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BRIEF REPORT

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Modified screening of *MYC* promotor region elements using the CRISPR library in ovarian cancer

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Abstract

Ovarian cancer remains one of the most lethal gynecological malignancies owing to its high recurrence rate and chemotherapeutic resistance. *MYC* is a well-known proto-oncogene that is frequently amplified in ovarian cancer and has been implicated in drug resistance. Previously, we established a new promoter–reporter system combined with a CRISPR activation library to identify unknown *MYC* regulators, and *M1AP* was identified as a novel *MYC* regulator. However, considering the insufficient explanation for the absence of guide RNA (gRNA) of *MYC*, this present study explored methods to prevent the gRNA of *MYC* itself from binding. This study first modified the promoter–reporter vector to improve its quality, then conducted CRISPR screening and analyzed candidate genes as *MYC* promoter regulators using next-generation sequencing in OVSAHO ovarian cancer cells. Eighty-six genes had ≥ 1000 reads, and Pearson's correlation coefficient analysis was performed on the cBioPortal of the Cancer Genomics database. Fourteen genes were identified as candidate *MYC* regulators with positive and significant correlations with *MYC*. Seven genes, including *CYP4v2, ASPH, ANP32D, PCED1A, ABI1, FUZ,* and *HOOK2,* demonstrated significantly higher luciferase activity than the control genes. Four genes, including *ABI1, PCED1A, HOOK2,* and *CYP4v2*, activated the *MYC* promoter, which showed over twofold higher activity than the control when overexpressed using a vector. In conclusion, four genes that activate *MYC* promoters were identified in an ovarian cancer cell line using the CRISPR library system with a modified promoter–reporter tool. These results will prove helpful in the development of novel treatment strategies for ovarian cancer.

Keywords Ovarian cancer, MYC, Oncogene, CRISPR, Luciferase assay

Introduction

The standard treatment for ovarian cancer involves aggressive surgical resection followed by chemotherapy. Ovarian cancer remains one of the most lethal gynecological malignancies worldwide [1] because of its high recurrence rate and resistance to chemotherapy despite advancements in surgical resection and novel therapeutic agents such as bevacizumab and poly ADP-ribose polymerase inhibitors that appeared in the last few decades [2].

MYC is a widely studied proto-oncogene that plays an essential role in the tumorigenesis of many human

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cancers [3, 4]. The Cancer Genome Atlas project analyzed molecular abnormalities in 489 high-grade serous ovarian carcinomas. Copy number analysis showed that *MYC* was frequently amplified in over 20% of tumors, which is the highest frequency among major malignant tumors [5]. Previous studies have suggested that *MYC* hyperactivation is associated with aggressive cancer outcomes [6], and *MYC* has been implicated in cisplatin drug resistance in ovarian cancer [7–9].

As a new therapeutic target in cancer, *MYC* inhibitors have been researched for decades; however, 'direct' *MYC* inhibitors have not been developed due to the complex structure of MYC protein [6]. Several recent studies have focused on small molecules of 'indirect' *MYC* inhibitors such as MYCi975 [10], 10,074-G5 [11], JQ1 [12], and degraders of BRD4 [13]. Although several studies have already reported these molecules indirectly inhibiting *MYC*, they have not yet been introduced into clinical practice. Therefore, we have focused on factors that control the transcriptional activity of *MYC* as a potential therapeutic strategy.

The CRISPR activation library is a powerful and useful tool for genetic screening through gain-of-function studies and has been widely used to elucidate biological mechanisms [14]. Previously we reported a new promoter–reporter system combined with a CRISPR activation library to identify unknown *MYC* regulators and this method was used to successfully identify *M1AP*, which activates endogenous *MYC* [15].

In previous screening [15], we used human embryonic kidney (HEK)293 T cells. Our screening system with pMYC-promoter-Dendra2 uses fluorescence signaling for cell sorting. The fluorescent signal of Dendra2, which is correlated with *MYC* expression, should be negative or weak without activation or perturbation. In the case of HEK293T, as the Dendra2 positive rate was approximately 20% without CRISPR activation, we sorted cells with a notably stronger fluorescent signal. *M1AP* was identified as a *MYC* promoter regulator, although gRNA for *MYC* was not. Next, we explored other cell lines that used for screening of the natural *MYC* promoter regulators in ovarian cancer using our system. We tested the OVSAHO, OVTOKO, OVISE, and OVCAR3 cell lines and selected the OVSAHO cell line because we consider Dendra2, which is weakly positive without activation, to be the optimal cell line to confirm that our promotor system functions optimally and for accurately screening using fluorescent signals (Supp. Fig. S1).

