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Overexpression and Implications of Melanoma-associated Antigen A12 in Pathogenesis of Human Cutaneous Squamous Cell Carcinoma

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Abstract. *Background/Aim: Melanoma-associated antigen A12 (MAGEA12) has recently been reported as a repressor of tumor-suppressor genes. This study aimed to investigate the implications of MAGEA12 expression in the pathogenesis of cutaneous squamous cell carcinoma (cSCC). Materials and Methods: MAGEA12 and p21 expression were investigated in 15 samples of normal skin and 111 of cSCC tissues by immunohistochemistry. The biological functions of MAGEA12 in cSCC were also investigated both in vitro and in vivo. Results: Expression of both MAGEA12 and p21 was significantly increased in cSCC. MAGEA12 expression showed a positive correlation, while p21 expression showed negative correlation with the recurrence-free survival of patients with cSCC. In addition, MAGEA12 knockdown significantly attenuated proliferative, migratory, invasive, and tumorigenic activities of cSCC cells and was negatively correlated with p21 expression both in vitro and in vivo. Conclusion: MAGEA12-mediated down-regulation of p21 may be involved in cSCC pathogenesis and MAGEA12 may serve as a molecular biomarker in cSCC.*

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As a common type of non-melanoma skin cancer, cutaneous squamous cell carcinoma (cSCC) constitutes more than 20% of all skin cancers. Although the associated survival rate is higher than for those of many other cancer types, the cSCC-specific death rate approaches that of melanoma-related mortality (1). There is still no effective therapeutic strategy for advanced cSCC, other than surgical treatment for early-stage cSCC. While numerous studies have been performed to identify genetic risk factors of cSCC, the diagnostic and therapeutic value of this research remains limited.

The melanoma-associated antigen gene A (MAGEA) family is a group of cancer-testis antigens. Twelve family members, MAGEA1 to MAGEA12, are included in the MAGEA family and share a MAGE homology domain (2, 3). MAGEA proteins are normally expressed in the testis, placenta, and fetal ovary, especially in immature cells such as spermatogonia, trophoblasts, and oogonia (4-6). In contrast, aberrant overexpression of MAGEA proteins has been found in many types of cancers in different organs, such as prostate (7), breast (8, 9), colon (10), brain (11), and lung (12-14), and was associated with poor patient prognosis. For example, in non-small cell lung cancer, MAGEA3 and MAGEA9 expression were shown to be significantly related to decreased patient survival (13, 15), and in ovarian cancer, expression of MAGEA1, -A9 and -A10 were significant indicators of a poor prognosis (16, 17). Moreover, in patients with gastric cancer, expression of MAGEA1 to -A6 was found in peritoneal wash after cancer resection, and was found to be associated with poor prognosis (18). The oncogenic activities of MAGEA family proteins have also been investigated both *in vitro* and *in vivo*. Findings have shown that expression of MAGEA3 and MAGEA6 is significantly positively correlated with *in vitro* cell viability and clonogenic activities of various types of cancer cell lines such as of the lung, breast, and colon (19). Moreover, in

thyroid cancer cell lines, MAGEA3 expression promoted cell motility and invasion *in vitro* and, more impressively, tumorigenic activity and metastatic ability were increased in MAGEA3-overexpressing cells *in vivo* (20).

There have been limited mechanistic studies regarding the function of MAGEA proteins. Some investigators have shown that members of the MAGEA family, such as MAGEA2, -A3, and -A6, can form ubiquitin ligase complexes with tripartite motif-containing (TRIM) proteins and thereby increase the ubiquitin ligase activity of TRIM against some tumor-suppressor genes, such as 5' adenosine monophosphate-activated protein kinase (AMPK) and p53, resulting in their proteasome-dependent degradation (19, 21, 22).

MAGEA12 shares amino acid sequence homology with other MAGEA family proteins, particularly driver oncogenes, such as *MAGEA3* and *MAGEA6* (23, 24). As a structural mimicker of MAGEA3 and MAGEA6, MAGEA12 was recently found to demonstrate oncogenic activity in some studies (23, 25). However, there is only limited information regarding MAGEA12 expression in relation to cancer progression.

In the present study, we comparatively investigated MAGEA12 and p21 protein expression in normal skin and cSCC tissue samples. The clinicopathological significance of MAGEA12 and p21 protein expression was investigated in cSCC patients with a median follow-up period of 11 months (range=1-156 months). The influence of MAGEA12 expression on the biological behavior of cSCC cell lines was also investigated *in vitro* and *in vivo*.

