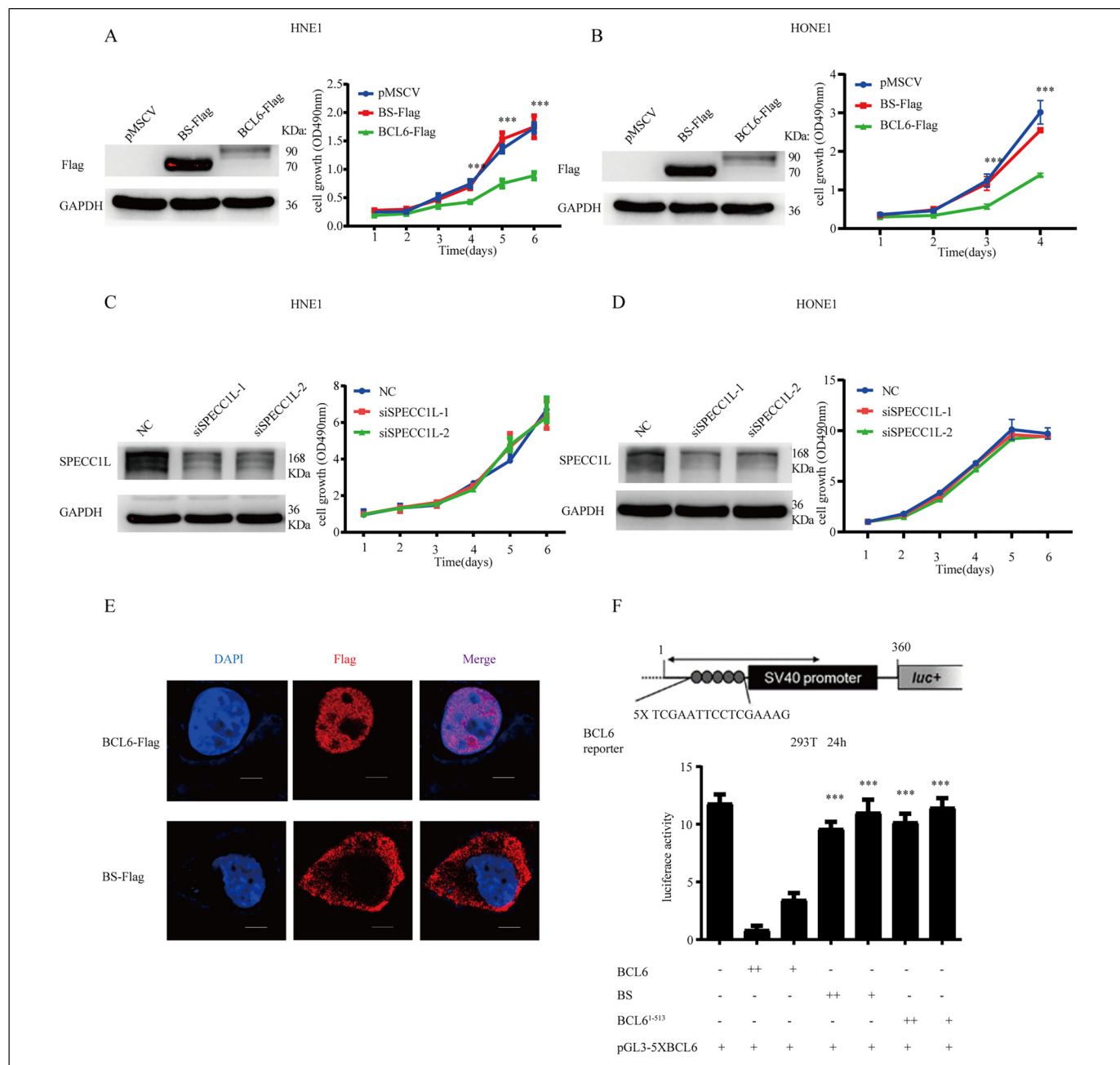
Figure 3. Effect of the fusion gene on the anticancer effect of BCL6 and the underlying mechanism. MTT assays of HNE1 cells (A) and HONE1 cells (B) stably expressing BCL6 or BS. MTT assays of HNE1 cells (C) and HONE1 cells (D) seeded 24 h after transfection with SPECC1L siRNA and then cultured for the indicated period. $n = 6$ wells per group. * refers to differences between vector and BCL6 or BS or between NC and siSPECC1L-1 or siSPECC1L-2 (* $P < .05$; ** $P < .01$; and *** $P < .001$ (two-way ANOVA followed by the Bonferroni post hoc test). (C) Immunofluorescence staining was performed to detect the localization of BCL6 and BS in NPC cells. Nuclei were counterstained with DAPI. (D) The transcriptional inhibition ability of BCL6, BS, and BCL6[1-513] was identified by a luciferase reporter assay. * refers to differences between BCL6 and BS or BCL6[1-513] (* $P < .05$; ** $P < .01$; and *** $P < .001$ (ANOVA followed by the Bonferroni post-hoc test). All experiments were repeated 3 times and representative results are shown.

