Identification of genes involved in the regulation of \( TERT \) in hepatocellular carcinoma

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Telomerase reverse transcriptase (\( TERT \)) promotes immortalization by protecting telomeres in cancer cells. Mutation of the \( TERT \) promoter is one of the most common genetic alterations in hepatocellular carcinoma (HCC), indicating that \( TERT \) upregulation is a critical event in hepatocarcinogenesis. Regulators of \( TERT \) transcription are, therefore, predicted to be plausible targets for HCC treatment. We undertook a genome-wide shRNA library screen and identified C15orf55 and C7orf43 as regulators of \( TERT \) expression in HepG2 cells. Promoter assays showed that C15orf55- and C7orf43- responsive sites exist between base pairs –58 and +36 and –169 and –59 in the \( TERT \) promoter, respectively. C15orf55 upregulates \( TERT \) expression by binding to two GC motifs in the SP1 binding site of the \( TERT \) promoter. C7orf43 upregulates \( TERT \) expression through Yes-associated protein 1. The expression levels of C15orf55 and C7orf43 also correlated with that of \( TERT \), and were significantly increased in both HCC tissues and their adjacent non-tumor tissues, compared to normal liver tissues from non-HCC patients. Analysis of 377 HCC patients in The Cancer Genome Atlas dataset showed that overall survival of patients with low levels of C15orf55 and C7orf43 expression in tumor tissues was better compared with patients with high levels of C15orf55 and/or high C7orf43 expression. These results indicate that C15orf55 and C7orf43 are involved in the incidence and progression of HCC by up-regulating \( TERT \). In conclusion, we identified C15orf55 and C7orf43 as positive regulators of \( TERT \) expression in HCC tissues. These genes are promising targets for HCC treatment.

KEYWORDS
C15orf55, C7orf43, hepatocellular carcinoma, telomerase reverse transcriptase, transcription

Abbreviations: AMOTL2, angiomotin-like protein 2; BRD4, bromodomain-containing protein 4; CLEC4C, C-type lectin domain family 4 member C; EGR1, early growth response protein 1; GABPA, GA-binding protein alpha chain; HCC, hepatocellular carcinoma; HIF1, hypoxia-inducible factor 1; LZTR1, leucine zipper like transcription regulator 1; SLC22A16, solute carrier family 22, member 16; TCGA, The Cancer Genome Atlas; TERT, telomerase reverse transcriptase; YAP1, Yes-associated protein 1.
1 | INTRODUCTION

Telomerase, consisting of TERT and telomerase RNA component, adds telomeric repeat DNA sequences (TTAGGG) at the end of eukaryotic linear chromosomes to protect DNA from end-to-end fusion, rearrangement, and translocation.\(^1\)\(^2\)\(^-\)\(^12\) Cell mitosis results in progressive shortening of telomeres and leads to cell senescence; therefore, immortal cells constitutively activate telomerase to maintain telomere length and escape from telomere-induced replicative senescence.\(^3\)\(^-\)\(^6\) A recent study clarified that increased expression of TERT also promotes immortalization by bypassing oncogene-induced senescence.\(^7\) This is consistent with the observation that TERT gene expression is activated in more than 90% of cancers, irrespective of tumor type.\(^8\)

Hepatocellular carcinoma is the third leading cause of cancer-related death.\(^9\) Hepatocellular carcinoma and cirrhotic premalignant lesions frequently show increased expression of TERT, whereas mature and senescent hepatocytes have low TERT expression.\(^6\)\(^,\)\(^10\)\(^-\)\(^13\) The difference in TERT expression between these 2 tissue types suggests that telomerase reactivation plays an important role in hepatocarcinogenesis.\(^14\) Indeed, TERT promoter mutation resulting in activation of TERT expression is the most frequent genetic alteration in HCC and is seen in 61% of HCC tissues and in 19% of premalignant nodules.\(^11\)\(^,\)\(^13\)\(^,\)\(^16\)\(^-\)\(^19\) These results indicate that TERT is a possible target for HCC therapy.\(^17\) However, a direct telomerase inhibitor was frequently associated with adverse events and failed to improve the prognosis of non-small-cell lung cancer patients in a phase II study.\(^18\) TERT expression is the primary determinant regulating the telomerase activity in cancer cells\(^12\) and a number of studies examining TERT expression in cancer have been published, most of which have investigated promoter mutations and chromosomal rearrangements.\(^19\) Suppression of TERT transcription is a reasonable approach to target telomerase; therefore, it is important to identify the genes that regulate TERT transcription.

