Identification of ZASC1 Encoding a Krüppel-like Zinc Finger Protein as a Novel Target for 3q26 Amplification in Esophageal Squamous Cell Carcinomas

Issei Imoto, Yasuhiro Yuki, Itaru Sonoda, Tetsuo Ito, Yutaka Shimada, Masayuki Imamura, and Johji Inazawa

Department of Molecular Cytogenetics, Medical Research Institute, Tokyo Medical and Dental University, Tokyo 113-8510, Japan [I. I., Y. Y., I. S., J. I.]; Core Research for Evolutional Science and Technology of Japan Science and Technology Corporation, Suita 565-0012, Japan [H. I.]; and Department of Surgery, Surgically Basic Medicine, Kyoto University Graduate School of Medicine, Kyoto 606-8507, Japan [T. I., Y. S., M. I.]

Abstract

Among the transcription factors that direct proliferation, differentiation, and death of cells, several Krüppel-like zinc finger molecules such as GLI1 and ZNF147 can become oncogenic when the genes encoding them are overexpressed by the DNA amplification mechanism. Here, we report the discovery of a novel member of this class, ZASC1, from a recently reported critical region of 3q26 amplification frequently observed in various squamous cell carcinomas, including esophageal squamous cell carcinomas (ESCs). The deduced 485-amino acid protein product of ZASC1 contained a putative nuclear localization signal; the exogenously transfected ZASC1 was translocated into cell nuclei. ZASC1 was frequently coamplified and subsequently overexpressed with PIK3CA in our panel of ESC cell lines and primary tumors. In patients with ESC, a higher mRNA expression level of ZASC1 appeared to be associated with shorter overall survival, and a multivariate analysis demonstrated that ZASC1 mRNA expression was an independent prognosticator. In addition, exogenous expression of ZASC1 promoted the growth of ESC cells, whereas down-regulation of ZASC1 expression by means of an antisense oligonucleotide suppressed the growth of ESC cells. Taken together, our results suggest that ZASC1 might be involved in the pathogenesis of ESC as one of the targets for 3q26 amplification, alone or in concert with other targets.

Introduction

Amplification of chromosomal DNA is one of the mechanisms capable of activating genes that are implicated in developing tumors. Several oncogenes, including CCND1, are known targets of gene amplification in ESCs\(^1\) (1). The advent of comparative genomic hybridization has made it possible to identify additional chromosomal amplicons that are likely to harbor previously unidentified genes associated with esophageal carcinogenesis (2, 3).

One of the regions most frequently amplified in ESC, but poorly characterized, is the long arm of chromosome 3; in most cases, the 3q26 band seems to lie in the center of the amplicon (2, 3). Amplification around 3q26 is found consistently in SCCs of the cervix, lung, and head and neck (4–8), suggesting that activation of certain genes in that region confers a selective advantage for cancer cells. However, the large physical size of overlapping amplified areas around 3q26, and variable minimal regions, make the precise definition of a common region to examine for target genes difficult. Recent studies of various SCCs have indicated that a few megabases around PIK3CA at 3q26.3 appear to constitute an apex within the 3q26 amplicon (7–9), in which amplification is associated with clinical outcome (7). Although PIK3CA is an outstanding candidate oncogene (10), independent targets may exist within the same amplicon.

Transcription factors represent an important category of molecules that can participate in cellular cascades favoring tumor development. Among them, several genes encoding Krüppel-like zinc finger proteins, such as GLI1 (11) and ZNF217 (12), have already been identified as being activated through gene amplification and involved in neoplastic transformation. Many additional members of Krüppel-like zinc finger proteins may be involved in the progression of human cancers (13), but their functional roles in carcinogenesis remain largely unknown. If the genes encoding them are seen to be located in amplified regions in tumors, these proteins will become novel targets for exploration and, potentially, therapeutic strategies.

Here, we report the identification of ZASC1, which encodes a novel Krüppel-like zinc finger protein. This gene lies 200 kb telomeric to PIK3CA on 3q26.3 and within the genomic sequence that was recently identified as one of the recurrently amplified sequences by restriction landmark genomic scanning in SCC of the lung (14), indicating it to be a separate target for 3q26 amplification in SCCs. The results of our experiments to characterize this novel gene suggest that, when ZASC1 is activated through 3q26 amplification, its consequent overexpression may be involved in the pathogenesis of ESC, either separately or in coordination with other target genes within the amplicon.

