Cell. Mol. Biol. (ISSN: 1165-158X)

Journal Homepage: www.cellmolbiol.org

Cellular and Molecular Biology

Original Article

hMAGEA2 as a potential diagnostic and therapeutic target for melanoma progression and metastasis

Junkoo Yi[1,2#], Chae Yeon Kim[3#], Hyeonjin Kim[3], Eun Gyung Kim[3], Do Yoon Kim[10], Yonghun Sung[5], Kang Dong Liu[6], Seong-Kyoon Choi[4], Zae Young Ryoo[7], Song Park[8,9*], Myoung Ok Kim[3*]

[1] *School of Animal Life Convergence Science, Hankyong National University, Anseong, 17579, South Korea*
[2] *Gyeonggi Regional Research Center, Hankyong National University, Anseong 17579, South Korea*
[3] *Department of Animal Science and Biotechnology, ITRD, Kyungpook National University, Sangju 37224, South Korea*
[4] *Core Protein Resources Center, DGIST, Daegu 41566, South Korea*
[5] *Preclinical Research Center KMEDIhub Daegu, 41061, South Korea*
[6] *China-US (Henan) Hormel Cancer Institute, Zhengzhou 450008, China*
[7] *School of Life Sciences, BK21 FOUR KNU Creative Bioresearch, Kyungpook National University, Daegu 41566, South Korea*
[8] *Division of Animal Science, Gyeongsang National University, Jinju 52828, South Korea*
[9] *Institute of Agriculture and Life Science (IALS), Gyeongsang National University, Jinju 52828, South Korea*
[10] *Gyeongsangbukdo Livestock Research Institute, Yeongju, Gyeongsang 36052, South Korea*

Article Info

Article history:

Received: March 13, 2024
Accepted: October 07, 2024
Published: October 31, 2024

Use your device to scan and read the article online

Abstract

The incidence of melanoma, a highly aggressive skin cancer, continues to increase worldwide, particularly among populations with lighter skin tones. The diagnostic challenge of melanoma lies in the absence of a distinctive clinical presentation, as its characteristics vary based on anatomical location, growth type, and histopathology. The melanoma-associated antigen (MAGE) gene family is differentially expressed in various human cancers, including melanoma. In this study, we explored the association between human MAGEA2 (hMAGEA2) expression and melanoma. Using a human melanoma tissue array, we confirmed that hMAGEA2 expression was higher in melanoma and metastatic melanoma than in normal tissues. Additionally, we used SK-MEL-5 and SK-MEL-28 cell lines to investigate the cellular and molecular mechanisms underlying melanoma progression and invasiveness. In SK-MEL-5 and SK-MEL-28 cells, hMAGEA2 overexpression accelerated cell proliferation. Conversely, the knockdown of hMAEGA2 reduced cell proliferation, colony formation, and migration significantly and induced arrest at the G2/M phase of the cell cycle. With respect to the molecular mechanism, the knockdown of hMAGEA2 decreased the phosphorylation of Akt, JNK, and p38 MAPK. Additionally, hMAGEA2 knockdown reduced tumor formation significantly at the in vivo level. Collectively, the robust correlation between hMAGEA2 and melanoma metastasis supports the potential utility of hMAGEA2 as both a diagnostic marker and novel therapeutic target for patients with melanoma metastasis.

Keywords: Cell growth, Cell-derived xenograft model, MAGEA, Melanoma.

1. Introduction

Malignant melanoma is a highly aggressive and metastatic skin cancer arising from the malignant transformation of melanocytes [1]. Patient prognosis is typically poor, with a 5-year survival rate of less than 15% [2, 3]. Our understanding of the pathobiology and genetics of melanoma has increased in recent years. This has led to the development of more effective clinical treatments, including targeted therapies and immunotherapies, both of which contribute to the regression of advanced disease. However, few treatments are effective in many patients [4]. The complex molecular mechanisms underlying the development and progression of malignant melanoma require further investigation.

Melanoma-associated antigens (MAGEs) are members of a gene family comprising more than 50 proteins. MAGEs can be classified into MAGE-I and MAGE-II based on their gene structure and tissue-specific expression patterns. MAGE-I family members (hereafter referred to as MAGE) are encoded by 28 genes clustered on the X chromosome, including MAGE-A, -B, and -C, which are typically expressed in cancer cells of various origins but not in adult tissues, except in germline cells in the placenta, ovaries, and testes [5]. In contrast, MAGE-II subgroups, including MAGE-D variants, do not show defined chromosomal clustering, are not cancer-specific, or are ubiquitously expressed in normal adult tissues and germline cells [6, 7]. MAGE proteins act as antitumor immune targets; therefore, these antigens are a focus of immunotherapy research for gastrointestinal carcinomas