In the present study, we undertook an unbiased screen of TERT promoter activators using a genome-wide shRNA library. This screening system succeeded in identifying 2 previously unrecognized activators of TERT transcription. These activators are expected to be novel therapeutic targets for HCC.

2 | MATERIALS AND METHODS

2.1 | Plasmids and lentiviruses

Short hairpin RNA expression vectors for C15orf55, C7orf43, F2, SLC22A16, CLEC4C, and LZTR1, and expression vectors for C15orf55 and C7orf43 and their negative controls (sh-empty and pcDNA6-empty) were used in this study. Firefly luciferase reporter vectors driven by multiple lengths of the TERT promoter are described in Figure S1. The lentiviruses were generated based on former shRNA vectors using Second Generation Packaging Mix (Applied Biological Materials, Richmond, Canada) and 293T cells. All constructs were verified by direct DNA sequencing.

2.2 | Cell lines and cell culture

HepG2 cells were maintained in DMEM supplemented with 10% FBS. HepG2 sublines were established by transfection of the cells with pcDNA6-Empty (OE-Empty), pcDNA6-C15orf55 (OE-C15orf55), and pcDNA6-C7orf43 (OE-C7orf43).

2.3 | Short hairpin RNA library screening

A lentivirus-based shRNA library (hGW shRNA Library Module 2; Cellecta inc., Mountain View, CA) was transduced into HepG2 cells, which were doubly transfected with both TERT promoter-driven GFP and an exogenous TERT expression vector to maintain steady growth of cells. Selection with puromycin and cell-sorting was carried out to detect cells that suppressed TERT promoter activity.

2.4 | Patient samples

Liver tumor tissue and adjacent nontumor liver tissue from 65 HCC patients were acquired following surgery for HCC between 2007 and 2011 at the Tottori University Hospital (Yonago, Japan). The study was approved by the ethical committee of Tottori University (1610A118). The data of 377 HCC patients in TCGA dataset (https://cancergenome.nih.gov/) were also examined.

2.5 | Statistical analysis

All statistical analyses were carried out using R software (version 3.1.3; www.r-project.org/). \(P < .05\) was considered to indicate significant difference. Additional information is provided in Appendix S1.

3 | RESULTS

3.1 | Identification of C15orf55 and C7orf43 as activators of the TERT promoter

To identify genes that activate TERT transcription, we carried out an shRNA screen of HepG2 human HCC cells that were genetically modified to express exogenous TERT to compensate for the decreased expression of endogenous TERT by shRNA (Figure 1A). One hundred clones with low TERT promoter activity were obtained after shRNA-transduction of HepG2 cells. From these clones, 6 genes were identified as potential activators of TERT transcription (Tables S1 and S2). The expression of CLEC4C was not high enough to be detected in HepG2 cells by RT-PCR; therefore, this gene was omitted from further analysis (data not shown). After confirmation of successful shRNA-induced downregulation of the genes F2, C15orf55, C7orf43, and LZTR1 (Figure 1B), shRNA-induced downregulation experiments selected C15orf55 and C7orf43 as activators.
of TERT transcription (Figure 1C, Table S3). The transient expression of C15orf55 induced significantly higher levels of TERT mRNA expression than control vector in all HCC cell lines including HepG2, HLE, HuH7, HuH6, and PLC/PRF/5 (Figures 1D and S2). C7orf43 also showed significantly higher TERT mRNA expression in these cell lines, except for that in HuH7 cells, in which TERT expression was not significantly, but slightly increased (Figures 1D and S2). Stably transfected OE-C15orf55 and OE-C7orf43 clones showed significantly higher TERT expression compared with OE-Empty (Figures 2A and S3). Telomerase activity was also increased in OE-C15orf55 and OE-C7orf43 cells compared with OE-Empty control cells (Figure 2B).

3.2 Effect of C15orf55 and C7orf43 on HepG2 cell proliferation

As TERT promotes cell proliferation through several pathways, we attempted to investigate the effect of C15orf55 and C7orf43 on cell proliferation. Both OE-C15orf55 and OE-C7orf43 clones grew faster in a plane culture, and formed larger colonies in a methylcellulose medium than OE-Empty clone did (Figures 3A and S4). The population-doubling time of OE-C15orf55 and OE-C7orf43 clones was shorter than that of OE-Empty (OE-C15orf55, 29.8 hours; OE-C7orf43, 30.0 hours, and OE-Empty, 37.1 hours; Figure 3A). The shRNA-mediated knockdown of C15orf55 or C7orf43 in HepG2 cells resulted in a significant reduction in the number of colonies, compared with that of control shRNA (Figure 3B,C).