The aim of the study was to assess inhibitors of *MYC* promoter activity as potential therapeutic targets in ovarian cancer, to enhance the quality of the novel promoter–reporter system 'pMYC-promotor-Dendra2' vector, conduct a comprehensive CRISPR screening, and identify candidate genes that regulate the *MYC* promoter in ovarian cancer cells using next-generation sequencing (NGS).

Materials and methods

Cell culture and preparing for SAM library screening

HEK 293 T cells were obtained from the JCRB Cell Bank (National Institutes of Biomedical Innovation, Health, and Nutrition, Osaka, Japan), and the human high-grade serous ovarian cancer cell lines OVSAHO, OVTOKO, OVISE, and OVCAR3 were purchased from the RIKEN Cell Bank (RIKEN Cell Bank, Tsukuba, Japan). HEK 293 T cells were maintained in Dulbecco's modified Eagle's medium (DMEM), and other cells were maintained in Roswell Park Memorial Institute (RPMI)−1640 medium with L-glutamine and phenol red (both purchased from Fuji Film, Osaka, Japan) with 10% fetal bovine serum (FBS) and penicillin–streptomycin with 5% CO_2 at 37 °C. Lentiviral induction was performed as previously described [16] using Lipofectamine 3000 reagent (Invitrogen, Waltham, MA, USA). The lentiviral plasmids used were lentiMPHv2 (#89,308; Addgene, Watertown, MA, USA) and lentidCAS-VP64_BLAST (#61,425; Addgene). The OVSAHO cell line was initially infected with lentiMPHv2 followed by treatment with hygromycin B (200 µg/mL), then infected with lentidCAS9-VP64 followed by treatment with blasticidin (10 µg/mL) for 2 weeks. MPH–dCas9–VP64-expressing OVSAHO cells (1×10^6 cells) were transfected with pMYC-promoter-Dendra2 or pMYC-promoter-Dendra2 Deletion using AMAXA Nucleofactor II, according to the manufacturer's instructions (#VCA-1003, program T-030, solution V; Lonza, Basel, Switzerland) and treated with 500 µg/mL of neomycin for 2 weeks. The OVSAHO cells were then subjected to SAM library screening.

Modifying the MYC promoter–reporter system and lentiviral plasmids

In a previous study, a promoter–reporter system termed "pMYC-promoter-Dendra2" was developed. This system harbors approximately 3 kb of the *MYC* promoter region upstream of the photoconvertible fluorescent protein, Dendra2 [15]. First, p*MYC*-promoter-Dendra2 was induced in the human ovarian carcinoma cell line OVSAHO, and the CRISPR activation library was used by applying the same protocol as that used in a previous study. In this plasmid, a region of approximately 260 bp from the *MYC* transcriptional start site was removed using restriction enzymes (ScaI and BamHI). Three gRNA binding sites for *MYC* are located in this region. The double-stranded DNA corresponding to this region, with all three gRNA binding sites deleted (gBlocks®, Integrated DNA Technologies, Coralville, IA, USA), was

generated into the pMYC-promoter-Dendra2. This modified pMYC-promoter-Dendra2 was termed "pMYC-promoter-Dendra2-Deletion." Dendra2-positive cells were collected using a cell sorter.

SAM library screening and gRNA identification

'CRISPR/Cas9 synergistic activation mediator (SAM)' is an engineered protein complex for the transcriptional activation of endogenous genes.'