Materials and Methods

Clinical samples. One hundred and eleven samples of cSCC were selected from patients diagnosed with cSCC from 2000 to 2010 from the Department of Pathology, Yonsei University Health System in Seoul, Korea and 15 normal skin tissues obtained from surgery were included for this study. All specimens were obtained from the Department of Pathology, Yonsei University Health System in Seoul, Korea. The clinicopathological characteristics of the patients are summarized in Table I. This study was approved by the Institutional Review Board for Bioethics of Yonsei University Health System, Severance Hospital (IRB 2018-0874-001).

Cell culture and establishment of MAGEA12-knockdown cSCC cells. Two human cSCC cell lines, HSC-1 and A431, were purchased from the Japanese Collection of Research Bioresources Cell Bank (Osaka, Japan) and the Korean Cell Line Bank (Seoul, Korea), respectively, for use in this study. Cell lines were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (Gibco Biosciences, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; Invitrogen, Waltham, MA, USA). pGFP-C-*shMAGEA12* lentiviral particle (OriGene, Rockville, MD, USA) was used to establish HSC-1 and A431 cells with stable *MAGEA12* knockdown (HSC-1-MAGEA12$^{\Delta}$ and A431-MAGEA12$^{\Delta}$, respectively). Silencing of *MAGEA12* was confirmed with immunocytochemistry. Lentiviral particles for pGFP-C-shLenti vector weve used to establish control stable cell lines (HSC-1-Mock and A431-Mock).

Influence of MAGEA12 knockdown on the biological behavior of cSCC cells. To investigate the implications of MAGEA12 expression on the biological behavior of the cSCC cells, proliferation, migration, and invasive ability, as well as tumorigenic activity, were comparatively investigated in mock and *MAGEA12$^{\Delta}$*-A431 and -HSC-1 cells. To determine the proliferative ability, cells were seeded in 6-well plates at a density of 2×10^4 and counted each day for 4 days after trypan blue staining. To investigate migration ability, cells were seeded in a 24-well plate at a density of 2.5×10^4. At 16 h after seeding, a scratch wound was made, and the relative closure was determined 24 h after the creation of the scratch wound. To determine cell invasive ability, cells were seeded at a density of 4×10^4 with culture medium containing 2% FBS in the upper chamber of a transwell insert (BD Biosciences, Bedford, MA, USA) coated with Matrigel (BD Biosciences, San Jose, CA, USA). Culture medium containing 20% FBS was added to the bottom chamber. After 34 h of culture, invading cells were stained using 0.25% crystal violet and counted under a microscope.

For *in vivo* xenograft analysis, A431-Mock or A431-*MAGEA12$^{\Delta}$* cells (5×10^6 cell/100 μl phosphate-buffered saline) were subcutaneously injected into the right flank of anesthetized 5-week-old female BALB/c-*nu/nu* mice (NARA Biotech, Seoul, Korea) (n=5/group). The tumor volume was calculated every 3 days with the following formula: width$^2\times$length$\times1/2$ (27). All mice were sacrificed after 21 days, and the tumor nodules were removed for analysis. All procedures for the animal study were performed under protocols approved by the Animal Care and Use Committee of the College of Medicine of Yonsei University (2017-0253).

Immunohistochemical staining. MAGEA12 (rabbit monoclonal IgG, working dilution 1/200; Abcam, Inc., Cambridge, MA, USA), p21 (mouse monoclonal IgG, working dilution 1/200; Abcam) and Ki-67 (mouse monoclonal IgG, working dilution 1/100; Dako Products, Santa Clara, CA, USA) antibodies were used as primary antibodies for immunochemical staining in the present study. As described in previous research (26), the tissue sections were deparaffinized and hydraded, and then incubated with a mixture of methanol and hydrogen peroxidase (40:1) to inhibit endogenous peroxidase activity. Primary antibody incubation was performed following an antigen retrieval process using antigen retrieval buffer (Dako Products).