3.3 C15orf55 and C7orf43 recognize different promoter sites to activate TERT expression

To identify the sites responsible for C15orf55- and C7orf43-induced activation of the TERT promoter, we generated luciferase reporter constructs containing 5’ to 1.2, 0.7, 0.4, 0.2, and 0.1 kb of the TERT promoter (#1 to #7). HepG2 cells were cotransfected with these
reporter vectors and C15orf55 or C7orf43 expression vectors, and luciferase activity was measured. Transfection of C15orf55 significantly activated promoters #1 to #7 of the TERT gene. C7orf43 transfection also activated promoters #1 to #6, but failed to activate promoter #7 (Figure 4A,B). A double transfection of C15orf55 and C7orf43 activated the TERT promoter to a greater extent than a single transfection of each one (Figure 4C), suggesting that C15orf55 and C7orf43 independently activate the TERT promoter at different sites. These results indicate that C15orf55- and C7orf43-responsive sites exist between base pairs −58 and +36, and −169 and −59 of the TERT promoter, respectively.

3.4 Involvement of SP1 in TERT promoter activation by C15orf55

The region of the TERT promoter responsible for C15orf55-induced activation (−58 to +36) contains 2 GC motifs, a region to which SP1 binds to induce TERT expression. To investigate whether C15orf55 regulates TERT transcription through SP1 on the GC motifs, 2 reporter vectors carrying 1 or the other of the GC motifs were constructed (Figure S1B). C15orf55 was shown to activate TERT expression through both GC motifs (Figure 5A). Furthermore, mithramycin, a competitive inhibitor of SP1 for binding to the GC motif, prevented C15orf55-mediated activation of the TERT promoter (Figure S5A).

The siRNA-mediated SP1 knockdown canceled C15orf55-induced activation of the TERT promoter (Figure S5B,C). Consistent with this observation, significantly more SP1 was recruited to the GC motifs in the TERT promoter in OE-C15orf55 cells than in OE-Empty cells (Figure 5B). These data indicate that C15orf55 activates TERT expression through SP1 on the promoter (Figure 5F).

3.5 Involvement of YAP1 in TERT promoter activation by C7orf43

Although our luciferase assay indicated that the region from −169 to −59 bp of the TERT promoter is responsible for C7orf43-induced activation of the promoter, only a few transcription factors that
of C7orf43 overexpression on YAP1 activity, the expression and nuclear localization of YAP1 were examined in OE-C7orf43 cells. As expected, the protein levels of YAP1 and AMOTL2 were upregulated, whereas C7orf43 did not affect YAP1 phosphorylation, which represses YAP1 activity through the cytosolic retention of YAP1 (Figure 5D). Nuclear localization of YAP1 was also significantly increased in OE-C7orf43, compared to OE-Empty cells, as indicated by immunofluorescent staining and immunoblotting of nuclear extracts (Figures 5E and S6). In addition, it was shown that C7orf43-induced activation of TERT promoter was canceled by siRNA-mediated knockdown of YAP1 (Figure S7). Our data indicate that C7orf43 activation of YAP1 is dominant over AMOTL2-mediated suppression of YAP1. Taken together, these data indicate that C7orf43 activates the TERT promoter via YAP1 (Figure 5F).