LentiSAMv2 (#61,597; Addgene), which includes 100 non-targeting control gRNAs, was transduced into HEK 293 T cells as previously described [15] and then transfected into the prepared OVSAHO cells (3×10^7 cells). After 2 weeks of selection with zeocin, Dendra2-positive cells were collected using a cell sorter with MoFlo XDP (Beckman Coulter, Brea, CA, USA). Twenty-five Dendra2-positive cells were collected in bulk in 24-well plates (SAM#1). After approximately 3 weeks of incubation, three colonies were formed. Genomic DNA (gDNA) was extracted using the QIAamp® DNA Micro Kit (#56,204; QIAGEN, Hilden, Germany). Polymerase chain reaction (PCR) was conducted on the extracted gDNA using KOD FX® with the following primers: (forward) 5'-TCG TCGGCAGCGTCAGATGTGTATAAGAGACAGCAT ACAGTGCTTTATATATCTTGTGGAAAGGACGAA ACACC −3' and (reverse) 5'- GTCTCGTGGGCTCGG AGATGTGTATAAGAGACAGTCGCCTAAGCCAAG TTGATAACGGACTAGCCTT-3'. During the second cell sorting (SAM#2), approximately 1,300 Dendra2-positive cells were sorted in bulk. Collected cells were immediately centrifuged and washed with phosphate-buffered saline (PBS). The analyzed sequences of the 13 colonies were used for further analyses. Using "p*MYC*-promoter-Dendra2-Deletion", gDNA was extracted from approximately 3,000 high Dendra2-positive cells (0.05% of all sorting cells, SAM #3) and PCR was performed for the gRNA region.

Before NGS of inserted gRNAs was conducted, high-fidelity blunt-end TOPO cloning (#450,245; Invitrogen) was performed using a section of the PCR product. NGS was then performed using the Illumina NovaSeq 6000 platform (Illumina, San Diego, CA, USA), Cutadapt was used to trim adapter sequences, and Trimmomatic was used to remove regions with low-quality scores (Hokkaido System Science, Hokkaido, Japan) [17].

Correlation between MYC and candidate genes in databases

The cBio Cancer Genomics Portal [18] was used to compare each candidate gene and *MYC* mRNA expression in ovarian serous cystadenocarcinoma (TCGA, Pan Cancer Atlas Studies) [19, 20].

Dual-luciferase reporter assay

The *MYC* promoter-deleted *MYC* gRNA-binding site fragment was integrated into the pGL4 vector (#E131A; Promega, Madison, WI, USA) using pMYC-promoter-Dendra2-Deletion. Plasmids containing gRNA from the 14 candidate genes (pE1–U6-gRNA–MS2) were constructed [21]. pGL4 (200 ng), pE1–U6-gRNA–MS2(200 ng), pT3.5 dCas9 VP64 MPH (300 ng), and pRL Renilla luciferase reporter vectors (25 ng) were co-transfected into the HEK 293 T cells (1×10^5 cells) with Lipofectamine 3000 using the CRISPR activation system. The samples were harvested 48 h after transfection. A dual-luciferase assay with overexpression vectors was performed using the top four genes based on the dual-luciferase assay results with the gRNAs of the 14 candidate genes. Each assay was performed in triplicate and repeated thrice. The luciferase activity values were standardized with the values of the non-target genes, which the gRNA array showed as ACGGAGGCTAAGCGT CGCA.

Four overexpression and one control vector were purchased from VectorBuilder (#VB900025-2135kct, #VB900144-4920tmz, #VB900017-5698awx, #VB900009-5211tdb, and #VB010000-9486rey, Neu-Isenburg, Germany). The overexpression vector (1 µg) was co-transfected into HEK 293 T cells with pGL4 (200 ng) and pRL (25 ng). Luciferase activity was measured using a dual-luciferase reporter assay system (#1910; Promega) and Lumat LB9507 (PerkinElmer, Waltham, MA, USA).

Western blotting

Cells collected for western blotting were lysed in a sodium dodecyl sulfate (SDS) buffer containing 25% 0.125 M Tris–HCl (pH 6.8), 20% glycerol, 4% SDS, and 10% 2- mercaptoethanol with bromophenol blue, and DNA was disrupted through sonication on ice. Samples were separated using 4–20% SDS–polyacrylamide gel electrophoresis (PAGE) gels (Bio-Rad) and electro-transferred to Immobilon polyvinylidene difluoride (PVDF) membranes (Millipore). The membranes were incubated in Bullet Blocking One (Nacalai Tesque) for 5 min at room temperature. Next, the membrane was incubated with the primary antibodies anti-*ABI1* (proteintech, 27,387–1-AP), anti-*FAM113A*(*PCED1A*) (Novus Biologicals, NBP1-55,521), anti-*HOOK2* (proteintech, 12,458–1-AP), and anti-*CYP4v2* (proteintech, 13,826–1-AP) overnight at 4 °C, all at a dilution of 1:1000. After washing with tris-buffered saline with Tween (TBS-T), the membrane was incubated with the second antibody, horseradish peroxidase (HRP)-labeled anti-rabbit IgG (GE Healthcare, NA934), for 1 h at room temperature at a dilution of 1:5000. Protein bands were visualized using

the Clarity Western enhanced chemiluminescence (ECL) substrate (Bio-Rad).

