


# Decreased carbonyl reductase 1 expression promotes tumor growth via epithelial mesenchymal transition in uterine cervical squamous cell carcinomas

Yuki Nishimoto | Akihiro Murakami | Shun Sato | Takuya Kajimura |  
Kengo Nakashima | Kazuyuki Yakabe | Kotaro Sueoka | Norihiro Sugino 

Department of Obstetrics and Gynecology,  
Yamaguchi University Graduate School of  
Medicine, Ube, Japan

## Correspondence

Norihiro Sugino, Department of Obstetrics  
and Gynecology, Yamaguchi University  
Graduate School of Medicine, Ube, Japan.  
Email: sugino@yamaguchi-u.ac.jp

## Funding information

This work was supported in part by Japan  
Society for the Promotion of Science  
KAKENHI grants no. 23592425, 23791845,  
26462525, 15K10719, and 16H07009 for  
Scientific Research from the Ministry of  
Education, Science, and Culture, Japan.

## Abstract

**Purpose:** Carbonyl reductase 1 (CBR1) is involved in cancer progression. Recently, the authors reported that the loss of CBR1 expression is associated with a poor prognosis in uterine cervical cancer. Here, we investigated whether the decreased CBR1 expression promotes cancer progression by inducing the epithelial mesenchymal transition (EMT).

**Methods:** Antisense constructs of *CBR1* complementary DNA (antisense clones) and the empty vectors (control clones) were transfected into human uterine cervical squamous cell carcinoma cell lines (SKG II and SiHa) and the proliferation and EMT marker expression of these clones were analyzed in vitro. In an in vivo study,  $10^7$  cells of the antisense and control clones were subcutaneously injected into nude mice and the tumorigenesis was observed for 8 weeks.

**Results:** With the decreased CBR1 expression, the proliferation of the antisense clones increased, accompanied by a decrease in epithelial markers (E-cadherin and cytokeratin) and an increase in mesenchymal markers (fibronectin, alpha-smooth muscle actin, and N-cadherin), which suggests EMT induction. In the in vivo study, the tumor volume in the antisense group was significantly larger than that in the control group.

**Conclusion:** Decreased CBR1 expression promotes tumor growth by inducing EMT in uterine cervical squamous cell carcinomas.

## KEYWORDS

cancer progression, carbonyl reductase 1, cervical squamous cell carcinoma, epithelial mesenchymal transition, tumorigenesis

## 1 | INTRODUCTION

Carbonyl reductase 1 (CBR1) is a nicotinamide adenine dinucleotide phosphate-dependent, mostly monomeric, cytosolic enzyme with a broad substrate specifically for carbonyl compounds.<sup>1,2</sup> Therefore,

it has been studied extensively in relation to its ability to reduce a variety of carbonyl compounds: antitumor anthracycline antibiotics, daunorubicin and doxorubicin, and prostaglandins.<sup>3,4</sup> Carbonyl reductase 1 is also present in a variety of organs, including the liver, kidney, breast, ovary, and vascular endothelial cells, and its primary

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2018 The Authors. *Reproductive Medicine and Biology* published by John Wiley & Sons Australia, Ltd on behalf of Japan Society for Reproductive Medicine.

function is considered to be to control fatty acid metabolism.<sup>5</sup> Interestingly, CBR1 has been shown to regulate the malignant behaviors of cancer cells. For example, the decreased expression of CBR1 has promoted cell proliferation activities and tumorigenesis in vivo, with the loss of E-cadherin expression and the activation of matrix metalloproteinases in ovarian, uterine cervical, or uterine endometrial cancers.<sup>6-10</sup> Thus, the decreased expression of CBR1 promotes tumor growth and metastatic activities and is closely associated with lymph node metastasis and a poor prognosis in ovarian, uterine cervical, and uterine endometrial cancers.<sup>7,8,10</sup> In contrast, the increased expression of CBR1 has suppressed cell proliferation activities and tumor growth in various in vitro and in vivo experiments in ovarian cancers.<sup>9-11</sup>

The epithelial mesenchymal transition (EMT) is involved in the progression of some malignant tumors.<sup>12-15</sup> With regards to EMT, cancer cells are transformed into fibroblast-like cells with decreased E-cadherin expression. The authors reported that the suppression of CBR1 expression stimulated the invasion of cancer cells, with the reduction of E-cadherin expression, in uterine cervical cancer.<sup>7,8</sup> In addition, the authors recently found that decreased CBR1 expression promotes the invasive activity of cancer cells in endometrial adenocarcinomas by inducing EMT.<sup>8</sup> Therefore, CBR1 seems to play an important role in cancer progression by regulating EMT in cervical cancer.

The authors previously reported that, in vitro, CBR1 suppression increased cancer cell invasion, accompanied by a decrease in E-cadherin expression.<sup>7</sup> However, it is unclear whether the CBR1 suppression actually promotes tumor growth in vivo, and if it does, by what mechanism. In order to answer these questions, here it has been investigated whether the decreased expression of CBR1 promotes tumor growth in uterine cervical squamous cell carcinomas and, if this is the case, whether the decrease in CBR1 expression is associated with EMT.

## 2 | MATERIALS AND METHODS

### 2.1 | Cell line and culture

SiHa and SKG II, which are human uterine cervical squamous cell carcinoma cell lines, were used. The SiHa cells were purchased from Cell Resource Center for Biomedical Research (Tohoku University, Sendai, Japan) and the SKG II cells were purchased from Health Science Research Resources Bank (Osaka, Japan). The SiHa cells were cultured in Eagle's minimal essential medium (Sigma-Aldrich, Tokyo, Japan) that was supplemented with 10% heat-inactivated fetal calf serum (FCS). The SKG II cells were cultured in Ham's F12 medium (Sigma-Aldrich) that was supplemented with 10% FCS. The cells were seeded at a density of  $5 \times 10^4$  cells/well in a six-well microtiter plate in the medium that was supplemented with 10% FCS. Then, they were incubated at 37°C in a humidified 5% CO<sub>2</sub> incubator for 7 days. The cells were trypsinized and counted with a cell counter (Vi-CELL XR; Beckman Coulter, Tokyo, Japan) at each point, as reported previously.<sup>16</sup>

### 2.2 | Gene transfection procedures

Stable clones were established in which the CBR1 expression was suppressed. The antisense construct was transfected into SiHa and SKG II cells, as reported previously.<sup>7,8,17,18</sup> The suppression of CBR1 was verified by Western blot analyses. A clone transfected with the empty vector was used as a control. In the preliminary experiments, the reagent that was used for transfection did not influence cell mobility or CBR1 expression.