3.6 C15orf55 and C7orf43 have clinical significance for tumorigenesis and patient survival

To determine the clinical importance of C15orf55 and C7orf43, gene expression was examined in HCC specimens collected by our institution. The clinical and pathological characteristics of 65 patients are shown in Table 1. The gene expression of C15orf55, C7orf43, and TERT is also shown (Figures 6, 7A-D, S8, and S9). Increased expression levels of C15orf55 and C7orf43 were seen in nontumor liver tissues as well as tumor tissues, compared with normal liver tissues from non-HCC patients (Figure 6A,B). In the nontumor liver tissues, expression of C15orf55 was significantly associated with a lower Child-Pugh score ($P = .046$; Figure 6C). Although it did not reach statistical significance, C7orf43 expression also showed a tendency to be associated with liver cirrhosis ($P = .197$; Figure 6D). The expression levels of C15orf55 and C7orf43 were significantly correlated with TERT expression levels both in nontumor and tumor tissues of HCC patients (Figure 7A-D). Although the detection rate of TERT mRNA in tumor and nontumor tissues appears to be low, TERT mRNA levels were also reported to be very low or undetectable in previous studies. Given the existence of the correlation in nontumor tissues in which the frequency of TERT promoter mutation is quite low, C15orf55 and C7orf43 could play a role in TERT transcription independently from the mutation. These data indicate that C15orf55 and C7orf43 are involved in tumor progression, and also in the reactivation of telomerase toward tumorigenesis in damaged livers without the TERT promoter mutation. In agreement with this notion, C15orf55 and C7orf43 activated the WT TERT promoter to the same extent as mutant promoters, including the C228T mutation (Figure S10).

To further investigate the impact of C15orf55 and C7orf43 on the prognosis of HCC patients, survival data from TCGA database, in which 377 patients are enrolled, were analyzed because the small population enrolled at our institution has limited statistical power to estimate patients' prognosis (Figure S11). The HCC patients with high levels of C15orf55 and/or C7orf43 expression tended to have a poorer prognosis compared with those with low expression levels of both C15orf55 and C7orf43 (Figures 7E,F and S11).
4 | DISCUSSION

Expression of TERT is critical for the reactivation of telomerase, which plays an important role in the development and proliferation of cancer cells through telomere protection and avoidance of senescence. However, the mechanism by which hepatocytes acquire telomerase reactivation at the initiation of cancer remains largely unclear. Interestingly, a small subpopulation of hepatocytes with elevated Tert expression and telomerase activity exists in the liver of healthy mice to drive the regeneration and renewal of the liver, indicating that telomerase reactivation could be an intrinsic function in progenitor hepatocytes rather than resulting from genomic change, such as mutation or methylation.

In this study in a HCC cell line, we identified two genes, C15orf55 and C7orf43, as activators of TERT transcription through SP1 and YAP1, respectively. Although the involvement of these genes in TERT transcription and in the development and progression of HCC has not been reported, we found that expression of C15orf55 and C7orf43 was upregulated and correlated with TERT expression in tumor tissues and surrounding liver tissues from HCC patients. The Cancer Genome Atlas database also showed that expression levels of C15orf55 and C7orf43 in HCC are significantly correlated with poor prognosis of HCC patients. Consistent with this observation, overexpression of either C15orf55 or C7orf43 enhanced the proliferation of HepG2 cells in accordance with tuberculosis therapy, ATP1A1 as a gene that regulates aurilide B cytotoxicity, and the deubiquitinating enzyme, USP5, which modulates cell cycle regulators. The shRNA screening system has also identified HIF1α as a TERT regulator using mouse ES cells.

In this study in a HCC cell line, we identified two genes, C15orf55 and C7orf43, as activators of TERT transcription through SP1 and YAP1, respectively. Although the involvement of these genes in TERT transcription and in the development and progression of HCC has not been reported, we found that expression of C15orf55 and C7orf43 was upregulated and correlated with TERT expression in tumor tissues and surrounding liver tissues from HCC patients. The Cancer Genome Atlas database also showed that expression levels of C15orf55 and C7orf43 in HCC are significantly correlated with poor prognosis of HCC patients. Consistent with this observation, overexpression of either C15orf55 or C7orf43 enhanced the proliferation of HepG2 cells in accordance with...
increased TERT and telomerase activity. Telomerase promotes cell proliferation by stabilizing telomeres. Moreover, TERT itself controls the activity and expression of cell proliferation-related factors such as pRB, E2F, β-catenin, and p65. Taking these reports into consideration, the upregulation of TERT is a critical mechanism underlying the C15orf55- and C7orf43-induced cell proliferation. Our findings suggest that C15orf55 and C7orf43 are previously unrecognized factors for the reactivation of telomerase in HCC. These genes are potential therapeutic targets for a novel telomerase-directed therapy. An intervention targeting C15orf55 and/or C7orf43 might be beneficial in preventing carcinogenesis and tumor progression.