Anticancer Mechanism of BCL6 in NPC

Through cell and animal experiments, we found that BCL6, a gene that plays an important oncogenic role in lymphoma, functions as a tumor suppressor in NPC; thus, identifying its mechanism in NPC was particularly important. Since the previous results demonstrated that BCL6 greatly affected the proliferation of NPC cells, we examined the expression of proteins in cell cycle-related pathways in cells with stable BCL6 or BS overexpression, or knockdown of BCL6 expression. The expression levels of p21 and p27 were significantly increased
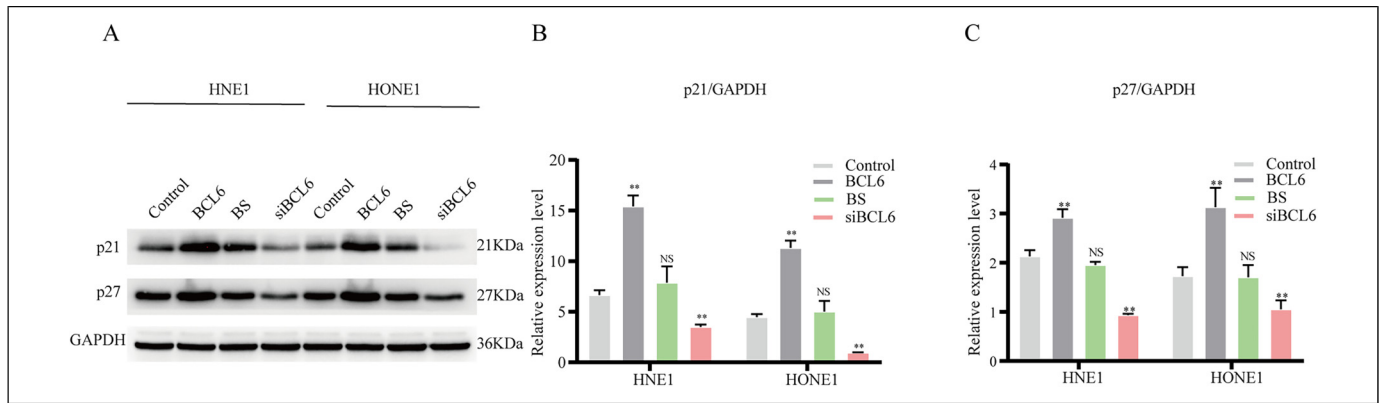
Figure 4. Representative Western blot images showing p21, p27, and GAPDH expression in HNE1 and HONE1 cells with BCL6 or BS overexpression, or with knockdown of BCL6 expression. The experiment was repeated 3 times and representative results are shown (A). The blots were quantified using Image Lab software (Bio-Rad, version 6.1), and paired *T*-test was used for statistical test (B and C).

in BCL6-overexpressing cell lines and significantly decreased after silencing of BCL6 but were not significantly changed after overexpression of the fusion gene BS (Figure 4). These results suggested that BCL6 can block the G1/S transition in NPC cells, thereby inhibiting cell proliferation, but the fusion gene loses this ability.

Discussion

In this study, a novel fusion gene, BS, was identified by transcriptome sequencing. In addition, we found that BCL6 functions as a tumor suppressor gene in NPC and that BS fusion resulted in loss of the DNA-binding domain of BCL6, which changed its cellular localization, thereby abolishing its anticancer function and ultimately promoting the development of NPC. Our results suggest that BS may play an important role in the occurrence and development of NPC.