Statistical analyses

Data were statistically analyzed using EZR software (version 1.36; Saitama Medical Center, Jichi Medical University, Saitama, Japan). The mean values of three or more groups were compared using one-way analysis of variance (ANOVA) with Dunnett's multiple comparison test. Statistical significance was set at $p < 0.05$.

Results

Cell sorting and modifying the MYC promoter–reporter system

First, CRISPR activation library was used for OVSAHO cells prepared with pMYC-promoter-Dendra2. To improve the quality of cell collection, we repeated the cell sorting procedure multiple times. The first sorting was termed SAM#1. As only three colonies advanced in SAM#1, we widened the gate of the flow cytometry and performed a second sorting (SAM#2). In comparison with a list of SAM human library annotations, contrary to our aim, only gRNA targeting *MYC* was identified (Table 1). This is because the gRNA for *MYC* itself bound directly to the *MYC* promoter in pMYC-promoter-Dendra2. In theory, a small number of gRNAs for other genes (such as *Gene X*) that activates the *MYC* promoter should also be detected, but as they were overwhelmingly small in number compared with the gRNAs for *MYC* itself, they were not detected. Therefore, we created a new reporter vector with deleted gRNA binding site of *MYC* and termed pMYC-promoter-Dendra2-Deletion (Fig. 1a). In this modified system, gRNAs targeting *MYC* cannot bind to this vector, enabling us to identify candidate genes (such as *Gene X*) that activate the *MYC* promoter.

Next, the CRISPR activation library was applied to the OVSAHO cells with "pMYC-promoter-Dendra2-Deletion" (SAM #3), and approximately 3,000 high-Dendra2-positive cells (0.05% of all sorting cells) were collected (Fig. 1b, c) and genes other than *MYC* were identified (Table 1). Finally, NGS was performed using these collected cells.

Table 1 Analysis of guide RNA sequences in sorted Dendra2-positive cells

SAM	Sample No	Sequence of gRNA	Gene name
#1	1	GGTGGGGAGGAGACTCAGCC	MYC
#1	2	GGTGGGGAGGAGACTCNGCC	MYC
#1	3	GGTGGGGAGGAGACTCAGCC	MYC
#2	1	NNNNNNNN	Not matched
#2	2	GGTGGGGAGGAGACTCAGCC	MYC
#2	3	GGTGGGGAGGAGACTCAGCC	MYC
#2	4	GGGTGGGGAGGAGACTCAGC	MYC
#2	5	GGGTGGGGAGGAGACTCAGC	MYC
#2	6	GAGTCTCCTCCCCACCCGGT	Not matched
#2	7	GGCTGAGTCTCCTCCCCACC	MYC
#2	8	GGCTGAGTCTCCTCCCCACC	MYC
#2	9	GGCTGAGTCTCCTCCCCACC	MYC
#2	10	GGCTGAGTCTCCTCCCCACC	MYC
#2	11	GGTGGGGAGGAGACTCAGCC	MYC
#2	12	GGCTGAGTCTCCTCCCCACC	MYC
#2	13	GGCTGAGTCTCCTCCCCACC	MYC

Correlation between MYC and candidate genes in databases

A total of 4,065,162 reads were counted and 13,595 types of gRNAs were detected during NGS. Applying cBioPortal [18], the Pearson correlation coefficient analysis was used to assess mRNA expression in *MYC* and the other genes, which had ≥ 1000 reads (totally 86 genes) (Supp. Fig. S2). From these 86 genes, the following 14 genes with positive and statistically significant correlations with *MYC* were selected as candidates: *CYP4V2, ORC4, RPGRIP1L, ADPRH, LGR6, PHB, ANP32D, STOML2, PCED1A, NEURL2, ABI1, TTC9C, FUZ,* and *HOOK2* (Fig. 1d, Table 2).

Dual-luciferase reporter assay using the 14 candidate genes

Seven genes, *CYP4v2, ASPH, ANP32D, PCED1A, ABI1, FUZ,* and *HOOK2,* showed significantly higher luciferase activity than the control (Fig. 2a). Next, we established cells overexpressing these four genes, which showed > twofold higher activity than the control, namely *ABI1* (NM_001178116), *PCED1A* (NM_001271168), *HOOK2* (NM_001100176), and *CYP4v2* (NM_207352)

(See figure on next page.)