C15orf55, also known as NUTM1, is a testis-specific protein with totally unknown function. However, it is not surprising that C15orf55 promotes carcinogenesis and proliferation of cancer cells because the fusion of C15orf55 with BRD4, which encodes a bromodomain-containing protein, is commonly found in a rare carcinoma arising in midline organs (OMIM: 608963). Although the domain corresponding to C15orf55 of the C15orf55-BRD4 fusion protein activated p300 histone acetyltransferase and chromatin remodeling, no attenuation of C15orf55-induced promoter activation was found in a reporter assay using C646, a specific inhibitor of CBP/P300 (data not shown). These data suggest that C15orf55 activates the TERT promoter in a CBP/P300-independent manner.

**TABLE 1** Characteristics of 65 patients who underwent surgery for hepatocellular carcinoma between 2007 and 2011

| Characteristic | n | Characteristic | n |
|---------------|---|---------------|---|
| Number of patients | 65 | Serosal invasion (n = 63) |
| Age, y | 70 (62-76) | Negative | 41 |
| Gender | | Positive | 22 |
| Male | 57 | Portal vein invasion (n = 63) |
| Female | 8 | Negative | 49 |
| Etiology | | Positive | 24 |
| HBV | 28 | Hepatic vein invasion (n = 63) |
| HCV | 13 | Negative | 59 |
| Non-B/non-C | 24 | Positive | 4 |
| Total bilirubin, mg/dL | 0.6 (0.5-0.8) | Bile duct invasion (n = 63) |
| Albumin, g/dL | 3.9 (3.7-4.2) | Negative | 61 |
| AST, IU/L | 34 (24-46) | Positive | 2 |
| ALT, IU/L | 28 (19-38) | TNM stage |
| AFP, ng/mL | 8.2 (3.4-71.4) | IA | 7 |
| Child-Pugh score | IB | 22 |
| 5 | 51 | II | 23 |
| 6 | 11 | IIIA | 7 |
| 7 | 3 | IIIB | 6 |
| Number of tumors | Fibrosis stage (f: n = 61) |
| 1 | 52 | 0 | 12 |
| 2 | 12 | 1 | 18 |
| ≥3 | 1 | 2 | 10 |
| Tumor size, cm | 4.0 (2.5-7.0) | 3 | 5 |
| Survival period, y | 4.3 (2.9-7.3) | 4 | 16 |
| Differentiation | Gene expression |
| Well | 2 | C15orf55 in tumor | 8.6 (1.0-98.0) |
| Moderate | 58 | C15orf55 in nontumor | 3.30 (0.04-37.38) |
| Poor/Undifferentiated | 5 | C7orf43 in tumor | 10.7 (1.7-92.2) |
| Capsular invasion (n = 63) | C7orf43 in nontumor | 2.2 (0.4-23.4) |
| Negative | 52 | TERT in tumor (n = 27) | 0.6 (0.1-1.1) |
| Positive | 11 | TERT in nontumor (n = 16) | 0.07 (0.01-0.31) |

Values for age, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), α-fetoprotein (AFP), tumor size, survival period, and gene expression are provided as the median and interquartile range. Relative gene expression levels of C15orf55 and C7orf43 to ACTB were normalized against that in normal liver. Relative gene expression levels of TERT to ACTB were normalized against that in tumor. HBV, hepatitis B virus; HCV, hepatitis C virus.
Instead, we found that SP1 is a critical transcription factor for C15orf55-induced activation of the TERT promoter. SP1 plays an important role in the transcriptional regulation of TERT. Although a number of proteins interact with SP1, little is known about a mechanism that regulates SP1 to activate the transcription of TERT. In this study, C15orf55 was identified as a novel upstream factor of SP1.

C7orf43, the other regulator of TERT transcription identified in our study, is also an uncharacterized protein. We found that C7orf43 activates YAP1, the main downstream effector of the Hippo signaling pathway, whose activation negatively regulates YAP1. YAP1 is suggested to be an oncogene in humans because its upregulation and nuclear localization have been observed in multiple types of human cancer. Moreover, knockdown or deletion of Hippo pathway components, such as Mst1/2, in liver lead to the activation of YAP1 and the development of HCC. Before this study, interaction between YAP1 and C7orf43 was unknown; however, an interactome analysis showed that C7orf43 interacts with AMOTL2. The latter suppresses YAP1 by tethering it in the cytoplasm. Note that AMOTL2 is also the target gene of YAP1 as a negative feedback regulator of the Hippo pathway. As shown in Figure SC, C7orf43 induced AMOTL2 expression, but YAP1 was still activated, suggesting that the negative feedback regulation might be abrogated by C7orf43. Therefore, we reason that C7orf43 functions as a suppressor of AMOTL2, thereby indirectly activating YAP1.