* Corresponding author.
E-mail address: cristaling9816@gnu.ac.kr (S. Park), ok4325@knu.ac.kr (M. O. Kim).
# These authors contributed equally
Doi: http://dx.doi.org/10.14715/cmb/2024.70.10.14

and other cancers. MAGE expression is an independent prognostic variable for metastasis and reflects the extent of relapse-free survival, comparable to Breslow thickness and ulceration in melanoma [8]. hMAGEA2 is overexpressed in various human cancers, and its overexpression has been correlated with a worse prognosis [9]. However, despite extensive research, the molecular mechanisms underlying the roles of human MAGEA2 (hMAGEA2) in the development and progression of melanoma remain poorly understood.

In the present study, SK-MEL-5 and SK-MEL-28 cell lines were used to explore the contribution of MAGEA2 to melanoma progression and invasiveness. These results suggest that hMAGEA2 is involved in the growth and invasiveness of melanoma cells and that its overexpression is correlated with metastasis. Therefore, hMAGEA2 may contribute to the progression of melanoma *in vivo* and is a potential diagnostic and novel therapeutic target.

2. Materials and Methods

2.1. Construction of a tissue microarray and immunohistochemistry

Tissue microarrays (TMAs) were constructed with 1 mm diameter cores punched from two or three distinct regions of each formalin-fixed paraffin-embedded tumor block. TMAs were assembled using a tissue arrayer (Beecher Instruments, Silver Spring, MD, USA). One section from each TMA was stained with hematoxylin and eosin and reviewed to confirm the presence of representative tumors. After deparaffinization, rehydration, and antigen retrieval, the hMAGEA2 protein was detected via immunohistochemistry using antibodies (sc-130164; Santa Cruz, Houston, TX, USA) and the Ultraview Universal DAB Detection Kit (Ventana, Tucson, AZ, USA). All sections were counterstained with hematoxylin. TMA sections were assessed for the intensity of staining and the percentage of cells with nuclear, cytoplasmic, and cell membrane staining. Briefly, staining was considered positive when moderate or strong immune reactivity was detected at an appropriate location above the cutoff point. A total of 45 malignant melanoma tissues, 20 normal skin tissues, and 10 adjacent cancerous skin tissues were analyzed.

2.2. Cell culture and transfection

The medium components for cell culture were purchased from Thermo Fisher Scientific (Grand Island, NY, USA). The human melanoma cell lines SK-MEL-5 and SK-MEL-28 were cultured in minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (P/S). For stable expression of hMAGEA2 in melanoma cell lines, the cells were seeded into 100 mm dishes. After 24 h, the cells were transfected with the pcDNA3.1-mock or pcDNA3.1-hMAGEA2 vector using FuGENE HD transfection reagent (Promega, Madison, WI, USA), following the manufacturer's instructions. To establish stable expression, the transfected melanoma cell lines were treated with G418 (1.5 mg/mL) for 7 days. The subcultures were treated with G418 every 3 days. All cell lines were maintained in an incubator at 37°C with 5% CO_2.

2.3 Cell proliferation assay

Cell proliferation was estimated using the CCK-8 assay (Cell Counting Kit-8; Dojindo Molecular Technologies, Inc., Kumamoto, Japan). The cells were seeded into 96-well plates (1×10^3 cells/well) and incubated for 0, 24, 48, 72, or 96 h. In total, 10 µL of CCK-8 solution was added to each well and incubated for an additional 1 h at 37°C. The optical density (OD) of each well was measured at 450 nm using a spectrophotometer (BioTek, Winooski, VT, USA).

2.4 Anchorage-independent colony formation assay

The effect of hMAGEA2 on anchorage-independent growth was investigated in melanoma cell lines. Cells (8×10^3 cells/mL), suspended in MEM supplemented with 10% FBS and 1% P/S, were added to the top layer of 0.3% agar over a base layer of 0.5% agar. The cultures were maintained at 37°C in a 5% CO_2 incubator for 2–3 weeks, and then colonies were counted under a microscope (Leica, Wetzlar, Germany) using Image-Pro Plus (v.6.1).

2.5. Wound healing assay

Wound healing assays were performed to evaluate cell motility. Melanoma cells were cultured in 6-well dishes at a density of 1×10^6 cells/well. A single scratch was created by scraping a p10 micropipette tip across the surface of a plate containing confluent cells. The cells were washed thrice with PBS to remove cell debris, and the assay medium was added. Images of the scratched area on each plate were obtained by light microscopy (Leica) at 10× magnification at 0, 12, and 24 h, and the distance between the gaps was evaluated using Image-Pro Plus (v.6.1).