For immunocytochemistry, 3×10^4 HSC-1-Mock, HSC-1-MAGEA12$^{\Delta}$, A431-Mock, and A431-MAGEA12$^{\Delta}$ cells were cultured on chamber slides (Thermo Fisher Scientific, Waltham, MA, USA) and then fixed with 95% ethanol. Endogenous peroxidase activity was inhibited with a mixture of methanol and hydrogen peroxidase (40:1), and 5% bovine serum albumin was used for blocking prior to primary antibody incubation. Real EnVision HRP Rabbit/Mouse Detection System (Dako Products) was used as the secondary antibody in this study. Visualization was performed using 3,3'-diaminobenzidine chromogen, and counterstaining was performed with hematoxylin. Rabbit IgG (R&D Systems GmbH, Wiesbaden-Nordenstadt, Germany) or mouse IgG (DakoCytomation A/S, Copenhagen, Denmark) were used as negative controls.

The weighted histoscore method was used to score the expression levels of MAGEA12, Ki-67, and p21 proteins (26). The staining intensity was divided into four categories: 0: Negative, 1: light brown, 2: brown, and 3: dark brown. The final histoscore was calculated based on the staining intensity and percentage of the cells, as follows: final score=(0× percentage of negative cells) + (1× percentage of light brown-stained cells) + (2× percentage of brown-

Table I. *The clinicopathological characteristics associated with study tissue samples.*

Clinicopathological variable	Value
Normal skin tissues	
Total no. of cases	15
Age, years	
Median age (range)	53 (21-87)
Gender, n (%)	
Male	6 (40)
Female	9 (60)
Site, n (%)	
Scalp	3 (20)
Face	6 (40)
Ear	3 (20)
Acral	3 (20)
SCC	
Total no. of cases	111
Age	
Median age (range)	74 (30-98)
Gender, n (%)	
Male	50 (45.0)
Female	61 (55.0)
Site, n (%)	
Scalp	14 (12.6)
Face	54 (48.6)
Ear	10 (9.0)
Lip	15 (13.5)
Acral	18 (16.2)
Size, cm	
Median size (range)	1.7 (0.3-4.5)
Differentiation, n (%)	
Well	41 (36.9)
Moderate	62 (55.9)
Poorly	8 (7.2)
Recurrence, n (%)	
Yes	18 (16.2)
No	93 (83.8)
Duration of follow-up, months	
Median (range)	11.0 (1.0-156.0)
Interval to recurrence, months	
Median (range)	11.0 (1.0-91.0)

SCC: Squamous cell carcinoma.

stained cells) + (3× percentage of dark brown-stained cells). For statistical analysis, patients were subdivided into groups with low (histoscore 0-100) and high (histoscore 101-300) expression.

Statistical analysis. The Mann–Whitney *U*-test was used in the present study to analyze the influence of *MAGEA12* knockdown on the biological behavior of cSCC cells. To analyze the association between expression of MAGEA12 and p21 and patient clinicopathological parameters, the chi-square test and Fisher's exact test were used. Kaplan–Meier analysis was performed for the association between expression of MAGEA12 and p21 with recurrence-free survival of the patients, and the significance of survival differences were analyzed using log-rank test. SPSS version 23 (IBM, Armonk, NY, USA) was used for statistical analysis. *p*-Values less than 0.05 were considered statistically significant.

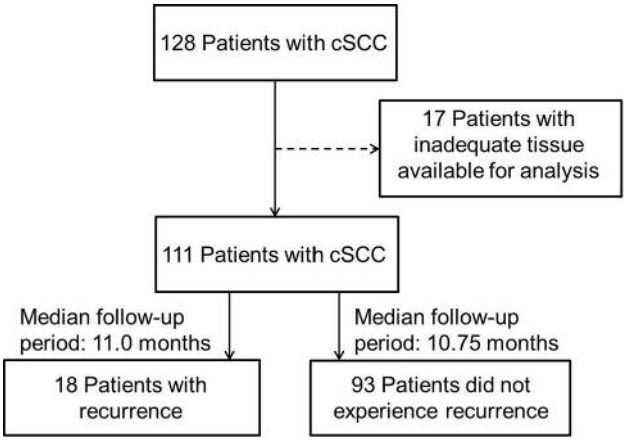

Figure 1. *Flow diagram for selection and outcome of patients with cutaneous squamous cell carcinoma (cSCC).*

Results

Patient characteristics. In total, 128 cases of cSCC were reviewed in the present study, and 17 cases were excluded on the basis of inadequate tissue available for analysis (Figure 1). As summarized in Table I, the median patient age at diagnosis was 74 years (range: 30-98 years), and the ratio of men to women was 1:1.2. The most common lesion site was the face (54, 48.6%). The median size of cSCC lesions was 1.7 cm, and the majority of patients had well or moderately differentiated histological type were (103/111, 92.8%). In the cSCC cohort, 18 (16.2%) patients experienced recurrence (median follow-up period: 11.0 months, range=1-91 months).