Materials and Methods

ESC Cell Lines and Primary Tumor Samples. All 31 ESC cell lines examined (KYSE series) had been established from surgically resected tumors (15). Data from comparative genomic hybridization analyses in 29 of those lines have been reported previously (3).

Primary tumor samples were obtained and frozen at the time of surgery from 31 ESC patients who were treated at the Kyoto University Hospital, with written consent from each patient in the formal style and after approval by the local ethics committee. None of the patients was treated with such preoperative therapies as radiation, chemotherapy, or immunotherapy. The median age of the patients was 64 years (range, 49–90). Tumor stages were classified according to tumor-node-metastasis classification of the International Union Against Cancer: stage I, 1 patient; stage II, 13 patients; stage III, 11 patients; stage IV, 6 patients. The clinicopathological investigation was made on the basis of guidelines for clinical and pathological studies on carcinoma of the esophagus established by the Japanese Society for Esophageal Diseases (16). The duration of overall survival was calculated for each patient from the date of primary surgery to the date of the last follow-up visit or death. The median follow-up periods were 52.7 months (range, 28.0–82.4) for 14 patients who...
are alive at the time of this writing. Total RNA was successfully extracted from all 31 tumors, and DNA from 14 of them.

Cloning and Sequencing of Human ZASC1. We used the genomic databases archived by the National Center for Biotechnology Information4 or the University of California Santa Cruz Biotechnology5 and a similarity-search program (BLAST6) to look for genes that might encode proteins containing C2H2-type Krüppel-like zinc finger motifs. Deduced amino acid sequences of all known genes, uncharacterized transcripts, ESTs that might be part of transcripts, and computer-predicted possible transcripts in the region of interest were reviewed and/or compared with the consensus sequence (13).

To obtain and confirm the cDNA sequences of transcripts of interest, we performed RT-PCR using a single-stranded cDNA synthesized by Superscript First-Strand Synthesis System (Invitrogen, Carlsbad, CA) from mRNA derived from ESC cell line KYSE-1170 and cDNA from a plasmid library as templates. For construction of the cDNA plasmid library, double-stranded cDNAs were produced from mRNA of KYSE-1170 with double-strand cDNA synthesis kits (Takara, Tokyo, Japan) and cloned into pBlueScript II SK vector (Stratagene, La Jolla, CA). PCR amplifications were performed using primers generated on the basis of EST sequences, genomic sequences, and/or universal T3 and T7 priming sites in the plasmid vector, and Pyrophosphate DNA polymerase (Takara) according to the manufacturer’s directions. Primer sequences are available on request. PCR products were subcloned and sequenced by a 377 ABI autosequencer (Applied Biosystems, Foster City, CA). Sequences were analyzed using BLAST, Motif7 and PSORT II8 programs. PCR products were subcloned and sequenced by a 377 ABI automatic sequencer (Applied Biosystems, Foster City, CA). Sequences were analyzed using BLAST, Motif7 and PSORT II8 programs.

FIC. Indirect FIC was performed as described previously (18). A plasmid construct encoding an epitope-tagged form of ZASC1 (pcDNA3.1-His-ZASC1) was assembled by cloning the full coding sequence of this gene in-frame along with Xpress epitope into the pcDNA3.1/Hisc vector (Invitrogen) and transfected into COS-7 cells using FuGENE6 (Roche Diagnostics, Tokyo, Japan) according to the manufacturer’s instructions. The cells were fixed with acetone/methanol (1:1 v/v) 48 h after transfection, and epitope-tagged ZASC1 protein was detected using monoclonal anti-Xpress antibody (Invitrogen) and FITC-conjugated antimouse secondary antibody (Medical & Biological Laboratories, Nagoya, Japan).

Southern and Northern blot Hybridizations. Southern and Northern blot analysis of genomic DNA and total RNA from each cell line, respectively, were performed as described previously (9, 18). To direct tissue-specific expression, a multiple tissue Northern blot (Human 12-tane MTN blot; Clontech, Palo Alto, CA) was used. Membranes were hybridized with [α-32P]-dCTP-labeled ZASC1 or PIK3CA cDNA probe or with a control probe (GAPDH).