### 2.3 | Western blot analyses

The cells were resuspended in radioimmunoprecipitation assay buffer (WAKO, Tokyo, Japan) and sonicated. The insoluble materials were removed by centrifugation at 20 000 g for 10 minutes at 4°C and the samples were boiled for five minutes after SDS sample buffer (New England BioLabs, Tokyo, Japan) was applied. The proteins (10 µg) were electrophoresed on 10% of SDS-polyacrylamide gel (PAGE). After SDS-PAGE, the proteins were transferred to a polyvinylidene difluoride membrane (New England BioLabs) with a semidry-type blotting system. After blocking the membrane with blocking solution, the blotted membrane was incubated with goat antihuman CBR1 polyclonal antibody (Abcam, Tokyo, Japan), rabbit antihuman E-cadherin monoclonal antibody (Abcam), mouse antihuman cytokeratin monoclonal antibody (Abcam), rabbit antihuman alpha-smooth muscle actin (αSMA) monoclonal antibody (Abcam), rabbit antihuman fibronectin polyclonal antibody (Abcam), and rabbit antihuman N-cadherin polyclonal antibody (Abcam) as the first antibodies (diluted at 1:1000 in the blocking solution). The membrane then was incubated with the peroxidase-conjugated secondary antibody. Last, the membrane was incubated in ECL-Western blotting detection reagents (GE Healthcare, Little Chalfont, UK) for five minutes and used to expose the Hyperfilm-ECL (GE Healthcare).

### 2.4 | Real-time polymerase chain reaction

The expression of a transcription factor of E-cadherin, *SNAIL*, was examined by semiquantitative real-time polymerase chain reaction (RT-PCR). The total RNA was isolated by using a RNeasy mini kit (QIAGEN, Tokyo, Japan). The complementary (c)DNA was synthesized from 1 µg of total RNA by using a QuantiTect Reverse Transcription Kit (Qiagen, Valencia, CA, USA). The RT-PCR was performed by using BIOTaq HS DNA Polymerase (BIOLINE, Tokyo, Japan), according to the manufacturer's protocol, with an amplifying primer pair for *SNAIL* (5'-ctccctgtcagatgaggacagt-3' and 5'-tccttggtgcagtatttgca-3') and glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) (5'-tgcaccaccaactgcttagc-3' and 5'-ggcatggactgtgcatgag-3') serving as an internal control. The thermal cycling conditions were 25 cycles (*GAPDH*) or 32 cycles (*SNAIL*) of 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 15 seconds, with an initial step of 95°C for 10 minutes. The PCR products were electrophoresed on a 2% agarose gel.

## 2.5 | In vivo experiments

This in vivo study was approved by the Committee for Ethics on Animal Experiments of Yamaguchi University Graduate School of Medicine, Ube, Japan (Certification No. 47-021). The SiHa cells ( $1 \times 10^7$  cells) were subcutaneously injected into female BALB/c nude mice (four weeks old). The tumor size was measured with calipers every week for 8 weeks after injection. The tumor volume was calculated according to the formula:  $V = 0.52 \times A^2 \times B$  (A, the smallest superficial diameter; B, the largest superficial diameter).<sup>19</sup>

## 2.6 | Immunohistochemistry

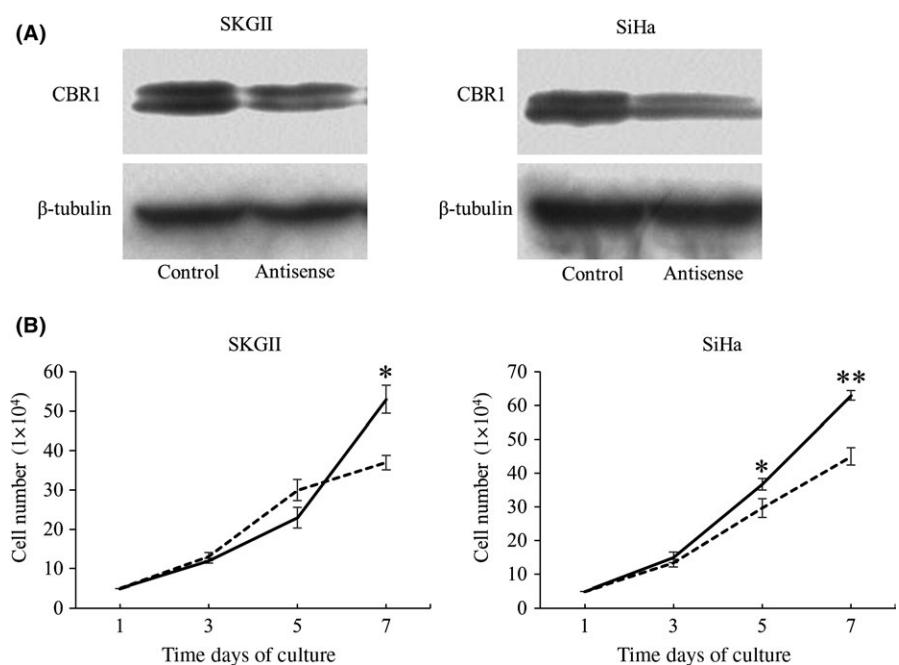
After eight weeks of injection, the mice were sacrificed and then the tumors were removed. Immunohistochemistry was performed by the streptavidin–biotin–peroxidase complex technique, as reported previously.<sup>7</sup> The tumor specimens were fixed in 10% buffered formalin and embedded in paraffin. The tissue sections (3  $\mu$ m thick) were deparaffinized and washed with cold phosphate-buffered saline (PBS). Endogenous peroxidase was blocked by incubating with  $H_2O_2$  (0.5% in methanol) for 50 minutes at room temperature (RT). Then, the sections were washed three times in cold PBS, incubated with normal goat serum (10%) to block non-specific binding, and sequentially incubated with anti-CBR1 (Abcam), anti-E-cadherin (Abcam), and anti- $\alpha$ SMA antibodies (Abcam) at 4°C overnight. Thereafter, the sections were washed and incubated for 30 minutes at RT with biotinylated antirabbit immunoglobulin (Ig)G + IgA + IgM by using the HISTIFINE SAB-PO kit (Nichirei, Tokyo, Japan). The sections were reacted with diaminobenzidine tetrahydrochloride chromogen mixture (Sigma-Aldrich). After counterstaining with hematoxylin, the slides were permanently mounted.