Expression of MAGEA12 and p21 in samples of normal skin and cSCC tissue. Both cytoplasmic expression of MAGEA12 and nuclear expression of p21 were frequently found in normal skin and cSCC tissue samples. Representative expression patterns for MAGEA12 and p21 in the tissue samples are shown in Figure 2A. All normal skin tissue samples showed both low expression of MAGEA12 and p21. In contrast, 42 (37.8%) and 44 (39.6%) cSCC tissues showed high MAGEA12 and high p21 expression, respectively (significantly different from normal tissue at $p=0.002$, and $p=0.001$, respectively) (Figure 2B).

In cSCC tissue samples, MAGEA12 expression was detected more often in older patients (47.3%) than in younger ones (28.6%) ($p=0.042$), and in poorly differentiated cSCC tissues (62.5%) than in well (39.0%) and moderately (33.9%) differentiated cSCC tissues, although these differences were not statistically significant ($p=0.285$). No significant association was found between MAGEA12 expression and other baseline clinicopathological parameters of patients with cSCC, such as

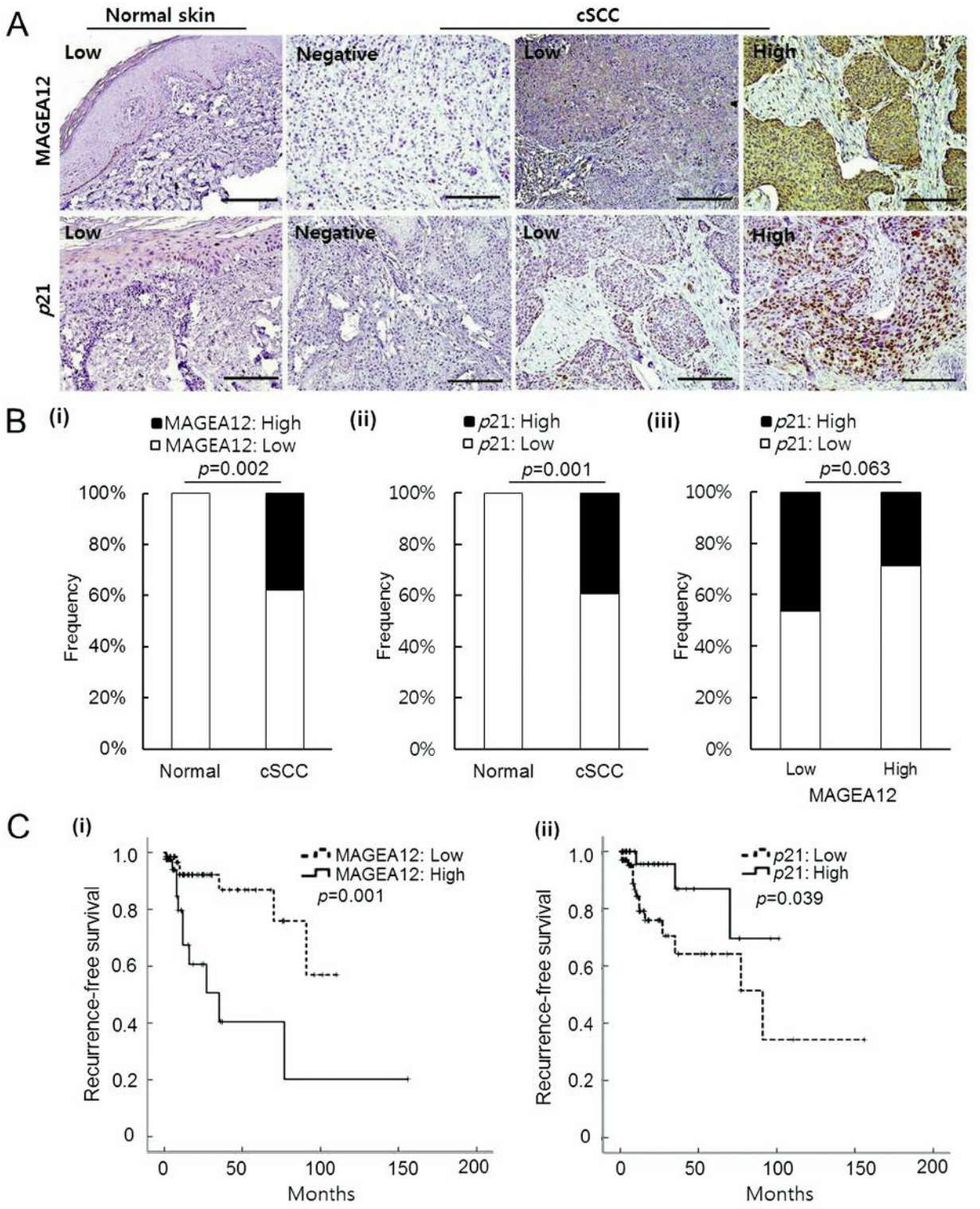

Figure 2. *Clinicopathological significance of melanoma-associated antigen gene A 12 (MAGEA12) and p21 expression in patients with cutaneous squamous cell carcinoma (cSCC). A: Representative expression patterns for MAGEA12 (upper panel) and p21 (lower panel) in normal skin and cSCC tissue sections. B: High expression of MAGEA12 and p21 was detected more often in cSCC tissues than normal skin tissues (i and ii). In cSCC tissues, a tendency was observed toward a lower frequency of high p21 expression in tissues with high MAGEA12 expression compared to that in tissues with low MAGEA12 expression (iii). C: In Kaplan–Meier analysis, MAGEA12 expression (i) was significantly positively correlated and p21 expression (ii) significantly negatively correlated with recurrence-free survival of patients with cSCC.*

