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Implication of Amyloid Precursor-like Protein 2 Expression in Cutaneous Squamous Cell Carcinoma Pathogenesis

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Abstract. *Background/Aim: Regulatory functions of amyloid precursor-like protein 2 (APLP2) expression in intracellular trafficking of major histocompatibility complex class I (MHC-I) and biological behavior of tumor cells have been reported in various types of malignancies but not in cutaneous squamous cell carcinoma (CSCC). This study aimed to investigate the role of APLP2 expression in the pathogenesis of CSCC. Patients and Methods: The expression of APLP2 and a key modulator of cancer immune escape, MHC-I, were determined in CSCC tissue samples obtained from 141 patients using immunohistochemistry. The regulatory effects of APLP2 expression on the biological behavior and surface expression of MHC-I in CSCC cells were investigated by trypan blue assay, Matrigel invasion assay, and in vivo xenograft analysis. Results: APLP2 immunoreactivity was high in 73 (51.8%) tissue samples from patients with CSCC and was significantly related to subcutaneous fat invasion and poor prognosis in our cohort. Moreover, proliferation of and invasion by CSCC cells were significantly reduced after APLP2 knockdown in CSCC cells both in vitro and in vivo. A significant association was found between APLP2 and membrane MHC-I expression in patients with CSCC. In vivo xenograft analysis showed that APLP2 knockdown increased membrane MHC-I expression in CSCC cells. Conclusion: APLP2 not only acts as an oncogene in CSCC progression but also as a possible modulator of cancer immune escape by influencing MHC-I expression on the cell surface. APLP2 may serve as a novel molecular biomarker and therapeutic target for patients with CSCC.*

Cutaneous squamous cell carcinoma (CSCC) is a common type of skin cancer and is categorized as having relatively poor prognosis, as most metastases are detected within 3 months of CSCC diagnosis, and more than 70% of patients with metastatic CSCC die from their disease within 3 years (1, 2). Designing effective therapeutic and follow-up plans for patients with CSCC remains a challenge for clinicians.

The etiology of CSCC involves various factors, including cumulative exposure to UV radiation, carcinogenic chemicals, immunosuppressive agents, and various genetic susceptibilities (3). The diversity of these risk factors may lead to various molecular alterations during CSCC progression. Many efforts are ongoing to identify specific biomarkers with a crucial impact on CSCC pathogenesis. However, no clinically efficient diagnostic or therapeutic biomarkers for CSCC have been identified.

A type I transmembrane glycoprotein, amyloid precursor protein (APP), was shown to have important functions in the pathogenesis of Alzheimer's disease through the neurotoxic effect induced by the amyloid β peptide that is present in APP (4). Homologs of APP, amyloid precursor-like protein 2 (APLP2) and APLP1 have frequently been found in mammals (5), and are known to play crucial functions in

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glucose and insulin homeostasis (6). Moreover, the roles of APLP2 expression in the biological behavior of neural stem cells, along with brain development, have also been indicated by some studies (7-9). Previous studies constructed dual gene knockout mouse models against *APP*, *APLP1*, and *APLP2* (10-12), and found a lethal phenotype in the *APP*/*APLP2* and *APLP1*/*APLP2* knockout groups but not in the *APP*/*APLP1* group, indicating that *APLP2* may be a major player in physiological processes among these family members (10).

Previous studies have shown that APLP2 overexpression can be frequently detected in various types of tumors, such as pancreatic, colorectal, and ovarian cancer, and that APLP2 expression may be implicated in cancer initiation and progression, largely by influencing the biological behavior of tumor cells, such as proliferation, migration, invasion, and survival (4, 13-15). Moreover, APLP2 can bind to major histocompatibility complex class I (MHC-I) molecules and promote the intracellular trafficking of MHC-I, thereby inhibiting membrane expression of MHC-I in various cell types, including cancer cells (16, 17).

MHC-I plays a pivotal role in the adaptive immune system by presenting immunogenic peptides to the surface of nucleated cells which can be recognized by cytotoxic $CD8^+$ T-cells (18). Loss of membrane expression of MHC-I is prevalent in many types of cancer and mediates a suppressive immune microenvironment by preventing immune surveillance of cancer cells by $CD8^+$ T-cells. The suppressive immune microenvironment is a major obstacle to effective immunotherapy of patients with cancer. Moreover, some studies have highlighted the promotory roles of aberrant MHC-I expression in the growth and metastasis of various types of cancer cell *in vivo* (19-22). In patients with various types of cancer, such as laryngeal cancer, prostate cancer, and glioblastoma, aberrant MHC-I expression is considered a prognostic indicator of cancer progression (23-25). Therefore, the molecular mechanisms underlying the alterations in MHC-I expression have received considerable attention in cancer research.