The immunohistochemical expression was quantified according to the authors' previous method.<sup>20</sup> a score was established corresponding to the sum of: (i) the percentage of positive cells (0, 0% immunopositive cells; 1, <50% positive cells; 2, >50% positive cells); and (ii) the staining intensity (0, absent; 1, weak; 2, moderate; 3, strong). The scoring was done on 10 randomly chosen areas at  $\times 200$  in the tissue sections from three tumor samples that had been obtained from each group. The scoring was performed by three independent observers. An observer-related mean was calculated for each tumor sample and the mean of the three observer-related means was used as a single observation.

## 2.7 | Microarray analysis and pathway analysis

The antisense cDNA to *CBR1* was transfected into SKG II (antisense) cells. A clone transfected with the empty vector was used as a negative control. The transcriptome of each clone was analyzed, as reported previously.<sup>21</sup> The total RNAs were isolated from cells by using a RNeasy mini kit (QIAGEN). The gene expression was analyzed by using a GeneChip Human Genome 2.0 ST Array (Affymetrix, Santa Clara, CA, USA), supporting 40 716 genes. The target cDNA was prepared from 250 ng of total RNA with the Ambion WT Expression kit (Ambion, Austin, TX, USA) and the GeneChip WT PLUS reagent kit (Affymetrix). Hybridization to the microarrays, washing, staining, and scanning were performed by using the GeneChip system (Affymetrix) that was composed of the Scanner 30007 G Workstation Fluidics 450 and the Hybridization Oven 645. The scanned image data were processed by using a gene expression analysis with the Partek Genomics Suite 6.5 software program (Partech, Munster, Germany). Those genes were extracted in which the expression in the antisense group was greater than 1.5-fold or less than two-thirds of that in the control group. Then, a pathway analysis was performed by using Ingenuity

**FIGURE 1** Establishment of clones with suppressed carbonyl reductase 1 (CBR1) expression. Antisense complementary DNA to *CBR1* was transfected into the uterine cervical cancer cell lines, SKG II and SiHa (antisense) cells. The control clone transfected with the empty vector was used as a negative control. A, The expression levels of CBR1 in the transfected cells were analyzed by Western blotting.  $\beta$ -tubulin was used as an internal control. B, The effects of CBR1 suppression on cancer cell proliferation. The number of cells was counted at each point. The values are shown as the mean  $\pm$  SE. \* $P < .05$  and \*\* $P < .01$ , compared to the control. (—), Antisense (---), control



Pathway Analysis (IPA) software (Ingenuity Systems, Redwood City, CA, USA).

## 2.8 | Statistical analyses

The significance of the difference between the two groups was analyzed by Tukey's test. A probability value of  $P < .05$  was considered to be significant. All the statistical analyses were performed by using the SPSS 5.0 J for Windows software package (SAS Institute, Inc., Cary, NC, USA).

## 3 | RESULTS

### 3.1 | Clones with suppressed carbonyl reductase 1 expression

In order to investigate the role of CBR1 on the malignant potential of squamous cell carcinoma cells, clones with suppressed CBR1 expression were established by transfecting antisense cDNA to *CBR1* into SKG II and SiHa cells. The cells that had been transfected with the empty vector were used as negative controls (control clones). As expected, SKG II and SiHa clones were obtained with decreased CBR1 expression (Figure 1A).

### 3.2 | Effect of carbonyl reductase 1 suppression on cell proliferation

The cell proliferation was significantly higher in the decreased CBR1 (antisense) group than in the control group on day 7 in the SKG II cells and on days 5 and 7 in the SiHa cells (Figure 1B).

### 3.3 | Effect of carbonyl reductase 1 suppression on the epithelial mesenchymal transition markers

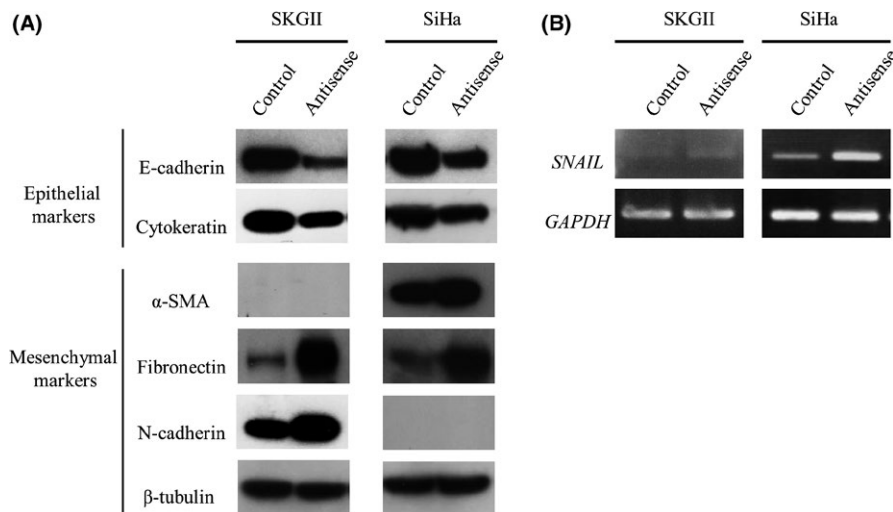
The expression levels of the epithelial markers, E-cadherin and cytokeratin, as measured by Western blotting, were lower in the decreased CBR1 (antisense) group, compared with the control group, in the SKG II and SiHa cells (Figure 2A). On the contrary, the expression level of the mesenchymal markers,  $\alpha$ SMA, fibronectin, and N-cadherin, were higher in the decreased CBR1 (antisense) group in the SKG II or SiHa cells (Figure 2A).