Fig. 1 CRISPR screening system using the OVSAHO ovarian cell line. **a** Schema of original and modified *MYC* promoter–reporter systems. Using original screening with pMYC-promoter-Dendra2, the gRNA targeting for *MYC* directly bound to *MYC* promoter (three gRNAs for *MYC* in pink). The gRNAs for *Gene X* should also be collected although, they were not detected likely due to small numbers. In modified screening with pMYC-promoter-Dendra2-Deletion, gRNA binding sites for *MYC* are deleted do detect other candidate genes *(*such as *Gene X)*. **b** Experimental model. The SAM library and pMYC-promoter-Dendra2/Deletion vectors were transfected into prepared OVSAHO cells, and Dendra2-positive cells were sorted using a cell sorter. **c** Cell sorting using FACS. Approximately 3,000 Dendra2-positive cells were collected. **d** Pipeline of candidate gene selection

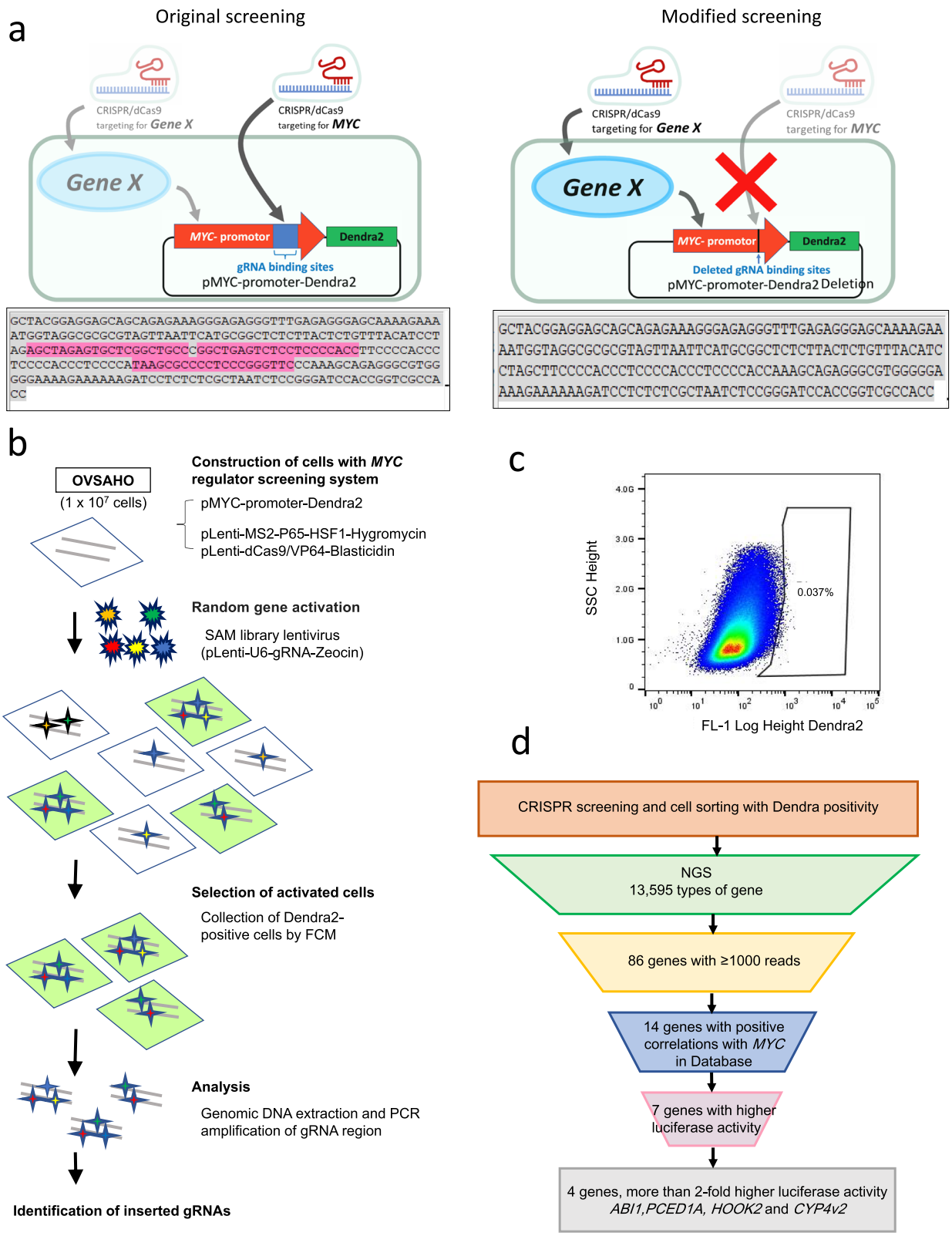

Fig. 1 (See legend on previous page.)