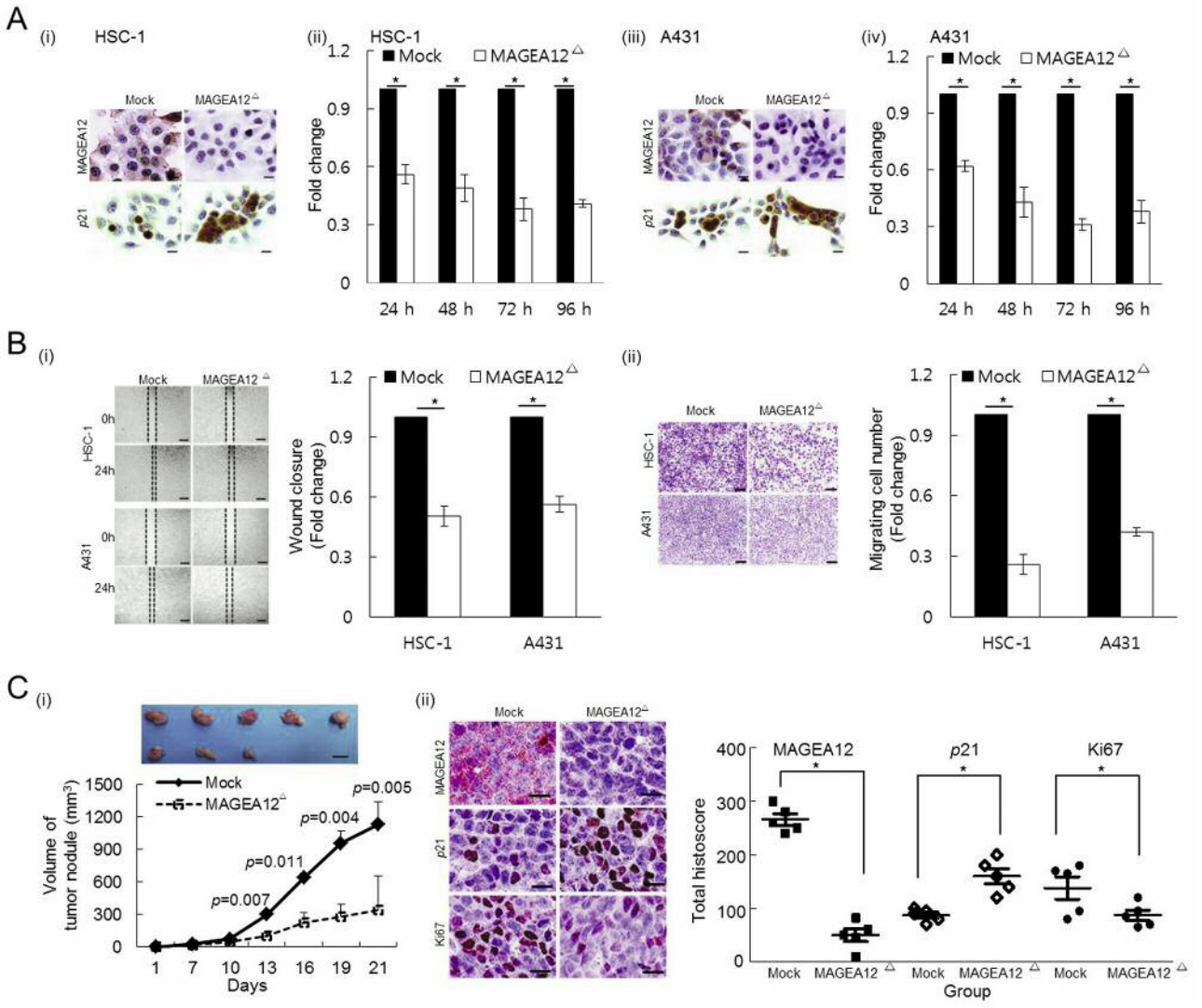

Figure 3. *The effect of melanoma-associated antigen gene A 12 (MAGEA12) knockdown on biological behavior of cutaneous squamous cell carcinoma (cSCC) cells. A: Prominantly reduced expression of MAGEA12 and increased expression of p21 was found in MAGEA12-transfected (MAGEA12$^{\Delta}$) HSC-1 (i) and A431 (iii) cSCC cells than in control cells (scale bar=10 μm). Proliferative ability was significantly reduced in MAGEA12$^{\Delta}$ cSCC cells compared to the control cells (ii and iv). B: Cell motility (i) and invasive ability (ii) were also significantly reduced in MAGEA12$^{\Delta}$ cSCC cells compared to the control cells (scale bar=100 μm). C: Knockdown of MAGEA12 expression in A431 cells attenuated tumorigenic activities in vivo. The volume of cSCC tumors was significantly smaller in the MAGEA12$^{\Delta}$-A431 group than in the A431-Mock group (i). Final histoscores for MAGEA12, Ki-67, and p21 expression were calculated for each group of tumor nodules. Markedly reduced expression of MAGEA12 and Ki-67 was observed in tumor nodules from the MAGEA12$^{\Delta}$-A431 group compared to the A431-Mock group. By contrast, p21 expression was markedly increased in tumor nodules from the MAGEA12$^{\Delta}$-A431 group compared to the A431-Mock group (ii) (scale bar=50 μm). *Significantly different at p<0.001.*

sex, lesion site, or tumor size. A significant association was observed between MAGEA12 expression and recurrence of cSCC in the present study: MAGEA12 expression was detected more often in patients with recurrence (61.1%) than in those without (33.3%) (p=0.026). In Kaplan–Meier analysis, MAGEA12 expression was significantly associated with poorer recurrence-free survival (p=0.001) (Table II) (Figure 2C i). The results of the present study showed that patients with high MAGEA12 expression (median survival: 6.7 months in the high-expression group *versus* 11.5 months in the low-expression group; p=0.001) exhibited a poor recurrence-free survival rate.

High p21 expression was detected more often in patients without recurrence (44.1%) than in those with (16.7%) (p=0.029) (Table II). Moreover, Kaplan–Meier analysis showed low p21 expression to be associated with poorer recurrence-free survival in patients with cSCC (p=0.039)

Table II. *Clinicopathological significance of expression of melanoma-associated antigen gene A 12 (MAGEA12) and p21 proteins in 111 patients with cutaneous squamous cell carcinoma.*