SNAIL is a transcriptional factor that suppresses E-cadherin and regulates EMT and is reported to be a useful predictor of the prognosis of several cancers.<sup>22-26</sup> The expression of *SNAIL* mRNA that was examined by the RT-PCR was higher in the decreased CBR1 (antisense) group, compared with the control group, in the SKGII and SiHa cells (Figure 2B).

There seems to be some differences in the expression of the EMT markers between the SiHa and SKGII cells. In fact, in the authors' previous report,<sup>12</sup> the apoptotic effect of the knock-down of heat shock protein 70 was more apparent in the SiHa cells than in the SKGII cells. Thus, the different expression of the EMT markers could be related to the different characteristics of the cells.

### 3.4 | In vivo effects of carbonyl reductase 1 suppression on tumor growth

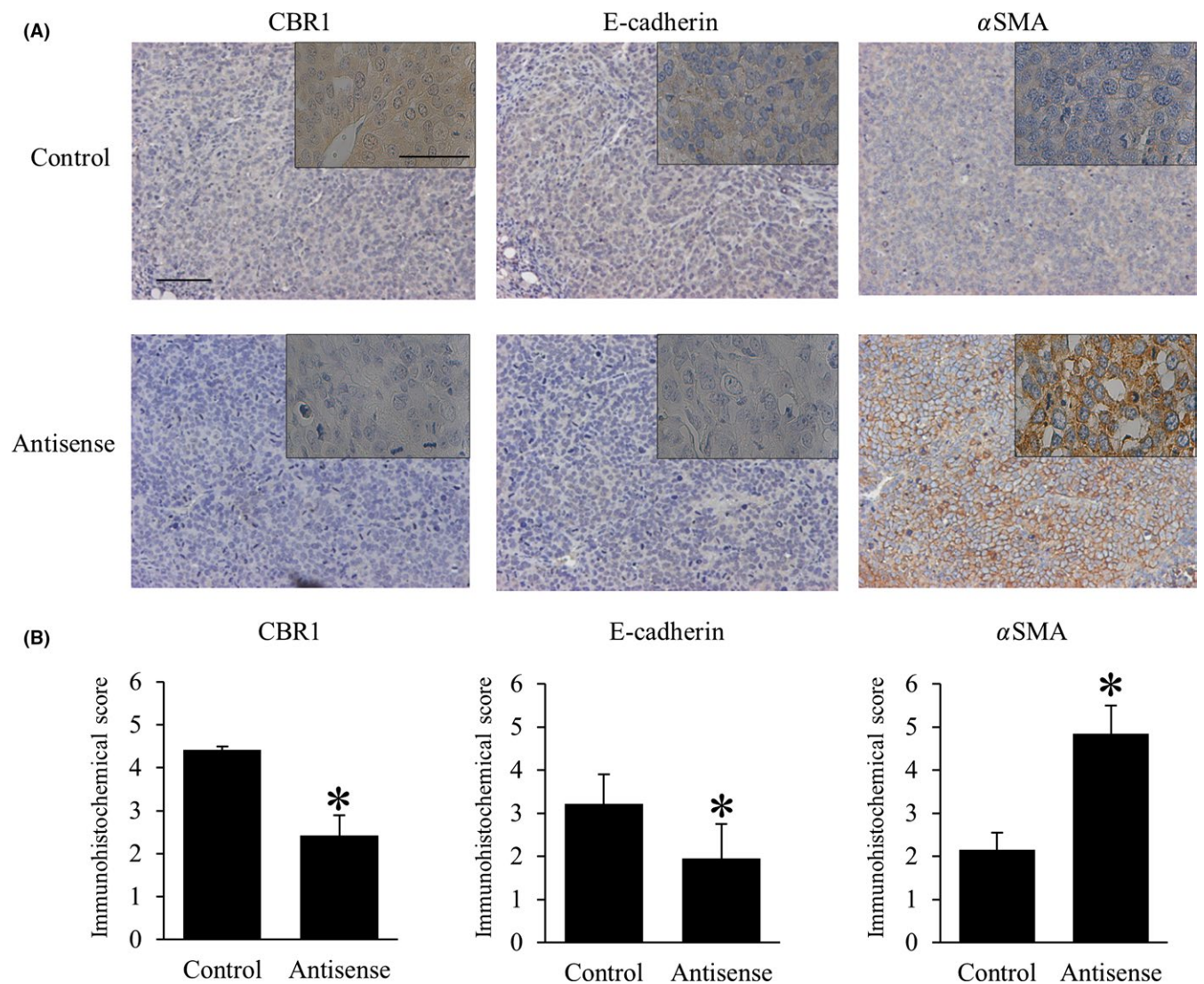
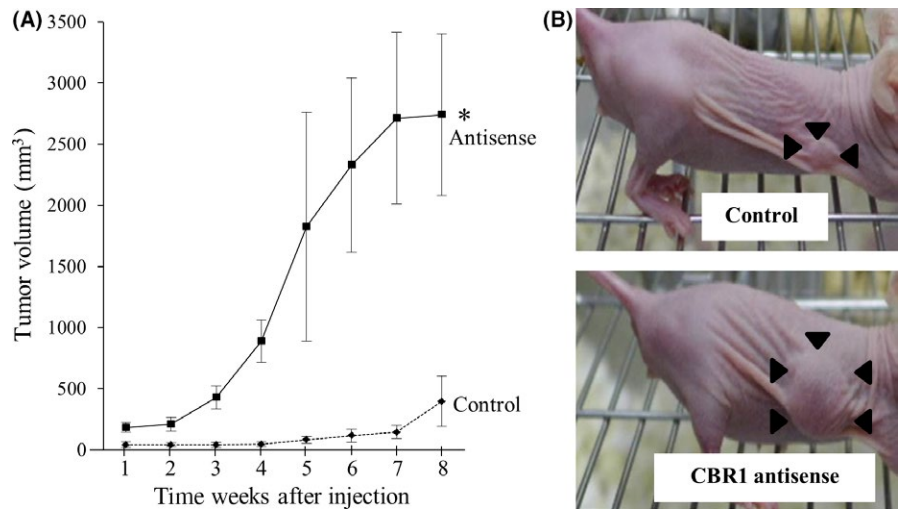
Tumors in the decreased CBR1 (antisense) group grew rapidly, compared with the tumors in the control group (Figure 3A). In the nude mice 8 weeks after cancer cell injection, the tumor size was significantly larger in the decreased CBR1 (antisense) group than in the control group (Figure 3B).