With the development of high-throughput sequencing technology, an increasing number of fusion genes have been found in various cancers. However, we are the first to discover that BCL6 can fuse with SPECC1L in NPC. We assume that the generation of the fusion gene may be related to the microhomologous sequence, since the sequence denoted by the dotted line in Figure 1D is the sequence shared by intron 6 of BCL6 and intron 1 of SPECC1L. Although microhomologous sequences constitute the basis of DNA repair, they are also a common cause of chromosomal translocation, and several fusion genes have been reported to have similar characteristics.[20] Due to the inclusion of microhomologous sequences, we hypothesized that the fusion gene BS might be present in multiple samples. Therefore, we expanded the scope of detection to detect the presence of BS in other NPC tissues, ESCC tissues, and HNC tissues and found that BS was present in 5.34% (11/206) of primary NPC biopsies, 2.13% (1/47) of HNC samples, and 0.70% of ESCC samples. This finding suggested that the fusion gene may exist in various cancers, such as breast cancer and lung cancer, a hypothesis requiring further verification in more relevant samples.

BCL6, a transcriptional repressor with a zinc finger domain, is well known as an oncogene in lymphoma and has been reported to be an oncogene in breast cancer and other cancers.[21,22] BCL6 was reported to be highly expressed in breast cancer cell lines, which promoted the survival of these cells, while targeting BCL6 with a peptidomimetic inhibitor induced apoptosis in these cells.[21] BCL6 induces EMT by enhancing the expression of the transcriptional repressor ZEB1, which binds to the E-cadherin promoter and inhibits E-cadherin transcription, thereby stimulating EMT in breast cancer cells to promote their invasion, migration, and growth.[22] However, surprisingly, this study indicated that BCL6 is a tumor suppressor in NPC, and the mechanism is worth exploring. It was initially found that BCL6 could inhibit the G1/S transition but could no longer localize to the nucleus after fusion with SPECC1L, resulting in loss of its transcriptional function. Therefore, we suspected that the gene profiles transcriptionally inhibited by BCL6 vary across cancers and that in NPC, BCL6 can transcriptionally inhibit the expression of certain oncogenes in the nucleus. Sarah R. Walker et al found through ChIP-seq that although some BCL6 binding sites in breast cancer cells overlapped with those identified in lymphoma cells, there might be a unique BCL6 binding pattern in breast cancer.[21] However, which genes are specifically regulated by BCL6 in NPC remains to be explored.

The SPECC1L gene encodes a coiled-coil domain-containing protein that may play a critical role in actin cytoskeleton reorganization during facial morphogenesis. A novel fusion gene, RET-SPECC1L, was found in uterine sarcoma tissue from an adult woman, and this fusion enabled the coiled-coil domain of SPECC1L to mediate ligand-independent autophosphorylation of the RET kinase domain, leading to activation of downstream signaling pathways that drive tumor cell proliferation.[23] Another novel SPECC1L-MET fusion was detected in circulating tumor DNA in a patient with lung adenocarcinoma following treatment ith erlotinib and osimertinib.[24] In addition, SPECC1L was found to be fused with ALK in an NSCLC

patient with pretreatment.[25] The above results indicate that SPECCL seems to have a tendency to fuse with other genes, but the underlying mechanisms remains to be clarified.

New proteins expressed by fusion genes may also have their own specific functions. For example, the fusion gene BCR-ABL1, which has been suggested to be the causative mutation of chronic myeloid leukemia (CML), has a new function; specifically, it has extremely strong tyrosine kinase activity. This activity leads to constitutive phosphorylation of a series of signaling proteins, affecting cell proliferation, differentiation, and apoptosis, thus promoting CML. However, the fusion gene that we found did not promote the proliferation of NPC cells after overexpression (Figure 3A and B). Thus, this fusion gene might not have an intrinsic cancer-promoting function.

One limitation of our study is that we did not explore the clinical significance of BS. Detecting the existence of BS in more patient tissues and analyzing its relationships with certain clinicopathological characteristics and the survival prognosis of patients will help to identify new diagnostic and therapeutic markers for NPC. This research focus will be completed in the future.

In conclusion, by performing RNA-seq on 18 NPC tissues, we discovered the fusion gene BS for the first time and confirmed its existence in various cancers. Gene fusion causes BCL6 to lose its inhibitory effect on the proliferation of NPC cells, thereby promoting the occurrence and development of NPC.

Conclusion

BCL6 is a tumor suppressor gene in NPC that loses its tumor suppressor function after fusion with SPECCL. BS is expected to become a new diagnostic marker for NPC. This conclusion warrants further study with more clinical samples.

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Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Approval

All of the tumor tissues were obtained from the patients, and all related procedures were performed with the approval of the Internal Review and Ethics Board of Sun Yat-sen University Cancer Center (approval number: B2022-412-01). All animal experiments were performed in accordance with a protocol approved by Sun Yat-sen University Cancer Center Animal Care and Use Committee (approval number: L102042015050A).

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Supplemental Material

Supplemental material for this article is available online.

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