There is no highly effective systemic treatment for CSCC; however, immunotherapy, such as that using programmed cell death-1 inhibitors, has recently emerged as an effective systemic treatment for CSCC. In particular, it is used to treat patients whose disease is at an advanced stage or are difficult to treat with surgery or radiation therapy (26, 27). Identifying possible modulators related to the formation of an immunosuppressive microenvironment may help improve the efficacy of immunotherapy in CSCC.

This study aimed to investigate the clinicopathological significance of APLP2 expression in a CSCC cohort and evaluated the influence of APLP2 expression on the biological behavior and status of membrane MHC-I expression in CSCC cells.

Table I. *Clinicopathological characteristics of study patients with cutaneous squamous cell carcinoma (n=141).*

Variable		Value
Age , years	Median (range)	75 (30-98)
Sex, n (%)	Male	67 (47.5)
	Female	74 (52.5)
Location, n (%)	Head and neck	119 (84.4)
	Trunk	18 (12.8)
	Extremities	2 (1.4)
	Acral	2 (1.4)
Tumor size	Mean±SD, cm	1.91±0.96
	≤2 cm	88 (62.4)
	>2 cm	53 (37.6)
Differentiation, n (%)	WD	66 (46.8)
	MD	64 (45.4)
	PD	11 (7.8)
Invasion to subcutaneous fat, n (%)	No	122 (86.5)
	Yes	19 (13.5)
Recurrence, n (%)	No	121 (85.8)
	Yes	20 (14.2)
Median histoscore (range)	APLP2	105 (0-210)
	MHC-I	0 (0-240)

APLP2: Amyloid precursor-like protein 2; MD: moderately differentiated; MHC-I: major histocompatibility complex class I; PD: poorly differentiated; SD: standard deviation; WD: well differentiated.

Patients and Methods

Patients in the CSCC cohort. The archived files of 145 patients with CSCC who underwent surgery at the Department of Dermatology between 2000 and 2018, Yonsei University Health System in Seoul, Korea were retrospectively reviewed for this study. Four patients with inadequate tissue available for analysis were excluded from this study. A total of 141 patients with CSCC were included in this study for whom follow-up was a median of 10.0 months (range=1.0-156.0 months). All surgical specimens were obtained from the Department of Pathology of Yonsei University Health System, Seoul, Korea. This study was approved by the Institutional Review Board of the Yonsei University Health System, Severance Hospital (approval no. 4-2018-0331).

The clinicopathological characteristics of the patients are summarized in Table I. Recurrence-free survival (RFS), the time from date of surgery to the time of recurrence or death, was considered as an endpoint of survival in our cohort.

Immunochemical staining. Immunohistochemical staining of CSCC tissue samples was performed as previously described (28). Antibodies to APLP2 (ab140624 rabbit monoclonal IgG, working dilution 1/200; Abcam, Cambridge, UK), MHC-I (ab134189 rabbit monoclonal IgG, working dilution 1/1,000; Abcam) and Ki-67 (M7240 mouse monoclonal IgG, working dilution 1/100; Dako Products, Santa Clara, CA, USA) were used as primary antibodies. Primary antibody incubation was performed after antigen retrieval and blocking for endogenous peroxidase activity. The REAL EnVision HRP Rabbit/Mouse Detection System (Dako) was used as the secondary antibody. Visualization was performed using the

chromogen 3,3'-diaminobenzidine and counterstaining was performed with hematoxylin. As previously described, the weighted histoscore method was used to score the total immunoreactivity for cytoplasmic and membrane expression of APLP2, membrane expression of MHC-I, and nuclear expression of Ki67 in the CSCC tissue sections. Based on the total histoscore, expression patterns of CSCC tissue samples for those antibodies were further divided into groups with low (0-100) and high (101-300) expression (29).

Cell culture and establishment of APLP2-knockdown CSCC cells. The CSCC cell lines HSC-1 and A431, were used in this study. HSC-1 was purchased from the Japanese Collection of Research Bioresources Cell Bank (Osaka, Japan), and A431 was purchased from the Korean Cell Line Bank (Seoul, Republic of Korea). As previously described (30), all cell lines were cultured in Roswell Park Memorial Institute 1640 medium (Gibco Biosciences, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; Invitrogen, Waltham, MA, USA). Plasmid green fluorescent protein-chloramphenicol-resistant (pGFP-C) short hairpin RNA Lenti Cloning Vector for APLP2 (OriGene, Rockville, MD, USA) were used to establish HSC-1 and A431 cells with stable *APLP2* knockdown (HSC-1-APLP2$^{\Delta}$ and A431-APLP2$^{\Delta}$, respectively).