**FIGURE 2** Effects of carbonyl reductase 1 (CBR1) suppression on epithelial mesenchymal transition (EMT) markers. Antisense complementary DNA to *CBR1* was transfected into the SKG II and SiHa (antisense) cells. The control clone transfected with the empty vector was used as a negative control. A, The expression levels of E-cadherin and cytokeratin as epithelial markers and of alpha-smooth muscle actin ( $\alpha$ SMA), fibronectin, and N-cadherin as mesenchymal markers were analyzed by Western blotting.  $\beta$ -tubulin was used as an internal control. The expression levels of  $\alpha$ SMA in the SKG II cells and N-cadherin in the SiHa cells were too low to be detected. B, As a transcription factor of EMT, *SNAIL* was examined by real-time polymerase chain reaction. Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) was used as an internal control



**FIGURE 3** In vivo effects of carbonyl reductase 1 (CBR1) suppression on tumor growth. Cells ( $1 \times 10^7$  cells) transfected with antisense complementary DNA to CBR1 (antisense group;  $n = 9$ ) or the empty vector (control group;  $n = 8$ ) were subcutaneously injected into female BALB/c nude mice (4 weeks old). A, The tumor size was measured every week for eight weeks after injection.  $*P < .01$ , compared to the control. B, Representative photographs of tumor growth 8 weeks after injection are shown



**FIGURE 4** Immunohistochemical analysis of carbonyl reductase 1 (CBR1) and epithelial mesenchymal transition-related markers in the tumors that can be seen in Figure 3. A, E-cadherin is an epithelial marker and alpha-smooth muscle actin ( $\alpha$ SMA) is a mesenchymal marker. The inserts indicate the high-power field. Scale bars: 100  $\mu$ m and 50  $\mu$ m in the low- and high-power fields, respectively. B, A quantitative analysis of the immunohistochemical expression of CBR1, E-cadherin, and  $\alpha$ SMA shows the mean  $\pm$  SE of three tumor samples.  $*P < .05$ , compared to the control

**TABLE 1** Top 20 genes that were upregulated in the carbonyl reductase 1 (CBR1)-suppressed cells

Gene symbol	Gene description	Fold change
<i>PLXDC2</i>	Plexin domain containing 2	13.61
<i>IFIT1</i>	Interferon-induced protein with tetratricopeptide repeats 1	10.24
<i>OAS2</i>	2'-5'-Oligoadenylate synthetase 2, 69/71 kDa	10.01
<i>OAS1</i>	2'-5'-Oligoadenylate synthetase 1, 40/46 kDa	9.32
<i>IFIH1</i>	Interferon-induced with helicase C domain 1	8.67
<i>PCDHB9/PCDHB10</i>	Protocadherin beta 9/protocadherin beta 10	8.55
<i>RN5S402</i>	RNA, 5S ribosomal 402	8.18
<i>IFI6</i>	Interferon, alpha-inducible protein 6	7.73
<i>IFITM1</i>	Interferon-induced transmembrane protein 1	7.59
<i>IFI44L</i>	Interferon-induced protein 44-like	7.34
<i>CCL2</i>	Chemokine (C-C motif) ligand 2	6.83
<i>LIPH</i>	Lipase, member H	6.79
<i>MX1</i>	Myxovirus (influenza virus) resistance 1, interferon-inducible protein p78 (mouse)	6.70
<i>CDH11</i>	Cadherin 11, type 2, osteoblast cadherin	6.45
<i>ANKRD1</i>	Ankyrin repeat domain 1 (cardiac muscle)	6.21
<i>FNDC1</i>	Fibronectin type III domain containing 1	6.05
<i>PSG5</i>	Pregnancy-specific beta-1-glycoprotein 5	6.01
<i>PRSS21</i>	Protease, serine, 21 (testisin)	5.89
<i>IFIT3</i>	Interferon-induced protein with tetratricopeptide repeats 3	5.62
<i>SNORA46</i>	Small nucleolar RNA, H/ACA box 46	5.40

The top 20 genes among the 457 genes that were upregulated in the CBR1-suppressed cells, as compared to the control (empty vector) cells, are shown.

### 3.5 | Immunohistochemistry

The immunohistochemical studies revealed that the tumors in the decreased CBR1 (antisense) group stained more weakly for CBR1 and E-cadherin than did the control group, while they stained more strongly for  $\alpha$ SMA than did the control group (Figure 4A). By quantitative analysis for the immunohistochemical expression, there were