Variable	No. of cases (%)	MAGEA12, n (%)		*p*-Value	p21, n (%)		*p*-Value
		Low	High		Low	High	
Age							
≤74 Years	56 (50.5)	40 (71.4)	16 (28.6)	0.042	34 (60.7)	22 (39.3)	0.939
>74 Years	55 (49.5)	29 (52.7)	26 (47.3)		33 (60.0)	22 (40.0)	
Gender							
Male	50 (45.0)	31 (62.0)	19 (38.0)	0.975	31 (62.0)	19 (38.0)	0.749
Female	61 (55.0)	38 (62.3)	23 (37.7)		36 (59.0)	25 (41.0)	
Site							
Scalp	14 (12.6)	6 (42.9)	8 (57.1)	0.107	10 (71.4)	4 (28.6)	0.831
Face	54 (48.6)	37 (68.5)	17 (31.5)		31 (57.4)	23 (42.6)	
Ear	10 (9.0)	4 (40.0)	6 (60.0)		7 (70.0)	3 (30.0)	
Lip	15 (13.5)	12 (80.0)	3 (20.0)		9 (60.0)	6 (40.0)	
Acral	18 (16.2)	10 (55.6)	8 (44.4)		10 (55.6)	8 (44.4)	
Size							
≤1.7 cm	56 (50.5)	36 (64.3)	20 (35.7)	0.642	34 (60.7)	22 (39.3)	0.939
>1.7 cm	55 (49.5)	33 (60.0)	22 (40.0)		33 (60.0)	22 (40.0)	
Differentiation							
Well	41 (36.9)	25 (61.0)	16 (39.0)	0.285	26 (63.4)	15 (36.6)	0.856
Moderate	62 (55.9)	41 (66.1)	21 (33.9)		36 (58.1)	26 (41.9)	
Poorly	8 (7.2)	3 (37.5)	5 (62.5)		5 (62.5)	3 (37.5)	
Recurrence							
No	93 (83.8)	62 (66.7)	31 (33.3)	0.026	52 (55.9)	41 (44.1)	0.029
Yes	18 (16.2)	7 (38.9)	11 (61.1)		15 (83.3)	3 (16.7)	

(Figure 2C ii). No significant associations were observed in the present study between p21 expression and baseline clinicopathological parameters of patients with cSCC, such as age, sex, lesion site, tumor size, or differentiation (Table II). Interestingly, a tendency was observed for a lower frequency of high p21 expression in tissues with high MAGEA12 expression (28.6%) compared to that in tissues with a low MAGEA12 expression (46.4%) (p=0.063) (Figure 2B).

Influence of MAGEA12 knockdown on biological behavior of cSCC cells. Compared to that in control cells, prominently reduced expression of MAGEA12 and increased expression of p21 protein was found in the *MAGEA12*$^{\Delta}$ cSCC cells (Figure 3A i and iii). Moreover, the proliferative, migratory, and invasive abilities of the cells were significantly reduced after *MAGEA12* knockdown in both HSC-1 and A431 cells.

The effect of *MAGEA12* knockdown on cSCC cell proliferation was investigated using the trypan blue assay. Compared to the findings in control cells, the relative number of *MAGEA12*$^{\Delta}$ HSC-1 cells significantly (p<0.001) decreased at 24, 48, 72, and 96 h after seeding (Figure 3A ii). The results were similar for A431 cells (p<0.001) (Figure 3A iv).

The effect of *MAGEA12* knockdown on cSCC cell migration was investigated with a wound-healing assay. *MAGEA12* knockdown significantly reduced the migratory ability of both *MAGEA12*$^{\Delta}$-HSC-1 and *MAGEA12*$^{\Delta}$-A431 cells compared to that the control cells as measured at 24 h after wound scratching (both p<0.001) (Figure 3B i). In addition, the influence of MAGEA12 knockdown on the invasive ability of cSCC cells was investigated using a matrigel invasion assay. The numbers of *MAGEA12*$^{\Delta}$-HSC-1 and *MAGEA12*$^{\Delta}$-A431 cells that traversed the membrane were found to be significantly reduced compared to the number of control cells (both p<0.001) (Figure 3B ii).

The effect of *MAGEA12* knockdown on cSCC cell tumorigenesis was investigated by nude mice xenograft analysis. All of the mice injected subcutaneously with A431-Mock cells had tumor nodules in the right flank. In contrast, only three mice in the *MAGEA12*$^{\Delta}$-A431 group had tumor nodules. Moreover, the volume of the tumors was significantly smaller in the A431 *MAGEA12*$^{\Delta}$ group than in the A431-Mock group (p=0.005) (Figure 3C i). According to immunohistochemical analysis, MAGEA12 expression was found in tumor nodules obtained from the A431-Mock group, and MAGEA12 expression was found to be significantly lower in the tumor nodules obtained from the *MAGEA12*$^{\Delta}$-A431 group. Interestingly, predominantly increased p21 expression and reduced Ki-67 expression was observed in tumor nodules from the *MAGEA12*$^{\Delta}$-A431 group compared to the A431-Mock group (all p<0.001) (Figure 3C ii).

Discussion

There are two types of MAGE family members, type I and type II MAGEs. Type II MAGE members have been detected throughout healthy tissues in humans, but have not been associated with tumors. In contrast, genes encoding type I MAGE members are located only on the X chromosome, their protein expression is known to be regulated by epigenetic modification, such as DNA methylation, and they are thereby rarely expressed in healthy tissues. On the other hand, epigenetic reprogramming of type I MAGE proteins occurs during tumorigenesis and results in DNA hypomethylation. Type I MAGE expression is tumor-specific, and so has been considered as a possible target for cancer immune therapies (28-30). MAGEA proteins are included in the type I MAGE family.