Table 2 Guide RNA sequences and read count of candidate genes in NGS

gRNA	Gene Name	Sequence	Count
sg015083	*CYP4V2*	CTGCGAGGTTGCTCTACGTG	1492
sg042467	*ORC4*	GACATTGTAGCGGGAGGTAC	1370
sg051675	*RPGRIP1L*	TTAGCACAGGAGAATTTCCC	1353
sg004415	*ASPH*	TCCAGTTTGTCTCGGTCCTT	1290
sg032538	*LGR6*	TATTTCTCACTTCCTACAAC	1282
sg044709	*PHB*	ACTCCCAAAAGGCTATGCAG	1242
sg002934	*ANP32D*	TAATAGATTTTGGGTGTGTT	1128
sg058828	*STOML2*	CTGTTCACGCCGAAGATCCC	1106
sg043681	*PCED1A*	TTTAGAAAAATCCGTGGTTT	1093
sg039172	*NEURL2*	CAGAGGTGAGCTGGCACCGG	1085
sg000482	*ABI1*	TGGGCGCATGCGCTTTGGAC	1049
sg022687	*FUZ*	CGCAGTCATCATCTTCAATC	1037
sg064502	*TTC9C*	GTGTCGCTGGTGGTTCACCT	1014
sg027117	*HOOK2*	TCCTCAAGATCCTTTTGAGA	1009

(Supp. Fig. S3). Using these cells the dual-luciferase reporter assay showed that these genes activated significantly higher luciferase activity (Fig. 2b). Furthermore, luciferase activity was significantly reduced in cells in which these four genes were knocked down (Fig. 2c). Then, quantitative PCR was performed to examine the mRNA expression of *MYC* in overexpression and knockdown of these four genes in OVSAHO cells; however, no significant correlation with *MYC* expression was observed (Supp. Fig. S4).

In addition to OVSAHO cells, we also validated other ovarian cancer cell lines, namely OVTOKO, OVISE, and OVCAR3 (Supp. Figure 5, 6). Only OVTOKO cells correlated with *MYC* expression, low *MYC* expression in *PCED1A* and *CYP4v2* knockdown cells (Supp. Fig. S5a) and high *MYC* expression in *HOOK2* and *CYP4v2* overexpression cells (Supp. Fig. S6a). Notably, no correlation was observed in the other cell lines.

Discussion

This study successfully identified four genes that activate the *MYC* promoter in an ovarian cancer cell line using the CRISPR library system with a modified promoter–reporter tool.

MYC is an important transcription factor that plays roles in cell growth, proliferation, and apoptosis in normal tissues, and its expression is strictly controlled. *MYC* is involved in the development and progression of many human cancers [3, 4] and previous studies have shown that hyperactivation of *MYC* is associated with aggressive cancer outcomes [6–9]. Owing to the essential role of *MYC* in malignant tumors, *MYC* inhibitors have been researched for decades as therapeutic targets; however, the MYC protein has been considered "undruggable" because of its structure [22, 23] and 'direct' *MYC* inhibitors have not yet been developed [6]. Several recent studies have focused on small molecules of 'indirect' *MYC* inhibitors. For example, MYCi975 disrupts MYC/MAX dimers and promotes MYC T58 phosphorylation and MYC degradation [10]. And the 10,074-G5 binds bHLH-ZIP domain of c-Myc, thereby preventing the formation of the c-MYC/MAX heterodimer [11]. Furthermore, as molecules targeting *MYC* transcription, BET inhibition by the bromodomain inhibitor JQ1 downregulates *MYC* transcription [12]. The degraders of bromodomain protein 4 (BRD4), which is a transcriptional and epigenetic regulator with intrinsic kinase and histone acetyltransferase activities, leads to the degradation of *MYC* transcription [13]. In addition, inhibitors of cyclin-dependent kinase (CDK) 9, which is a key transcriptional regulator, modulate the expression and activity of *MYC* [24]. Although several studies have been conducted, these molecules have not yet been applied in clinical practice.