2.6. Cell cycle analysis

Cells (1×10^5) were seeded in 60-mm dishes and cultured for 48 h. The cells were harvested by trypsinization, fixed cell with 70% ethanol at -20°C overnight, and re-suspended with 250 µL of 0.6% Triton X-100 solution after washing with pre-cold PBS. Then, the cells were incubated with RNaseA (200 µg/mL) for 30 min at room temperature, and a cell cycle analysis was conducted using a BD FACSCalibur Flow Cytometer (BD Biosciences, San Jose, CA, USA).

2.7. Lentiviral production and infection

Lentiviral hMAGEA2 shRNA vectors (sh-hMAGEA2 sequence: 5′-GATAATCGTCCTGGCCATAAT-3′) for the knockdown of hMAGEA2 were purchased from Sigma-Aldrich (St. Louis, MO, USA). Oligonucleotides were cloned into the pLKO.1 lentiviral vector. HEK293T cells were co-transfected with pLKO.1-scramble or pLKO.1-shMAGEA2 and pMDLg/pRRE, pMD2.G, or pRSV-Rev using FuGENE HD transfection reagent (Promega). SK-MEL-5 and SK-MEL-28 cells were infected with lentiviruses encoding shRNA using 8 µg/mL protamine sulfate (Sigma). After 48 h, cells were selected with puromycin (1 µg/mL) for 4 days to establish the stable knockdown of hMAGEA2 in the SK-MEL-5 and SK-MEL-28 cell lines.

2.8. Western blotting

Cell lysates were prepared using a lysis buffer (150 mM NaCl, 1% NP-40, 50 mM Tris-HCl, 1 mM PMSF, protease inhibitor, and dephosphorylated inhibitor tablet mixture). After centrifugation, the supernatant was extracted as total cellular protein, and the concentration was measured using a BCA Quantification Kit (Thermo Fisher Scientific). Proteins were separated via SDS-PAGE and transferred onto a polyvinylidene difluoride membrane (Millipore,

Billerica, MA, USA). The membranes were blocked with 5% milk in 1× TBST for 1 h and then incubated with primary antibodies overnight at 4°C. The membranes were then incubated with the corresponding secondary antibodies after washing with 1× TBST. Protein bands were visualized using an enhanced chemiluminescence (ECL) detection reagent (GE Healthcare Life Sciences, Chicago, IL, USA), and protein expression was visualized using Da Vinci software.

2.9. Xenograft mouse model

All experimental protocols were approved by the Animal Care and Use Committee of the Kyungpook National University. Athymic nude mice (6 weeks; mean body weight, 18 g) were obtained from Orient (Seoul, Korea). Animals were acclimated to the facility for one week before the study and had free access to food and water. Tumor volume was calculated from two measurements of the diameter of the tumor base using the following formula: tumor volume (mm^3) = (length × width × height × 0.5). Mice were monitored until the tumors reached 1 cm^3 in total volume and were euthanized for further studies.

2.10. Statistical analysis

All quantitative results are expressed as mean values ± SD. Mean values in different groups were compared using a two-tailed independent samples *t*-test, and P-values of <0.05 were considered significant.

3. Results

3.1. Tissue microarray analysis

To investigate whether hMAGEA2 was overexpressed in human melanoma tissues and whether MAGE expression increased with metastasis, we measured the expression levels of hMAGEA2 in human melanoma tissues using TMAs. The expression levels of hMAGEA2 were significantly higher in human melanoma cancer tissues than in adjacent cancer and normal tissues ($P < 0.01$) (Fig. 1A, 1B). Additionally, hMAGEA2 expression was significantly higher in melanoma with metastasis than in melanoma without metastasis ($P < 0.01$) (Fig. 1C). These findings suggested that hMAGEA2 is associated with melanoma metastasis and tumorigenesis.

3.2. Cell proliferation was affected by hMAGEA2 in melanoma cell lines with overexpression and knockdown

To investigate the functions of hMAGEA2 in melanoma, we established SK-MEL-5 and SK-MEL-28 cell lines transfected with a plasmid vector that did not express hMAGEA2 (mock vector) or a vector that drove the expression of hMAGEA2. An immunoblot analysis showed that the expression of hMAGEA2 was significantly higher in SK-MEL-5 and SK-MEL-28 cell lines with transfection than in mock-transfected cell lines (Fig. 1D). Cell proliferation was measured using the CCK-8 proliferation assay. Proliferation was measured at 0, 24, 48, 72, and 96 h in stably transfected SK-MEL-5 and SK-MEL-28 cell lines. Proliferation was higher in cell lines overexpressing hMAGEA2 than in control cells (Fig. 1E). Our results demonstrated that the overexpression of hMAGEA2 in SK-MEL-5 and SK-MEL-28 cells increased cell proliferation.