In the present study, we investigated for the first time, as far as we are aware, the implications of MAGEA12 expression in cSCC pathogenesis. We found that MAGEA12 expression was significantly higher in cSCC tissues compared to normal skin tissue samples. Our results are consistent with previous studies which showed that *MAGEA12* mRNA expression was significantly increased in cancer tissues compared with the corresponding normal tissues in oral squamous cell carcinoma, gastric cancer, and lung cancer (23, 25, 31). Similarly to other members of the MAGEA family, MAGEA12 may also play an important role in cancer progression.

The influence of MAGEA12 expression on the biological behavior of cancer cell lines was recently investigated in prostatic carcinoma and colorectal cancer cell lines both *in vitro* and *in vivo* (25). It was shown that numbers of viable tumor cells and clonogenic activities of the cells decreased in both prostate and colorectal cancer cells after knockdown of *MAGEA12*. Moreover, both tumor volume and tumor weight were lower in *MAGEA12*-knockdown cells than in control cells in *in vivo* tumor xenograft analysis. Consistent with these findings, the proliferative, migratory, and invasive, as well as tumorigenic, activities of cSCC cells were significantly reduced after *MAGEA12* knockdown in the present study. Moreover, in the surgical cSCC tissue samples, MAGEA12 expression was significantly negatively associated with poor recurrence-free survival. These results are in accordance with those of other investigators showing a significant association between *MAGEA12* mRNA expression and poor prognostic outcome in patients with lung and gastric cancer by The Cancer Genome Atlas database analysis (25).

Recently, MAGEA12-mediated p21 ubiquitination has been proposed as one of the critical underlying molecular mechanisms of the oncogenic activities of MAGEA12. Investigators have demonstrated that *MAGEA12* knockdown in prostate and colorectal cancer cell lines led to increased expression of p21 protein, but not *p21* mRNA (25). As a tumor-suppressor gene, *p21* can regulate tumor growth *via* interaction with proliferating nuclear antigen (PCNA) and cyclin-dependent kinases (CDKs). p21 competes for PCNA binding with various proteins that mediate DNA synthesis, and p21, thus, inhibits DNA synthesis (32). Moreover, p21 can induce cell-cycle arrest *via* inhibition of the activity of CDK1 and CDK2 (33). Along these lines, some investigators have demonstrated increased apoptotic activity and G_2/M phase arrest of cancer cells after *MAGEA12* knockdown (25). In the present study, we also found that *MAGEA12* knockdown cells showed reduced proliferative activity as well as increased p21 protein expression both *in vitro* and *in vivo*. Moreover, in cSCC tissue samples, high p21 protein expression was detected more often in patients with low MAGEA12 expression, although this finding was not statistically significant. The molecular mechanism related to the oncogenic effect of MAGEA12 expression in cSCC progression may not be limited only to regulation of p21 expression. Further study is needed to investigate the precise molecular mechanisms underlying the function of MAGEA12 in cSCC pathogenesis.

In summary, we found that *MAGEA12* knockdown significantly reduced proliferation, migration, and invasion of cSCC cells *in vitro*. Moreover, markedly increased p21 protein expression and significantly reduced tumorigenic activity were observed after *MAGEA12* knockdown *in vivo*. In cSCC tissue samples, MAGEA12 expression was significantly related to poorer recurrence-free survival of patients with cSCC. Although the finding was not of statistical significance, MAGEA12 expression in cSCC tissues tended to be negatively correlated with *p21* protein expression. MAGEA12 may play a critical role in cSCC pathogenesis, and, thus, may serve as an indicator of poor prognosis, as well as a molecular biomarker in patients with cSCC. Furthermore, MAGEA12-mediated p21 down-regulation may be one of the molecular mechanisms underlying the oncogenic activities of MAGEA12 in cSCC pathogenesis.

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Conflicts of Interest

The Authors declare that they have no competing interests in regard to this study.

Authors' Contributions

Guohua Zhao, Jung Yoon Bae, Zhenlong Zheng, and Hae Seok Park involved and performed most of the experiments. Kee Yang Chung, Mi Ryung Roh and Zhehu Jin conceived the study and wrote the paper. All Authors read and approved the article.

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