Real-Time Quantitative PCR and RT-PCR. Quantification of genomic DNA and mRNA of ZASC1 in primary tumors was performed using a real-time fluorescence detection method as described previously (19, 20). Real-time quantitative PCR was performed using LightCycler (Roche Diagnostics) with DNA polymerase (Takara) and cloned into pBlueScript II SK vector (Stratagene, La Jolla, CA). PCR amplifications were performed using primers generated for each sample. PCR amplifications were performed in duplicate for each sample.

Colony Formation Assay (Anchorage-dependent Growth Assay). Plasmid expressing ZASC1 (pcDNA3.1-His-ZASC1), its complementary strand (pcDNA3.1-His-antisense), or the empty vector (pcDNA3.1-His) were transfected using FuGENE6 (Roche Diagnostics) into KYSE-410 and KYSE-790 cells for colony formation assay as described by Lin et al. (21), with minor modification. After 2 weeks of incubation with G418 (1.0 mg/ml), cells were fixed with 70% ethanol and stained by crystal violet solution.

5 Internet address: http://genome.ucsc.edu/.
7 Internet address: http://motif.genome.ad.jp/.
8 Internet address: http://cokie.imcb.osaka-u.ac.jp/nakaip/psort.html.

ASO Experiments. ASO experiments were performed as described previously (20). We synthesized the following oligonucleotides containing OPTs (Espep Oligo Service Co., Tsukuba, Ibaragi, Japan): ZASC1-AS, nucleotides 446–463 of ZASC1 cDNA (GenBank accession no. AB097862) in the antisense direction; ZASC1-IV, an inverse control for ZASC1-AS; and ZASC1-SC, a scrambled control for ZASC1-AS. OPTs were delivered into cells using Oligo-Teste (Invitrogen) according to the manufacturer’s protocol.

To evaluate expression of ZASC1, 4 × 105 cells were plated on 6-cm dishes, and their mRNA levels were determined 24 h after transfection by real-time quantitative RT-PCR. For measurements of cell growth and DNA synthesis, 2 × 103 cells were seeded in 96-well plates. Viable cells were assessed 48, 72, and 96 h after transfection of various concentrations of OPTs by the colorimetric water-soluble tetrazolium salt (WST) assay (cell counting kit-8; Dojindo Laboratories, Kumaomoto, Japan), whereas DNA synthesis was assessed 48, 72, and 96 h after transfection of OPTs by BrdUrd incorporation using a cell proliferation ELISA, BrdUrd colorimetric kit (Roche Diagnostics). For flow cytometric analysis, cells were trypsinized 48 h after transfection of OPTs, fixed in 70% ethanol overnight, and incubated in a staining buffer (0.1% Triton X-100 in PBS, 40 units/ml RNase A, and 20 μg/ml propidium iodide) on ice for 1 h. Cells were analyzed for DNA content using a FACScan cytomter and Cell Quest software (Becton Dickinson, San Jose, CA). Experiments were repeated twice, each performed in triplicate.

Statistical Analysis. Relationships between relative copy number ratios and expression levels of ZASC1 were analyzed using Spearman’s test to calculate CCs and associated Ps. Possible correlation between the variables of the analyzed primary ESC tumors and gene expression status was tested by the χ2/Fisher’s exact test. Ps of survival were calculated by the Kaplan-Meier method, and statistical differences between groups were evaluated by log-rank tests. Univariate and multivariate prognostic effects were evaluated under the Cox proportional-hazard model. One-way ANOVA with subsequent Scheffé’s tests were used to determine the significance of differences in multiple comparisons. P < 0.05 was required for significance in each case.