Expression of C15orf55 and C7orf43 was upregulated in nontumor tissues and in tumor tissues of HCC, and was correlated with TERT expression. Because hepatocytes in the nontumor tissue have a much lower mutation rate in the TERT promoter than HCC cells, this finding indicates that both C15orf55 and C7orf43 induce TERT transcription in hepatocytes in cirrhotic livers in a mutation-independent manner. Consistently, a reporter assay showed that C15orf55 and C7orf43 significantly activated the WT TERT promoter (without C228T mutation) (Figure S10). However, activation of the WT TERT promoter by C7orf43 was significantly lower than that of the C228T mutant promoter, suggesting that C7orf43 also activates the TERT promoter in a mutation-dependent manner. This is consistent with C7orf43 activating both fragments #3 and #6, the latter containing the C228T mutation-dependent GABPA-binding site. Although mutation of the TERT promoter plays critical roles in carcinogenesis, a recent study by Chiba et al showed that TERT promoter mutation does not necessarily confer sufficient activation to telomerase to immortalize precancerous cells in the early step of carcinogenesis. Their finding indicates that additional factors that increase TERT transcription are required to fully activate telomerase...
Our data suggest that C15orf55 and C7orf43 might be critical factors for the reactivation of telomerase in HCC, in addition to the promoter mutation. Consistent with our in vitro observations, C15orf55 and C7orf43 had a negative impact on patients’ prognosis. Our findings indicate that upregulation of these genes induces tumor progression through telomerase reactivation, which strongly associates with poor prognosis of HCC patients. Therefore, it is worth investigating whether C15orf55 or C7orf43 has tumorigenic potential independent of TERT. The mechanism of how these genes activate SP1 and YAP1 could provide useful information for a novel HCC therapy.

A recent study by Lin et al showed that a small population of hepatocytes expressing high levels of TERT (TERT high) in healthy liver is involved in hepatocyte renewal. Based on these results, further studies to reveal the precise mechanisms in which these genes act could lead to the development of a novel telomerase inhibitor. Interestingly, it is also possible that C15orf55 and C7orf43 themselves promote cell proliferation in a TERT-independent manner, because C15orf55 and C7orf43 activate SP1 and YAP1, respectively, both of which have various functions for cell proliferation other than TERT.

**FIGURE 7** Expression of C15orf55 and C7orf43 correlates with TERT expression and patient survival. A-D, Gene expression and TERT expression in nontumor (A,C) and tumor (B,D) in patients with hepatocellular carcinoma. E,F, Survival curves of hepatocellular carcinoma patients from The Cancer Genome Atlas database. Overall survival rates (A), and recurrence-free survival rates (B) are shown for patients with low C15orf55 and C7orf43 expression (C15low and C7low), and high C15orf55 and/or high C7orf43 expression (C15high and/or C7high). Relative gene expression levels of TERT to ACTB were normalized against that in the tumor.

and to immortalize the cells. Our data suggest that C15orf55 and C7orf43 might be critical factors for the reactivation of telomerase in HCC, in addition to the promoter mutation.
upregulated in TERT\textsuperscript{high} hepatocytes, including SP1 (1.67 ± 0.18 fold change compared with TERT\textsuperscript{low} hepatocytes), AMOTL2 (1.65 ± 0.26), and the target gene of YAP1, CYR61 (1.45 ± 0.31).\textsuperscript{33} In combination with their report, the present study indicates that C15orf55 and C7orf43 function as intrinsic TERT activators in hepatocytes. Investigation of C15orf55 and C7orf43 expression and activation of SP1 and YAP1 in TERT\textsuperscript{high} cells could provide information for the prevention of hepatocarcinogenesis.

In conclusion, the present study identified 2 novel activators of TERT expression through the TERT promoter. Our results provide new insights into the molecular background of telomerase reactivation in HCC as well as in hepatocytes. Functional analysis of the factors related to C15orf55 and C7orf43 should be undertaken to further our understanding of the molecular mechanisms of hepatocarcinogenesis. C15orf55 and C7orf43 are also promising targets for the treatment and prevention of HCC.

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CONFLICT OF INTEREST

We declare the following conflict of interest: GS has more than 5% of the total shares of KanonCure Inc. The other authors have no competing interests.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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