Furthermore, to investigate the effects of hMAGEA2 downregulation, we established SK-MEL-5 and SK-MEL-28 cells in which hMAGEA2 was suppressed using lentiviral infection. An immunoblot analysis of sh-hMAGEA2-infected melanoma cells revealed that the expression of endogenous hMAGEA2 was lower than that in the cells expressing sh-mock (Fig. 1F). The results of a CCK-8 proliferation assay indicated that the knockdown of hMAGEA2 decreased the proliferation of SK-MEL-5 and SK-MEL-28 cells significantly (Fig. 1G). Overall, these data demonstrated that hMAGEA2 plays an important role in melanoma cell proliferation.

3.3. Knockdown of hMAGEA2 inhibits the migration of melanoma cells

An analysis of clinical pathology data indicated that hMAGEA2 is involved in tumor metastasis. To confirm whether hMAGEA2 is involved in the migration of SK-MEL-5 and SK-MEL-28 cells, a wound-healing assay was performed. First, we constructed hMAGEA2 knockdown SK-MEL-5 and 28 cells and measured the serum-induced migration of metastatic SK-MEL-5 and 28 cells. sh-hMAGEA2 decreased serum-induced cancer cell migration (Fig. 2A). SK-MEL-5 and 28 sh-hMAGEA2 cell migration was significantly lower than that in SK-MEL-5 and -28 sh-MOCK cells (Fig. 2B and C). These results suggested that hMAGEA2 plays a key role in melanoma metastasis.

3.4. Knockdown of hMAGEA2 suppresses anchorage-independent colony formation of melanoma cells

The effect of hMAGEA2 suppression on the ability of

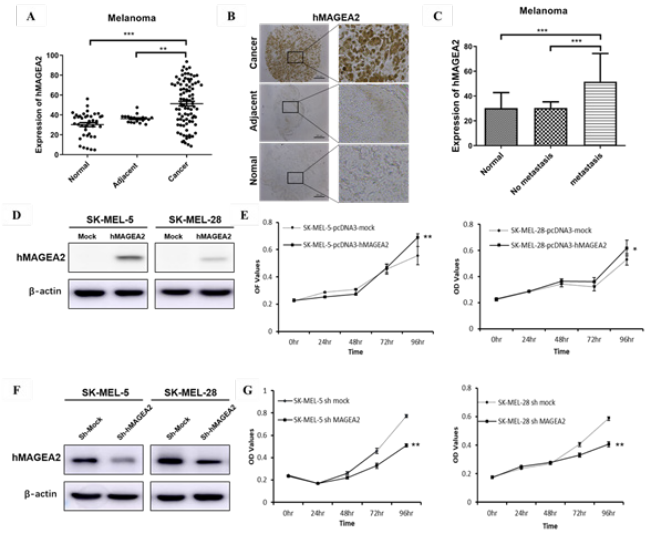

Fig. 1. hMAGEA2 is highly expressed in human melanoma tissues, hMAGEA2 overexpression increases proliferation, and hMAGEA2 knockdown decreases proliferation of SK-MEL-5 and SK-MEL-28 cell lines. (A) Expression of hMAGEA2 in human melanoma tissues. (B) Immunostaining of hMAGEA2 in human melanoma tissues. (C) Expression of hMAGEA2 in melanoma with or without metastasis. (D) Stable hMAGEA2 overexpression in SK-MEL-5 and SK-MEL-28 cell lines; β-actin was used as a loading control. (E) Proliferation was higher in melanoma cells that overexpress hMAGEA2 relative than in the control. (F) hMAGEA2 protein expression in SK-MEL-5 and SK-MEL-28 cell lines in the presence of mock or short hairpin RNA translation inhibitors. β-actin was used as a loading control. (G) Proliferation was suppressed in cells containing short hairpin RNAs specific for hMAGEA2 inhibition relative to that in cells containing nonspecific RNAs (mock). Data are expressed as means ± SD; *P < 0.05, **P < 0.01, ***P < 0.001, relative to control.