Results and Discussion

Identification of a Novel Gene, ZASC1, from the Critical Region of 3q26 Amplification. Because one of the apices in the 3q26 amplicon has been mapped recently within a few megabases around PIK3CA (7), we considered it feasible to search for Krüppel-like zinc finger proteins using their highly conserved motifs in silico. The critical region reported by Singh et al. (7) and information archived in the YAC-conig map database (San Antonio Genome Center9) directed our search for all possible transcripts that are located within the 2.3-megabase region between WI-4037 (YAC966F9) and WI-5357 (YAC823D8). We identified only two transcripts encoding elements of Krüppel-like zinc finger proteins, WIG1 (GenBank accession no. NM_022470) and LOC51193 (GenBank accession no. NM_016331). The WIG1 gene is induced by wild-type p53 (22), and its overexpression inhibits cell growth (23); the WIG1 is, therefore, assumed to function as a tumor suppressor. Because the LOC51193, in contrast, had never been characterized, we focused our efforts on characterizing this putative suppressor. Notably, the LOC51193 or genomic sequence AC007862 containing this transcript was recently identified as a recently amplified sequence in SCC of the lung (14), strongly suggesting that the LOC51193 locates within the core of the 3q26 amplicon. On the basis of the sequences present in LOC51193 and in Integrated Molecular Analysis and Gene Expression EST clones 3912907 and 4794621, we designed primers to amplify all coding elements as well as untranslated regions. Possible exons were connected by RT-PCR using a single-stranded cDNA template, whereas 5′ and 3′ untranslated regions were determined by RT-PCR using a plasmid cDNA library as template and universal primers along with gene-specific primers. In this manner, we assembled a 3016-bp cDNA sequence encoding a...
putative 485-amino-acid protein with a predicted molecular mass of 56.05 kDa. We designate the novel gene ZASC1 (GenBank accession no. AB097862), to indicate its nature as a Krüppel-like zinc finger protein amplified in ESCs. The genomic structure of ZASC1 was determined by comparing its cDNA sequence to the corresponding genomic sequence using the BLAST program. The ZASC1 gene consists of at least seven exons, and its genomic extent is approximately 12.5 kb (Fig. 1A). As one would have predicted from the database, FISH using a ZASC1-containing bacterial artificial chromosome as a probe produced clear doublet signals on chromosomal band 3q26.3 in normal human lymphocytes (Fig. 1B).

The deduced amino acid sequence indicated that the ZASC1 contains nine C2H2-type zinc finger motifs in its COOH-terminal portion (Fig. 1A). Krüppel-like zinc finger proteins are divided into two classes according to the number of zinc fingers present (24). Proteins in the first category contain fewer than five zinc fingers; these molecules are generally identified as transcriptional regulators involved in cell proliferation and differentiation. In contrast, the biological functions of proteins with more than five zinc fingers are largely unknown, and ZASC1 belongs to this second class. Its N-terminal 205 amino acids show no similarity with any other proteins and contain no motifs recognized by the databases. The remaining 280 amino acids toward the COOH-terminal, containing all zinc fingers, showed the most homology with ZFX (GenBank accession no. NM_003410) and ZFY (GenBank accession no. NM_003411), but identities were only 29% for both molecules. Therefore, it is difficult to speculate about the function of ZASC1 on the basis of its structure. It will be of great interest to determine whether the N-terminal sequence contains activities regulating transcription and whether the zinc fingers interact with specific DNA sequences and/or proteins. Because ZASC1 contains putative DNA-binding motifs and the PSORT II program predicted its nuclear localization, we determined the subcellular localization of transiently transfected ZASC1 by adding an epitope tag in COS-7 cells.

This approach confirmed that ZASC1 was present exclusively in cell nuclei (Fig. 1C). No detectable staining was observed in cells transfected with the parental plasmid as a control (data not shown). Although the subcellular localization should be confirmed by investigating the presence of endogenous ZASC1 using specific antibody, our result suggested that ZASC1 might function as a nuclear transcription factor.

Northern blots analysis of mRNA derived from various human tissues was performed to gain insight into the spatial distribution of ZASC1 mRNA. As shown in Fig. 1D, a single 4.8-kb transcript was expressed in most human tissues represented in the blot. Expression was abundant in heart and skeletal muscle and relatively high in kidney, liver, and pancreas.

Amplification and Subsequent Overexpression of ZASC1 in ESCs. Because ZASC1 appears to lie within the critical region of 3q26 amplification of SCCs (7), we confirmed the presence of ZASC1 in this amplicon in our panel of ESC cell lines. As shown in Fig. 2, A and B, ZASC1 was amplified in many of these cell lines. ZASC1 was always amplified in the same lines as PIK3CA, already considered a potential target for 3q26 amplification (10); PIK3CA lies only 200 kb away from the ZASC1 gene, on the centromeric side. We observed frequent amplification of ZASC1 in primary ESC tumors as well (Fig. 2D). The commonly accepted criterion for describing a putative target for amplification is that amplification of the gene in question leads to its overexpression (9, 20). On that basis, we compared expression levels of ZASC1 with its amplification status in ESC cell lines and identified consistent overexpression of ZASC1 in lines with its amplification (Fig. 2B). Interestingly, the expression of ZASC1 was up-regulated by 10% serum after 24 h-serum starvation (Fig. 2C), suggesting a role for this gene in the regulation of cell growth. The positive and significant correlation between the copy number ratio and expression status of ZASC1 also pertainied in primary ESC tumors (CC = 0.919 and P = 0.006; Fig. 2D). These lines of evidence...
 supported the view that ZASC1 might represent an independent target for 3q26 amplification in ESCs.