**TABLE 2** Top 20 genes that were downregulated in the carbonyl reductase 1 (CBR1)-suppressed cells

Gene symbol	Gene description	Fold change
<i>ABI3BP</i>	ABI family, member 3 (NESH) binding protein	0.12
<i>FAM163A</i>	Family with sequence similarity 163, member A	0.14
<i>INHBA</i>	Inhibin, beta A	0.19
<i>LINC00052</i>	Long intergenic non-protein coding RNA 52	0.23
<i>ERP27</i>	Endoplasmic reticulum protein 27	0.24
<i>NPR2</i>	Natriuretic peptide receptor B/guanylate cyclase B (atrio-atriuretic peptide receptor B)	0.27
<i>CDH1</i>	Cadherin 1, type 1, E-cadherin (epithelial)	0.27
<i>TNIK</i>	TRAF2 and NCK interacting kinase	0.27
<i>ANPEP</i>	Alanyl (membrane) aminopeptidase	0.28
<i>AK5</i>	Adenylate kinase 5	0.29
<i>VGLL1</i>	Vestigial like 1 (Drosophila)	0.29
<i>MAGED1</i>	Melanoma antigen family D, 1	0.29
<i>SPOCK1</i>	Sparc/osteonectin, cwcv- and kazal-like domains proteoglycan (testican) 1	0.29
<i>NEGR1</i>	Neuronal growth regulator 1	0.30
<i>GRHL2</i>	Grainyhead-like 2 (Drosophila)	0.31
<i>FAM27E3</i>	Family with sequence similarity 27, member E3	0.31
<i>RAI2</i>	Retinoic acid-induced 2	0.33
<i>FBXO32</i>	F-box protein 32	0.34
<i>NTRK2</i>	Neurotrophic tyrosine kinase, receptor type 2	0.35
<i>TMLHE</i>	Trimethyllysine hydroxylase, epsilon	0.35

The top 20 genes among the 269 genes that were downregulated in the CBR1-suppressed cells, as compared to the control (empty vector) cells, are shown.

significant differences in the expression of CBR1, E-cadherin, and  $\alpha$ SMA between the control group and the antisense group (Figure 4B). These findings suggest that the growth of the tumors in the decreased CBR1 (antisense) group involved EMT.

### 3.6 | Microarray analysis and pathway analysis

In order to investigate how CBR1 regulates EMT, a microarray analysis and IPA analysis were performed. The suppression of the CBR1 upregulated 457 genes and downregulated 269 genes. The top 20 upregulated and downregulated genes are shown in Tables 1 and 2, respectively. The downregulated genes included E-cadherin, while the

**TABLE 3** Top 15 canonical pathways for the aberrantly expressed genes, by carbonyl reductase 1 (CBR1) suppression

Ingenuity canonical pathway	P-value
Interferon signaling	$7.24 \times 10^{-6}$
Hepatic fibrosis/hepatic stellate cell activation	$2.75 \times 10^{-4}$
Role of pattern recognition receptors in recognition of bacteria and viruses	$3.39 \times 10^{-4}$
Wingless-related integration site/ $\beta$ -catenin signaling	$4.57 \times 10^{-4}$
Activation of IRF by cytosolic pattern recognition receptors	$5.13 \times 10^{-4}$
Extracellular signal-regulated kinase 5 signaling	$2.51 \times 10^{-3}$
Oncostatin M signaling	$2.82 \times 10^{-3}$
Integrin-linked kinase signaling	$3.47 \times 10^{-3}$
Aryl hydrocarbon receptor signaling	$3.63 \times 10^{-3}$
Transforming growth factor $\beta$ signaling	$3.80 \times 10^{-3}$
P2Y Purigenic receptor signaling pathway	$4.47 \times 10^{-3}$
Unfolded protein response	$4.68 \times 10^{-3}$
Neurotrophin/transfer RNA-Lys signaling	$4.90 \times 10^{-3}$
Antiproliferative role of TOB in T cell signaling	$6.31 \times 10^{-3}$
Thyroid cancer signaling	$6.46 \times 10^{-3}$

The 726 aberrantly expressed genes (457 upregulated and 269 downregulated genes) by CBR1 suppression were analyzed by an ingenuity pathway analysis (IPA). Among the 59 canonical pathways that were obtained from the IPA, the top 15 pathways are shown. IRF, interferon regulatory factor; TOB, transducer of ErbB.

upregulated genes included fibronectin. An IPA analysis of the 726 upregulated and downregulated genes identified many potential pathways, the top 15 of which are shown in Table 3. Two of the pathways involve Wnt/ $\beta$ -catenin signaling and transforming growth factor- $\beta$  (TGF- $\beta$ ) signaling, which are involved in EMT.

## 4 | DISCUSSION

The authors previously reported that CBR1 suppression changed the morphology of cervical cancer cells, enhanced their invasive activity, and decreased their expression of E-cadherin *in vitro*.<sup>7,8</sup> In the present study, CBR1 suppression increased the proliferation activity of cancer cells, with both a decrease in epithelial markers (E-cadherin and cytokeratin) and an increase in mesenchymal markers (fibronectin,  $\alpha$ SMA, and N-cadherin), as well as an increase in a transcription factor of E-cadherin (SNAIL), which are characteristic features of EMT.<sup>27-31</sup> Furthermore, cancer cells with suppressed CBR1 expression showed a high level of activity of tumorigenesis *in vivo* (Figure 3). These results suggest that CBR1 suppression promotes tumor growth by inducing EMT in squamous cell carcinoma cells.

The present results are in agreement with previous reports on ovarian cancer and uterine endometrial cancer: a close relationship between the decreased CBR1 expression and lymph node metastasis or a poor prognosis has been found in ovarian cancer.<sup>19</sup> Cancer cells with the decreased CBR1 expression have a high level of metastatic activity.<sup>6</sup> The authors' previous reports also showed that suppression of the CBR1 expression promoted malignant behaviors by inducing EMT and that the loss of CBR1 expression was associated with a poor prognosis in uterine endometrial cancers.<sup>8</sup>

It is unclear how CBR1 regulates EMT. In this study, it was found that CBR1 suppression stimulated the intracellular signaling pathways of TGF- $\beta$  and Wnt/ $\beta$ -catenin (Table 3), both of which have been reported to play roles in regulating EMT.<sup>32-40</sup> The authors speculate that CBR1 regulates EMT by interacting with the TGF- $\beta$  and Wnt/ $\beta$ -catenin pathways. It would be interesting to investigate in follow-up studies whether TGF- $\beta$  actually induces EMT through a Wnt/ $\beta$ -catenin pathway in uterine cervical cancer cells.