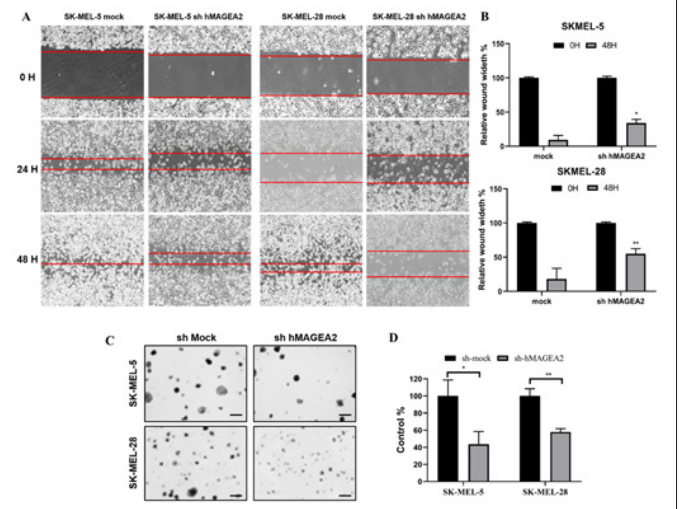

Fig. 2. Knockdown of hMAGEA2 inhibits motility and anchorage-independent colony formation in melanoma cell lines. (A) Motility of SK-MEL-5 sh-MOCK cells and SK-MEL-5 sh-hMAGEA2 cell lines was evaluated using wound-healing assays. Cells were plated in complete medium at a confluent density and scratched with a micropipette tip. Average rates of wound closure were calculated from three independent experiments. Bar, 300 μm. (B) Graphs of relative wound width after 48 h in the SK-MEL-5 cell line (upper panel) and relative wound width after 48 h in the SK-MEL-28 cell line (lower panel) in a wound healing assay. (C) Images of colonies formed by the indicated cell lines transfected with the indicated knockdown vectors. (D) Relative colony numbers in the indicated two cell lines transfected with the indicated knockdown vectors. Data are expressed as means ± SD; *P < 0.05, **P < 0.01.

melanoma cells to form colonies on soft agar was also measured. Colony formation was decreased in sh-hMAGEA2 melanoma cells relative to that in sh-MOCK control cells (Fig. 2C). In the SK-MEL-5 and SK-MEL-28 cell lines, the number of colonies formed was significantly decreased by the knockdown of hMAGEA2 (Fig. 2D). Our results demonstrated that the knockdown of hMAGEA2 suppressed colony formation on soft agar. Overall, these data demonstrated that hMAGEA2 is crucial for the anchorage-independent growth of melanoma cells.

3.5. Overexpression and knockdown of hMAGEA2 affect cell cycle progression in melanoma cells lines

The effect of hMAGEA2 on cell cycle progression was analyzed using flow cytometry. The results suggested that the overexpression of hMAGEA2 induced G1 phase distribution in the two cell lines (Fig. 3A and C). The knockdown of hMAGEA2 resulted in G2/M phase arrest in these two cell lines (Fig. 3B and D). Together, these findings indicate that hMAGEA2 is associated with cell cycle progression.

3.6. Knockdown of hMAGEA2 reduces the phosphorylation of AKT, JNK and p38 MAPK in melanoma cell lines

The MAPK and PI3K/AKT signaling pathways are targets for the effective treatment of advanced-stage melanoma [10-12]. To explore the molecular mechanism of action of hMAGEA2 in melanoma, western blotting was performed using the SK-MEL-5 cell line with hMAGEA2 knockdown. In previous study, we have demonstrated that hMAGEA2 regulates cell proliferation through the AKT and ERK1/2 pathways in breast cancer [13]. Therefore, we hypothesized that hMAGEA2 affects the growth of melanoma cell lines via these pathways. As expected, phospho-AKT was significantly downregulated and phospho-JNK and phospho-p38 were decreased following hMAGEA2 knockdown in SK-MEL-5 cells. However, phospho-ERK expression remained unchanged, in contrast to our previous results (Fig. 4).

3.7. Knockdown of hMAGEA2 decreases xenograft tumor formation *in vivo*

Finally, to investigate growth rates *in vivo*, we performed a xenograft tumor formation assay. The rate of growth in tumors derived from the SK-MEL-5 cell line with hMAGEA2 knockdown was substantially lower than that in mock-transfected cells (Fig. 5A, B, C). These findings suggest that the knockdown of hMAGEA2 suppresses tumor formation *in vivo*.

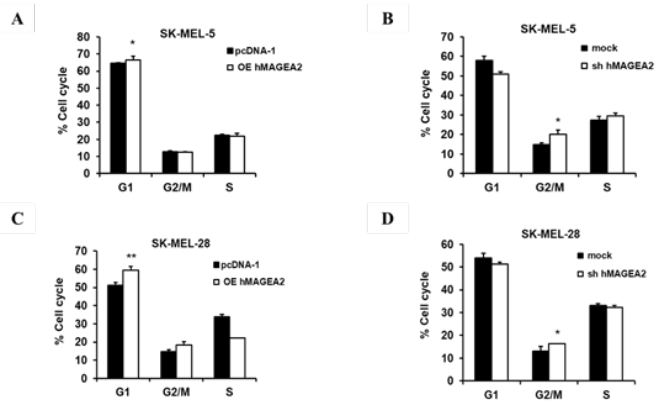

Fig. 3. hMAGEA2 affects melanoma cell cycle progression. (A) and (C) Percentage of cells in G1 phase in the hMAGEA2 overexpression group was higher than that in the sh-MOCK group in the two cell lines. (B) and (D) Knockdown of hMAGEA2 increases the percentage of cells in G2/M phase in two cell lines. Data are presented as means ± SD; *P < 0.05, **P < 0.01.