In our previous research using HEK293T cells, although we used conventional pMYC-promoter-Dendra2 which harbor *MYC* gRNA binding sites, *MYC* was not detected. We successfully identified *M1AP* as a novel regulator, and in *M1AP*-overexpressed cells, the dual-luciferase assay showed a significant increase in promoter activity and *MYC* mRNA and protein expressions [15]. In contrast, in our current study, *MYC* gRNA was selected, but not *M1AP*. In OVSAHO cells overexpressing four genes, identified with the modified screening system "pMYC-promoter-Dendra2-Deletion", the dual-luciferase assay showed a significant increase of promoter activity; however, increasing of *MYC* mRNA expression was not observed (Supp. Fig. S4). As no differences in the experimental strategy were present, other than the cell line, we concluded that the difference of the cellular context of the cell lines is the reason.

In the seven hits genes, searching using PubMed database system, only one study referenced the relation with *MYC* expression [25]. Yao et al. reported that overexpressed ASPH increased cell viability by regulating c-Myc and cyclin-D1 expressions in hepatoma cells [26], although no reference to ovarian cancer was made. A search of the STRING database did not reveal any interactions between *MYC* and the other gene products [27]. Furthermore, based on the National Center for Biotechnology Information (NCBI) database, no transcriptional functions of the four selected genes have been described [28], and this study is the first to report the molecular function of enhancing *MYC* transcriptional activity.

Although these candidate genes activated the *MYC* promoter, this study had certain limitations. We performed