The inhibitory effect of CBR1 on ovarian cancer growth is also reported to be mediated by the activation of the tumor necrosis factor receptor (TNFR) pathway.<sup>11</sup> However, this study's microarray and IPA analyses did not detect TNFR signaling. It is hard to explain why CBR1 does not interact with TNFR signaling in uterine cervical cancer cells, while it does in ovarian cancer cells. The difference could be related to that the intracellular signaling pathways that are involved in cancer progression differ among cancers.

It is unclear whether the CBR1 action is mediated by its enzyme activity. The authors found in preliminary experiments that E-cadherin expression was decreased by a CBR1 inhibitor that inhibits CBR1 enzyme activities in cervical cancer cells, suggesting that CBR1 at least partially works as an enzyme.

The present study suggests that CBR1 can be a new target molecule controlling uterine cervical cancer. Increasing CBR1 expression could be a new strategy for the treatment of uterine cervical cancer. Although the effect of the overexpression of CBR1 on the malignant behaviors of uterine cervical cancer cells was not investigated in this study, the authors previously reported that the overexpression of CBR1 (induced by transfection of sense cDNAs into the SiHa cells) increased E-cadherin expression and decreased the secretion of matrix metalloproteinases.<sup>7</sup> Thus, it is expected that increasing CBR1

expression would inhibit tumorigenesis in squamous cell carcinoma cells. Interestingly, clofibric acid, which is a peroxisome proliferator-activated receptor  $\alpha$  ligand and is used for the treatment of hyperlipidemia, increased CBR1 expression and inhibited tumor growth in human ovarian cancer.<sup>41</sup> Thus, clofibric acid appears to be a promising agent for the treatment of uterine cervical cancer.

At present, uterine cervical cancers are the most common cause of death among young women. Although there are effective vaccines for human papillomavirus, it is still hard to treat patients with advanced cancers and recurrent diseases. This study might provide a novel therapeutic strategy that targets CBR1 for uterine cervical cancer.

## DISCLOSURES

**Conflict of interest:** The authors declare no conflict of interest. **Human rights statement and informed consent:** All the authors contributed significantly and were in agreement with the content of the manuscript. This article does not contain any study with human participants that was performed by any of the authors. **Animal studies:** All the institutional and national guidelines for the care and use of laboratory animals were followed.

## ORCID

Norihiro Sugino  <http://orcid.org/0000-0001-8874-7931>

## REFERENCES

- Penning TM, Drury JE. Human aldo-keto reductases: function, gene regulation, and single nucleotide polymorphisms. *Arch Biochem Biophys.* 2007;464:241-250.
- Mindnich RD, Penning TM. Aldo-keto reductase (AKR) superfamily: genomics and annotation. *Hum. Genomics.* 2009;3:362-370.
- Miura T, Nishinaka T, Terada T. Different functions between human monomeric carbonyl reductase 3 and carbonyl reductase 1. *Mol Cell Biochem.* 2008;315:113-121.
- Gonzales-Covarrubias V, Ghosh D, Lakhman SS, Pendyala L, Blanco JG. A functional genetic polymorphism on human carbonyl reductase 1 (CBRV881) impacts on catalytic activity and NADPH binding affinity. *Drug Metab Dispos.* 2007;35:973-980.
- Wermuth B, Bohren KM, Heinemann G, vonWartburg JP, Gabbay KH. Human carbonyl reductase. Nucleotide sequence analysis of a cDNA and amino acid sequence of the encoded protein. *J Biol Chem.* 1988;263:16185-16188.
- Ismail E, Al-Mulla F, Tsuchida S, et al. Carbonyl reductase: a novel metastasis-modulating function. *Cancer Res.* 2000;60:1173-1176.
- Murakami A, Fukushima C, Yoshidomi K, et al. Suppression of carbonyl reductase expression enhances malignant behavior in uterine cervical squamous cell carcinoma: carbonyl reductase predicts prognosis and lymph node metastasis. *Cancer Lett.* 2011;311:77-84.
- Murakami A, Yakabe K, Yoshidomi K, et al. Decreased carbonyl reductase 1 expression promotes malignant behaviours by induction of epithelial mesenchymal transition and its clinical significance. *Cancer Lett.* 2012;323:69-76.
- Osawa Y, Yokoyama Y, Shigeto T, Futagami M, Mizunuma H. Decreased expression of carbonyl reductase 1 promotes ovarian cancer growth and proliferation. *Int J Oncol.* 2015;46:1252-1258.
- Umamoto M, Yokoyama Y, Sato S, Tsuchida S, Al-Mulla F, Saito Y. Carbonyl reductase as a significant predictor of survival and lymph node metastasis in epithelial ovarian cancer. *Br J Cancer.* 2001;85:1032-1036.
- Miura R, Yokoyama Y, Shigeto T, Futagami M, Mizunuma H. Inhibitory effect of carbonyl reductase 1 on ovarian cancer growth via tumor necrosis factor receptor signaling. *Int J Oncol.* 2015;47:2173-2180.
- Bates RC, Mercurio AM. The epithelial-mesenchymal transition (EMT) and colorectal cancer progression. *Cancer Biol Ther.* 2005;4:365-370.
- Ahmed N, Abubaker K, Findlay J, Quinn M. Epithelial mesenchymal transition and cancer stem cell-like phenotypes facilitate chemoresistance in recurrent ovarian cancer. *Curr Cancer Drug Targets.* 2010;10:268-278.
- Lamouille S, Xu J, Derynck R. Molecular mechanisms of epithelial-mesenchymal transition. *Nat Rev Mol Cell Biol.* 2014;15:178-196.
- Thiery JP, Acloque H, Huang RY, Nieto MA. Epithelial-mesenchymal transitions in development and disease. *Cell.* 2009;139:871-890.
- Yoshidomi K, Murakami A, Yakabe K, Sueoka K, Nawata S, Sugino N. Heat shock protein 70 is involved in malignant behaviors and chemosensitivities to cisplatin in cervical squamous cell carcinoma cells. *J Obstet Gynaecol Res.* 2014;40:1188-1196.
- Murakami A, Nakagawa T, Kaneko M, et al. Suppression of SCC antigen promotes cancer cell invasion and migration through the decrease in E-cadherin expression. *Int J Oncol.* 2006;29:1231-1235.
- Nakagawa T, Murakami A, Torii M, Nawata S, Takeda O, Sugino N. E-cadherin increases squamous cell carcinoma antigen expression through phosphatidylinositol-3 kinase-Akt pathway in squamous cell carcinoma cell lines. *Oncol Rep.* 2007;18:175-179.
- Yakabe K, Murakami A, Kajimura T, et al. Functional significance of transgelin-2 in uterine cervical squamous cell carcinoma. *J Obstet Gynaecol Res.* 2016;42:566-572.
- Yakabe K, Murakami A, Nishimoto Y, Kajimura T, Sueoka K, Sugino N. Clinical implications of human leukocyte antigen class I expression in endometrial cancer. *Mol Clin Oncol.* 2015;3:1285-1290.
- Maekawa R, Taketani T, Mihara Y, et al. Thin endometrium transcriptome analysis reveals a potential mechanism of implantation failure. *Reprod Med Biol.* 2017;16:206-227.
- Battle E, Sancho E, Franci C, et al. The transcriptional factor snail is a repressor of E-cadherin gene expression in epithelial tumour cells. *Nat Cell Biol.* 2000;2:84-89.
- Vernon AE, LaBonne C. Tumor metastasis: a new twist on epithelial-mesenchymal transitions. *Curr Biol.* 2004;14:719-721.
- Kim MA, Lee HS, Lee HE, Kim JH, Yang HK, Kim WH. Prognostic importance of epithelial-mesenchymal transition-related protein expression in gastric carcinoma. *Histopathology.* 2009;54:442-445.
- Dave N, Guaita-Esteruelas S, Gutarra S, et al. Functional cooperation between Snail1 and twist in the regulation of ZEB1 expression during epithelial to mesenchymal transition. *J Biol Chem.* 2011;286:12024-12032.
- Cano A, Perez-Moreno MA, Rodrigo I, et al. The transcriptional factor snail controls epithelial-mesenchymal transitions by repressing E-cadherin expression. *Nat Cell Biol.* 2000;2:76-83.
- Liu F, Zhou Y, Zhou D, et al. Whole DNA methylome profiling in lung cancer cells before and after epithelial-to-mesenchymal transition. *Diagn Pathol.* 2014;9:66-70.
- Zhang JX, Mai SJ, Huang XX, et al. MIR-29c mediates epithelial-to-mesenchymal transition in human colorectal carcinoma metastasis via PTP4A and GNA13 regulation of  $\beta$ -catenin signaling. *Ann Oncol.* 2014;25:2196-2204.
- Peinado H, Marin F, Cubillo E, et al. Snail and E47 repressors of E-cadherin induce distinct invasive and angiogenic properties in vivo. *J Cell Sci.* 2004;117:2827-2839.
- Hur K, Toiyama Y, Takahashi M, et al. MicroRNA-200c modulates epithelial-to-mesenchymal transition (EMT) in human colorectal cancer metastasis. *Gut.* 2013;62:1315-1326.