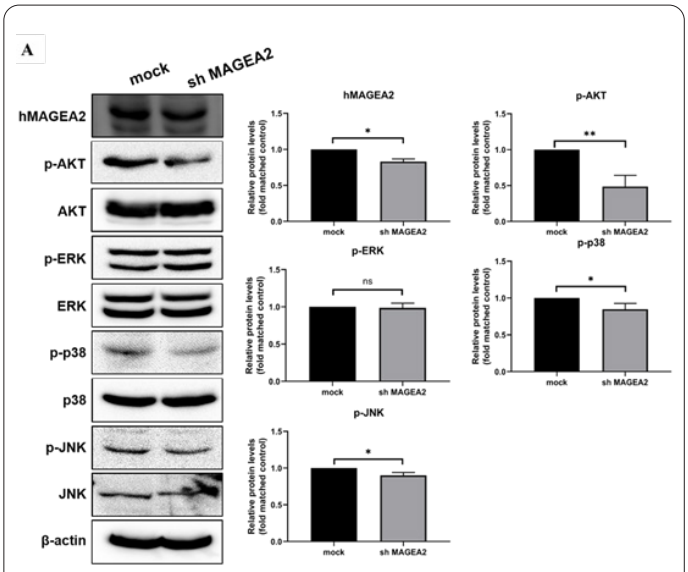

Fig. 4. Knockdown of hMAGEA2 reduces the phosphorylation of AKT, JNK, and p38 significantly. (A) Effects of the knockdown of hMAGEA2 in SK-MEL-5 cells on signaling pathways were assessed using a western blot analysis followed by densitometric quantification. Data are presented as means ± SD; *P < 0.05, **P < 0.01.

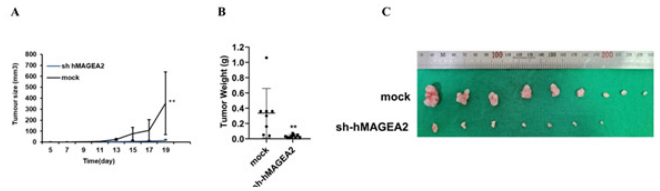

Fig. 5. Knockdown of hMAGEA2 inhibits tumor formation *in vivo*. (A) Growth curve of xenograft tumor formation after injection of SK-MEL-5 cells with or without the knockdown of hMAGEA2 into BALB/c nude mice. Data are presented mean ± SD; *P < 0.05, **P < 0.01.