To elucidate the significance of ZASC1 expression in primary ESC, we then determined the mRNA expression level of ZASC1 in 31 primary tumors and compared them with clinicopathological features and clinical outcome. Tumors with ZASC1 mRNA expression greater than the median value (33.5; data not shown) were defined as ESC with high ZASC1 expression, whereas those with ZASC1 mRNA expression less than or equal to the median value were defined as ESC with low ZASC1 expression. There was no statistically significant association between ZASC1 mRNA expression and any of clinicopathological variables listed in Table 1 ( \( \chi^2 \)/Fisher’s exact test; gender, \( P > 0.999 \); age, \( P = 0.1012 \); histological type, \( P = 0.6758 \); depth of invasion, \( P = 0.4308 \);

Table 1 Cox proportional hazard regression analysis for overall survival

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate</th>
<th>Multivariate&lt;sup&gt;c&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>( n )</td>
<td>RR (95% CI)</td>
</tr>
<tr>
<td>Gender</td>
<td>Male 25</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Female 6</td>
<td>0.1915 (0.70-5.84)</td>
</tr>
<tr>
<td>Age</td>
<td>( \leq 55 ) 4</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>( &gt;55 ) 27</td>
<td>0.7221 (0.30-5.73)</td>
</tr>
<tr>
<td>Histologic type</td>
<td>Well 7</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Moderately 17</td>
<td>0.1774 (0.67-8.87)</td>
</tr>
<tr>
<td></td>
<td>Poorly 7</td>
<td>0.2344 (0.56-11.09)</td>
</tr>
<tr>
<td>Depth of invasion</td>
<td>T1, T2 13</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>T3, T4 18</td>
<td>0.7056 (0.48-3.12)</td>
</tr>
<tr>
<td>Lymphnode metastasis</td>
<td>Negative 11</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Positive 20</td>
<td>0.026 (1.22-23.81)</td>
</tr>
<tr>
<td>Stage</td>
<td>I, II 14</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>III, IV 17</td>
<td>0.0515 (0.99-8.13)</td>
</tr>
<tr>
<td>Lymphatic invasion</td>
<td>Negative 0</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Positive 22</td>
<td>0.048 (1.02-5.88)</td>
</tr>
<tr>
<td>Venous invasion</td>
<td>Negative 10</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Positive 21</td>
<td>0.046 (1.03-20.00)</td>
</tr>
<tr>
<td>ZASC1 mRNA expression&lt;sup&gt;d&lt;/sup&gt;</td>
<td>( \leq ) Median 16</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>&gt; Median 15</td>
<td>0.011 (1.37-11.36)</td>
</tr>
</tbody>
</table>

\( ^{c} \) The best model for a given patient cohort was obtained by successively eliminating the least statistically significant variables from the full Cox model containing all nine variables until only statistically significant \( (P < 0.05) \) variables were left.

\( ^{d} \) mRNA expression was determined by a real-time quantitative RT-PCR, as described in “Materials and Methods.”