31. Peinado H, Olmeda D, Cano A. Snail, Zeb and bHLH factors in tumour progression: an alliance against the epithelial phenotype? *Nat Rev Cancer*. 2007;7:415-428.
32. Zavadil J, Bottinger EP. TGF-beta and epithelial-to-mesenchymal transitions. *Oncogene*. 2005;24:5764-5774.
33. Lamouille S, Derynck R. Cell size and invasion in TGF-beta-induced epithelial to mesenchymal transition is regulated by activation of the mTOR pathway. *J Cell Biol*. 2007;178:437-451.
34. Deckers M, van Dinther M, Buijs J, et al. The tumor suppressor Smad4 is required for transforming growth factor beta-induced epithelial to mesenchymal transition and bone metastasis of breast cancer cells. *Cancer Res*. 2006;66:2202-2209.
35. Morizane R, Fujii S, Monkawa T, et al. miR-34c attenuates epithelial-mesenchymal transition and kidney fibrosis with ureteral obstruction. *Sci Rep*. 2014;4:578:1-9.
36. Valcourt U, Kowanetz M, Niimi H, Heldin C, Moustakas A. TGF-beta and the Smad signaling pathway support transcriptomic reprogramming during epithelial mesenchymal cell transition. *Mol Biol Cell*. 2005;16:1987-2002.
37. Ghahhari NM, Babashah S. Interplay between microRNAs and WNT/ $\beta$ -catenin signaling pathway regulates epithelial-mesenchymal transition in cancer. *Eur J Cancer*. 2015;51:1638-1649.
38. Liu QQ, Chen K, Ye Q, Jiang XH, Sun YW. Oridonin inhibits pancreatic cancer cell migration and epithelial-mesenchymal transition by suppressing Wnt/ $\beta$ -catenin signaling pathway. *Cancer Cell Int*. 2016;16:57.
39. Gu Y, Wang Q, Guo K, et al. TUSC3 promotes colorectal cancer progression and epithelial-mesenchymal transition (EMT) through WNT/ $\beta$ -catenin and MAPK signaling. *J Pathol*. 2016;239:60-71.
40. Zhang L, Liu H, Mu X, Cui J, Peng Z. Dysregulation of Fra1 expression by Wnt/b-catenin signalling promotes glioma aggressiveness through epithelial-mesenchymal transition. *Biosci Rep*. 2017;37:1-12.
41. Yokoyama Y, Xin B, Shigeto T, et al. Clofibrilic acid, a peroxisome proliferator-activated receptor  $\alpha$  ligand, inhibits growth of human ovarian cancer. *Mol Cancer Ther*. 2007;6:1379-1386.

**How to cite this article:** Nishimoto Y, Murakami A, Sato S, et al. Decreased carbonyl reductase 1 expression promotes tumor growth via epithelial mesenchymal transition in uterine cervical squamous cell carcinomas. *Reprod Med Biol*. 2018;17:173-181. <https://doi.org/10.1002/rmb2.12086>