4. Discussion

MAGEA is an 11-member subfamily of the broader family of MAGE proteins, which is characterized by a high MAGE homology domain [14]. Although the MAGEA cluster is activated in a wide range of cancers, including bladder, ovarian, and pancreatic cancer as well as melanoma, studies have focused on developing cancer vaccines based on immune targets. However, the exact role of MAGEA remains unclear. Recent evidence suggests that individual MAGE proteins function in specific pathways critical for tumor progression. Furthermore, compared with the MAGED to MAGEL gene clusters, the MAGEA to MAGEC clusters have been studied more extensively [14]. The expression of MAGEA3 stimulates cell cycle progression, migration, and invasion in thyroid cells *in vitro,* and these characteristics are associated with aggressive tumor behavior [15]. Silencing of MAGEB in murine melanoma cells suppresses melanoma growth *in vivo* [16]. Moreover, complex formation by KAP1, MAGE-A, MAGE-B, and MAGE-C inhibits p53-dependent apoptosis in MAGE-positive cells [16]. In the present study, we selected *MAGEA2* as a candidate gene for melanoma diagnosis and treatment. The suppression of MAGEA2 in lung cancer cells reduces their growth rate and survival substantially [17]. MAGEA2 overexpression is associated with poor prognosis in patients with glioma [18]. Glazer et al. reported that MAGEA2 could stimulate the growth of normal oral keratinocytes, whereas the knockdown of MAGEA2 in head and neck squamous cell carcinoma cells suppressed growth [19]. Thus, MAGEA2 is a potential target for cancer therapy. We found that hMAGEA2 was expressed in melanoma tissues (Fig. 1A, B) and that the expression of hMAGEA2 was associated with metastasis (Fig. 1C). Furthermore, hMAGEA2 knockdown affected cell proliferation, anchorage-independent colony formation, and migration (Figs. 2-3). These results suggested that hMAGEA2 is involved in cell growth. Mechanistically, the overexpression of hMAGEA2 triggered the cell cycle in the G1 phase, whereas the suppression of hMAGEA2 resulted in cell cycle arrest in the G2/M phase (Fig. 3). In addition, the MAPK and AKT pathways are complementarily regulated in melanoma; although the AKT and ERK1/2 pathways play an important role in cell proliferation when hMAGEA2 is overexpressed [13], the activation of signaling pathways differs depending on the cell type. This is in agreement with our observation that the phosphorylation levels of AKT, JNK, and p38 decreased in response to hMAGEA2 knockdown (Fig. 4). Recently, MAGEA2 has been shown to silence downstream targets of p53 activation via the MAGEA2-p53 complex in melanoma [20]. MAGEA proteins can activate specific RING-type ubiquitin E3 ligases [21, 22], thereby affecting ubiquitin signaling in cancer cells. Therefore, additional signaling pathways involving hMAGEA2 in cancer should be a focus of further research. CDX models can provide important information for translation from preclinical to clinical studies. The present study showed that hMAGEA2 knockdown reduced xenograft tumor formation in BALB/c nude mice (Fig. 5). Taken together, our results suggest that hMAGEA2 plays a key role in melanoma progression and metastasis and is a potential prognostic and therapeutic target in melanoma.

5. Conclusions

The results of this study show that hMAGEA2 plays a role in the growth and invasiveness of melanoma cells and is correlated with melanoma metastasis. In addition, the effects of hMAGEA2 in melanoma were mediated by the phosphorylation of AKT, JNK, and p38. Therefore, hMAGEA2 contributes to melanoma progression and may serve as a novel diagnostic and therapeutic target for treating metastatic melanoma.

Abbreviations

FBS, fetal bovine serum; MAGE, Melanoma-associated antigen; MEM, minimal essential medium; TMA, tissue microarray

Conflict of interest statement

The authors declare no conflicts of interest.

Consent for publication

All authors consented to the publication of this article.

Ethics approval and consent to participate

All animal experiments were approved by the Animal Care and Use Committee of the Kyungpook National University.

Availability of data and material

The datasets used in the present study are available from the corresponding author on reasonable request.

Authors' Contributions

JY, CYK, HK, EGK conducted the experiments and wrote the paper; JY, CYK, HK, EGK, DYK, YS, KDL, SKC and SP analyzed and organized the data; SP, ZYR, MOK conceived, designed the study and revised the manuscript.

Funding

This research was supported by a National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIT) (NRF-2022R1C1C2002699).

References

1. Miller AJ, Mihm Jr MC (2006) Melanoma. N Engl J Med 355 (1): 51-65. doi: 10.1017/S146239941100202X
2. Pacheco I, Buzea C, Tron V (2011) Towards new therapeutic approaches for malignant melanoma. Expert Rev Mol Med 13: e33. doi: 10.1017/S146239941100202X
3. Lujambio A, Lowe SW (2012) The microcosmos of cancer. Nature 482 (7385): 347-355. doi: 10.1038/nature10888
4. Shah DJ, Dronca RS Latest advances in chemotherapeutic, targeted, and immune approaches in the treatment of metastatic mela-