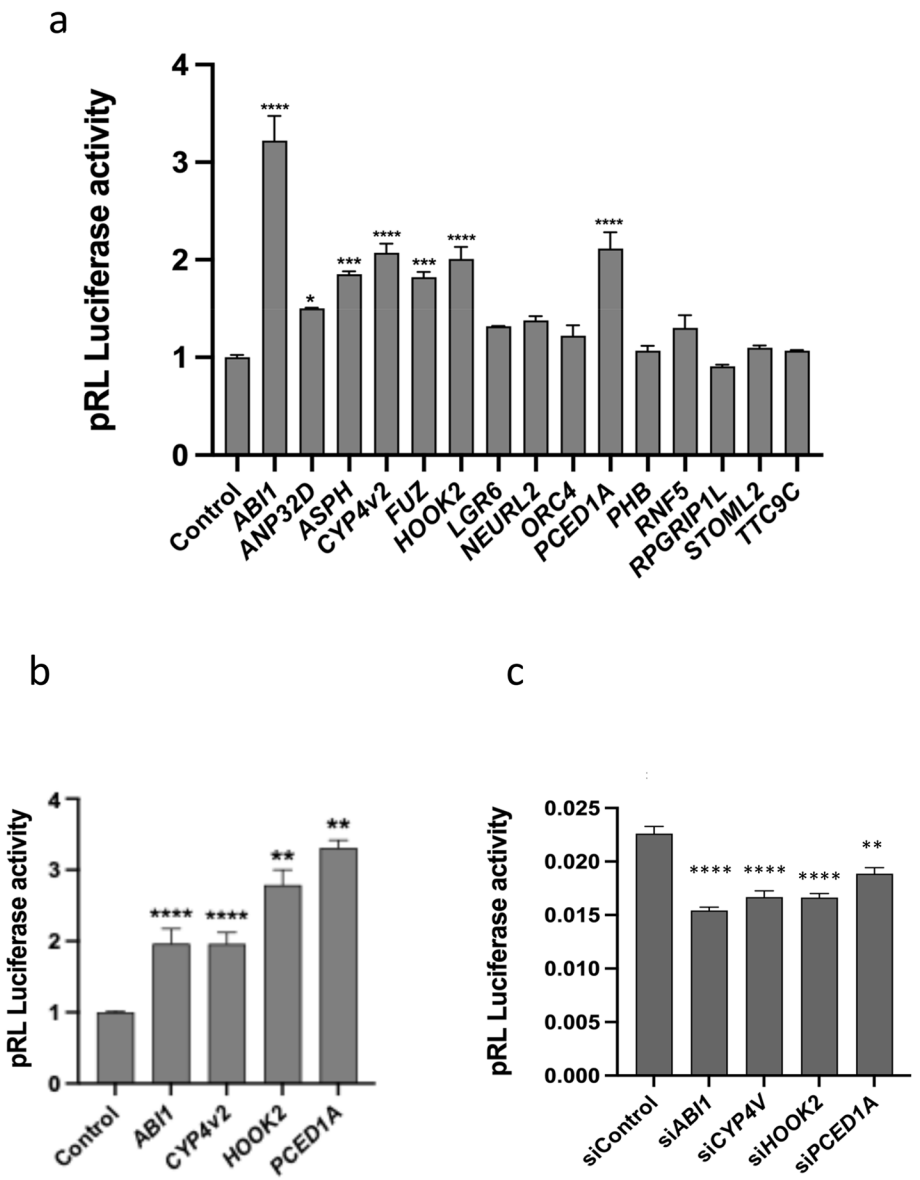

Fig. 2 Dual-luciferase reporter assay results. All experiments were performed in biological and technical triplicates. **a** Dual-luciferase reporter assay of candidate genes. Fourteen genes with a positive correlation with *MYC* on cBioPortal were selected as candidate genes based on the NGS results. **b** Dual-luciferase reporter assay with overexpression of the four genes. **c** Dual-luciferase reporter assay with knockdown of the four genes. The mean values of three or more groups were compared using a one-way ANOVA with Dunnett's multiple comparison test. *$p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, and **** $p \leq 0.0001$. Data are represented as the mean ± standard deviation

overexpression and knockdown of four genes in the ovarian cancer cell lines OVSAHO, OVTOKO, OVISE, and OVCAR3. Only OVTOKO correlated with *MYC* and the expression of specific genes, though, no correlation was observed in other cell lines. Possible reasons for this result can include: i) several signaling pathways tightly regulate *MYC* expression, and knockdown of one gene alone may not be sufficient to control *MYC* expression; ii) mRNA may be strongly post-transcriptionally regulated; iii) these candidate genes may not be overexpressed in the ovarian cancer cell lines; and iv) there may be more suitable criteria for selecting candidate genes from NGS results.

In conclusion, this study identified four genes that activate the *MYC* promoter in ovarian cancer cell lines using the CRISPR library system. Identifying these molecules provides insights into the molecular pathways and mechanisms that regulate *MYC* expression in ovarian cancer.

This understanding could help in unraveling the complex biology of *MYC*-driven cancers and potentially assist in identifying other nodes in these pathways that can be targeted therapeutically. However, more candidates should be explored based on the present findings considering the cellular context. Further research is also required to establish more accurate screening techniques to elucidate the comprehensive regulation system of *MYC* expression.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13048-025-01644-z.

Supplementary Material 1. Supplementary Figure S1. Flow cytometry results of Dendra 2-positive rate in HEK293T and ovarian cancer cell lines OVSAHO, OVTOKO, OVISE and OVCAR3.

Supplementary Material 2. Supplementary Figure S2. Pearson correlation coefficient analysis in cBioPortal at mRNA expression between MYC and the other identified genes. (a) CYP4V2, (b) ORC4 , (c) RPGRIP1L , (d) ASPH , (e) LGR6 , (f) PHB , (g) ANP32D , (h) STOML2 , (i) PCED1A , (j) NEURL2 , (k) ABI1 , (l) FUZ , (m) TTC9C , and (n) HOOK.

Supplementary Material 3. Supplementary Figure S3. Western blotting with overexpression of the four genes.

Supplementary Material 4. Supplementary Figure S4. qPCR results of MYC expression in OVSAHO cells. (a) Knockdown (b) Overexpression.

Supplementary Material 5. Supplementary Figure S5. qPCR results of MYC expression in knockdown of the four genes. (a) OVTOKO (b) OVISE and (c) OVCAR3.

Supplementary Material 6. Supplementary Figure S6. qPCR results of MYC expression in overexpression of the four genes (a) OVTOKO (b) OVISE and (c) OVCAR3.

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Authors' contributions
AY and YT conceived and performed the experiment, analyzed the data, prepared figures and tables and approved the submitted draft. MK conceived and designed this study, analyzed the data and approved the submitted draft. KS made interpretation of data and approved the submitted draft. SI and MI performed the experiments, prepared figures and approved the submitted draft. MO and HN analyzed the data, reviewed the paper and approved the submitted draft.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
No applicable.

Consent for publication
No applicable.

Competing interests
The authors declare no competing interests.

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