Fig. 2. A, representative FISH images with the ZASC1 probe hybridized to cells from ESC lines KYSE-1170 and KYSE-1190 showed remarkable copy number increases. B, representative results from Southern (top) and northern (bottom) blots using ZASC1, PIK3CA, or a control GAPDH probe in 16 ESC cell lines. Asterisks indicate cell lines showing the amplification of ZASC1. Note that ZASC1 was always coamplified with PIK3CA, and expression levels of both genes were correlated with their copy number status. C, the mRNA expression of ZASC1 is up-regulated by serum in ESC cells (KYSE-140). Cells were serum-starved for 24 h and subsequently treated with 10% serum for the indicated times. Serum clearly increased the levels of ZASC1 mRNA 8 h after treatment. D, amplification and consequent overexpression of ZASC1 in primary ESC tumors. The genomic copy number ratio of ZASC1 normalized on the basis of \( \beta\)-2-microglobulin was divided by that of normal cells and recorded as a relative copy number (top). Expression levels of ZASC1 normalized on the basis of GAPDH were recorded as relative expression levels (bottom). Asterisks indicate cases showing clear increase (>2.0) in relative copy number of ZASC1 (7 of 14 cases, 50%). Significant correlation (CC = 0.919 and \( P = 0.006 \)) between copy numbers and relative expression levels of ZASC1 was observed. E, Kaplan-Meier curve for overall survival rates of 31 patients with ESC. The overall survival rate for tumors with high ZASC1 mRNA expression (greater than the median value) was significantly lower than that for tumors with low ZASC1 mRNA expression (less than or equal to the median value; \( P = 0.0065 \), log-rank test).
lymph node metastasis, \( P = 0.9013 \); stage, \( P = 0.5761 \); lymphatic invasion, \( P = 0.6887 \); venous invasion, \( P = 0.2602 \). As shown in Fig. 2D, however, Kaplan-Meier curves using ZASC1 mRNA expression as a categorical variable demonstrated that patients with high ZASC1 expression had a significantly shorter overall survival time than did those with low ZASC1 expression (\( P = 0.0008 \); log-rank test). Univariate analysis by use of Cox proportional hazards model demonstrated that lymph node metastasis, lymphatic invasion, venous invasion, and ZASC1 mRNA expression correlated with overall survival (Table 1). When the data were stratified for multivariate analysis using Cox backward elimination survival model, ZASC1 expression remained significant at \( P = 0.0112 \) (hazard ratio, 4.03) with stage and lymphatic invasion for overall survival (Table 1). Our results provided the first evidence that the elevated expression of gene other than \( \pi K^{3}C A \) (25) within 3q26 amplicon can be correlated with shorter overall survival and an independent prognosticator, although amplification of 3q26 was reported to correlate with tumor progression and/or clinical outcome in SCCs (7, 26). A more definitive study, including prospective study, will be necessary to determine whether ZASC1 is indeed a reliable clinical predictor of outcome for individual patients with ESC.

Effect of ZASC1 on Growth of ESC Cells. To disclose a potential role of ZASC1 in esophageal tumorigenesis, we investigated the functional consequences of ZASC1 overexpression in cell growth in vitro. We first performed a colony formation assay (21) in KYSE-410 and KYSE-790, which exhibited no amplification and low expression of ZASC1 (Fig. 2A). Transiently transfected pcDNA3.1-His-ZASC1 produced markedly more colonies than did control plasmids (pcDNA3.1-His-antisense and pcDNA3.1-His; Fig. 3A). This result was confirmed by three independent experiments, each performed in duplicate.

To assess the growth-promoting role of ZASC1, we next down-regulated ZASC1 with an ASO targeting this gene in ESC cells that had exhibited its overexpression. In KYSE-1170 cells, ZASC1-AS, but not the control OPTs (ZASC1-IV or SC) or the transfecting agent alone (mock), induced a significant decrease in the level of ZASC1 mRNA (Fig. 3B), resulting in a significant inhibition of cell growth (Fig. 3C). ZASC1-AS exhibited the most striking antisense effects among five ASOs targeting different regions of ZASC1 in our preliminary experiments (data not shown). Antisense effects were obtained with either 300 or 1000 nm OPT, 48–96 h after transfection (Fig. 3B and data not shown). The same effects were observed in KYSE-270 and KYSE-960 (data not shown). Correlated with the reduced number of viable cells, DNA synthesis determined by BrdUrd incorporation was also suppressed (Fig. 3D). Flow cytometric analysis showed an increase in the sub-G1 population of cells treated with ZASC1-AS compared with those treated with ZASC1-SC or mock 48 h after transfection (Fig. 3E), indicating that the growth inhibition brought about by ZASC1-AS treatment might also lead to the induction of cell death.

These findings suggest that ZASC1 overexpression tends to promote the growth of ESC cells, although the molecular mechanisms underlying this effect remain unknown. Functional roles in the development and progression of tumors have been reported for several Krüppel-like zinc finger proteins, such as ZNF217 and ZK7 (12, 27). ZNF217 promotes immortalization of mammary epithelial cells by inducing increases in telomerase activity and resistance to the growth inhibition caused by transforming growth factor-\( \beta \) (12), whereas ZK7 inhibits apoptosis induced by radiation or chemotherapeutic reagents (27). Like ZASC1, however, the detailed mechanisms remain unknown. Additional investigation will be necessary to clarify the intracellular functions and molecular targets of ZASC1, because it is possible that inactivation of ZASC1 may lead to the regression of ESC or other SCCs, and it may become an optimal molecular target for cancer therapy.

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References

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