noma. In: Mayo Clin Proc, 2014. vol 4. Elsevier, pp 504-519. doi: 10.1016/j.mayocp.2014.02.002
5. Sang M, Wang L, Ding C, Zhou X, Wang B, Wang L, Lian Y, Shan B (2011) Melanoma-associated antigen genes–an update. Cancer Lett 302 (2): 85-90. doi: 10.1016/j.canlet.2010.10.021
6. Tseng H-Y, Chen LH, Ye Y, Tay KH, Jiang CC, Guo ST, Jin L, Hersey P, Zhang XD (2012) The melanoma-associated antigen MAGE-D2 suppresses TRAIL receptor 2 and protects against TRAIL-induced apoptosis in human melanoma cells. Carcinogenesis 33 (10): 1871-1881. doi: 10.1093/carcin/bgs236
7. Oya H, Kanda M, Takami H, Hibino S, Shimizu D, Niwa Y, Koike M, Nomoto S, Yamada S, Nishikawa Y (2015) Overexpression of melanoma-associated antigen D4 is an independent prognostic factor in squamous cell carcinoma of the esophagus. Dis Esophagus 28 (2): 188-195. doi: 10.1111/dote.12156
8. Svobodová S, Browning J, MacGregor D, Pollara G, Scolyer RA, Murali R, Thompson JF, Deb S, Azad A, Davis ID (2011) Cancer–testis antigen expression in primary cutaneous melanoma has independent prognostic value comparable to that of Breslow thickness, ulceration and mitotic rate. Eur J Cancer 47 (3): 460-469. doi: 10.1016/j.ejca.2010.09.042
9. Brasseur F, Rimoldi D, Liénard D, Lethé B, Carrel S, Arienti F, Suter L, Vanwijck R, Bourlond A, Humblet Y (1995) Expression of MAGE genes in primary and metastatic cutaneous melanoma. Int J Cancer 63 (3): 375-380. doi: 10.1002/ijc.2910630313
10. Savoia P, Fava P, Casoni F, Cremona O (2019) Targeting the ERK signaling pathway in melanoma. Int J Mol Sci 20 (6): 1483. doi: 10.3390/ijms20061483
11. Madhunapantula SV, Robertson GP (2011) Therapeutic implications of targeting AKT signaling in melanoma. Enzyme Res 2011. doi: 10.4061/2011/327923
12. Cheng Y, Zhang G, Li G (2013) Targeting MAPK pathway in melanoma therapy. Cancer Metastasis Rev 32: 567-584. doi: 10.1007/s10555-013-9433-9
13. Park S, Sung Y, Jeong J, Choi M, Lee J, Kwon W, Jang S, Park SJ, Kim H-S, Lee M-H (2017) hMAGEA2 promotes progression of breast cancer by regulating Akt and Erk1/2 pathways. Oncotarget 8 (23): 37115. doi: 10.18632/oncotarget.16184
14. Simpson AJ, Caballero OL, Jungbluth A, Chen Y-T, Old LJ (2005) Cancer/testis antigens, gametogenesis and cancer. Nat Rev Cancer 5 (8): 615-625. doi: 10.1038/nrc1669
15. Liu W, Cheng S, Asa SL, Ezzat S (2008) The melanoma-associated antigen A3 mediates fibronectin-controlled cancer progression and metastasis. Cancer Res 68 (19): 8104-8112. doi: 10.1158/0008-5472.CAN-08-2132
16. Yang B, O'Herrin SM, Wu J, Reagan-Shaw S, Ma Y, Bhat KM, Gravekamp C, Setaluri V, Peters N, Hoffmann FM (2007) MAGE-A, mMage-b, and MAGE-C proteins form complexes with KAP1 and suppress p53-dependent apoptosis in MAGE-positive cell lines. Cancer Res 67 (20): 9954-9962. doi: 10.1158/0008-5472.CAN-07-1478
17. Ujiie H, Kato T, Lee D, Hu H-P, Fujino K, Kaji M, Kaga K, Matsui Y, Yasufuku K (2017) Overexpression of MAGEA2 has a prognostic significance and is a potential therapeutic target for patients with lung cancer. Int J Oncol 50 (6): 2154-2170. doi: 10.3892/ijo.2017.3984
18. Meng Q, Luo G, Liu B, Sun Y, Yan Z (2018) Melanoma-associated antigen A2 is overexpressed in glioma and associated with poor prognosis in glioma patients. Neoplasma 65 (4): 604-609. doi: 10.4149/neo_2018_170625N440
19. Glazer CA, Smith IM, Bhan S, Sun W, Chang SS, Pattani KM, Westra W, Khan Z, Califano JA (2011) The role of MAGEA2 in head and neck cancer. Arch Otolaryngol Head Neck Surg 137 (3): 286-293. doi: 10.1001/archoto.2011.2
20. Monte M, Simonatto M, Peche LY, Bublik DR, Gobessi S, Pierotti MA, Rodolfo M, Schneider C (2006) MAGE-A tumor antigens target p53 transactivation function through histone deacetylase recruitment and confer resistance to chemotherapeutic agents. Proc Natl Acad Sci 103 (30): 11160-11165. doi: 10.1073/pnas.0510834103
21. Gao Y, Mutter-Rottmayer E, Greenwalt AM, Goldfarb D, Yan F, Yang Y, Martinez-Chacin RC, Pearce KH, Tateishi S, Major MB (2016) A neomorphic cancer cell-specific role of MAGE-A4 in trans-lesion synthesis. Nat Commun 7 (1): 12105. doi: 10.1038/ncomms12105
22. Doyle JM, Gao J, Wang J, Yang M, Potts PR (2010) MAGE-RING protein complexes comprise a family of E3 ubiquitin ligases. Mol Cell 39 (6): 963-974. doi: 10.1016/j.molcel.2010.08.029