Western blot analysis. Total protein extraction from pGFP-C-shAPLP2- and mock-transfected CSCC cells was performed using cell lysis buffer (Cell Signaling Technology, Danvers, MA, USA). The proteins were resolved using sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes (Bio-Rad, Hercules, CA, USA). Primary antibodies against APLP2 (ab140624 rabbit monoclonal IgG, working dilution 1/1,000, Abcam), extracellular-regulated kinase 1/2 (ERK1/2) (#9102 rabbit polyclonal IgG, working dilution 1/1,000; Cell Signaling Technology), Thr202/Tyr204 phosphorylated (p)-ERK1/2 (#9101 rabbit polyclonal IgG, working dilution 1/1000; Cell Signaling Technology), AKT serine/threonine kinase 1 (AKT) (#4685 rabbit monoclonal IgG, working dilution 1/1000; Cell Signaling Technology), and S473 p-AKT (ab81283 rabbit monoclonal IgG, working dilution 1/1000; Abcam) antibodies were used as for western blot analysis. Anti-rabbit IgG (working dilution 1/5000; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) was used as the secondary antibody, and the membranes were visualized using an enhanced chemiluminescence detection system (Pierce Biotechnology Inc., Rockford, IL, USA).

Influence of APLP2 knockdown on the biological behavior of CSCC cells. Trypan blue assay, Matrigel invasion assay, and *in vivo* xenograft analyses were performed to investigate the effects of *APLP2* knockdown on the proliferation, invasion, and tumorigenic activities of CSCC cells.

For the trypan blue assay, cells were seeded in a six-well plate at a density of 1×10^4 per well and counted after trypan blue staining at 36 and 72 h.

For the Matrigel invasion assay, a Transwell insert (BD Biosciences, Bedford, MA, USA) coated with Matrigel® (BD Biosciences, San Jose, CA, USA) was used in the upper chamber. Cells were seeded in the upper chamber at a density of 3×10^5 per chamber for HSC-1 and 2×10^5 per chamber for A431 cells with culture media containing 2% FBS. A 24-well plate containing culture medium supplemented with 20% FBS was used in the bottom chamber. After 36 h of culture, cells that had traversed the membrane were counted under a microscope after treatment with 0.25% crystal violet. Average numbers of cells that had traversed the membrane in three independent experiments were calculated for each group. Each experiment was conducted in triplicate.

Female BALB/c mice (16±2 g, 4 weeks of age) were provided by Central Lab Animal, Inc. (Seoul, Republic of Korea). Xenograft analysis was performed with two different experimental groups, and each group consisted of five mice. For *in vivo* xenograft analysis, 2×10^6 cells of each CSCC cell type were suspended in a 100 μl phosphate-buffered saline and were subcutaneously injected into the calvaria of the mice. Tumor volume was calculated as previously described (31). After 21 days, some of the tumor nodules had fixed to the bone surface and cannot be manipulated independently. In this study, this result was considered as a sign of invasion, and all mice were sacrificed after 21 days by CO_2 asphyxiation. Animal studies were approved by the Animal Ethics Committee of Yonsei University College of Dentistry (2019-0252).

Statistical analysis. The Mann–Whitney *U*-test was used for *in-vitro* and *in-vivo* studies to analyze the influence of APLP2 expression on the biological behavior of CSCC cells. Chi-squared and Fisher's exact tests were used to analyze the association between APLP2 expression and MHC-I, as well as clinicopathological parameters. Kaplan–Meier and Cox regression analyses were performed to investigate the prognostic significance of APLP2 expression in patients with CSCC. SPSS version 26 (IBM, Armonk, NY, USA) was used for all the statistical analyses. Statistical significance was at $p<0.05$ significant.

Results

Clinicopathological significance of APLP2 expression in the CSCC cohort. Both cytoplasmic and membranous expression of APLP2 was detected in 126 (89.4%) patients with CSCC. Immunoreactivity for APLP2 was high in 73 (51.8%) CSCC tissue samples and low in 68 (48.2%). Representative patterns of low and high APLP2 expression in CSCC tissue samples are shown in Figure 1A. None of the baseline clinical variables, including age, sex, lesion site, tumor size, or histological grade, were significantly associated with tissue APLP2 expression in patients with CSCC. A high expression of APLP2 was detected more often in patients with subcutaneous fat invasion (73.7%) than in those without it (48.4%) (p=0.049) (Table II). Moreover, patients with a high expression of APLP2 had shorter RFS than patients with a low expression of APLP2 (mean survival duration: 12.3 *vs*. 26.8 months, respectively; p=0.009) (Figure 1B). In multivariate analysis using clinicopathological factors as variables and both APLP2 and MHC-I expression as cofactors, poorly differentiated histological grade (hazard ratio=7.683, 95% confidence interval=1.714-34.439; p=0.008), high MHC-I expression (hazard ratio=6.247, 95% confidence interval=2.166-18.012; p=0.001) and high APLP2 expression (hazard ratio=4.282, 95% confidence interval=1.188-15.432; p=0.026) were found to have independent effects, worsening RFS (Table III).

APLP2 expression is significantly associated with membranous MHC-I expression in CSCC tissues. Loss of membranous

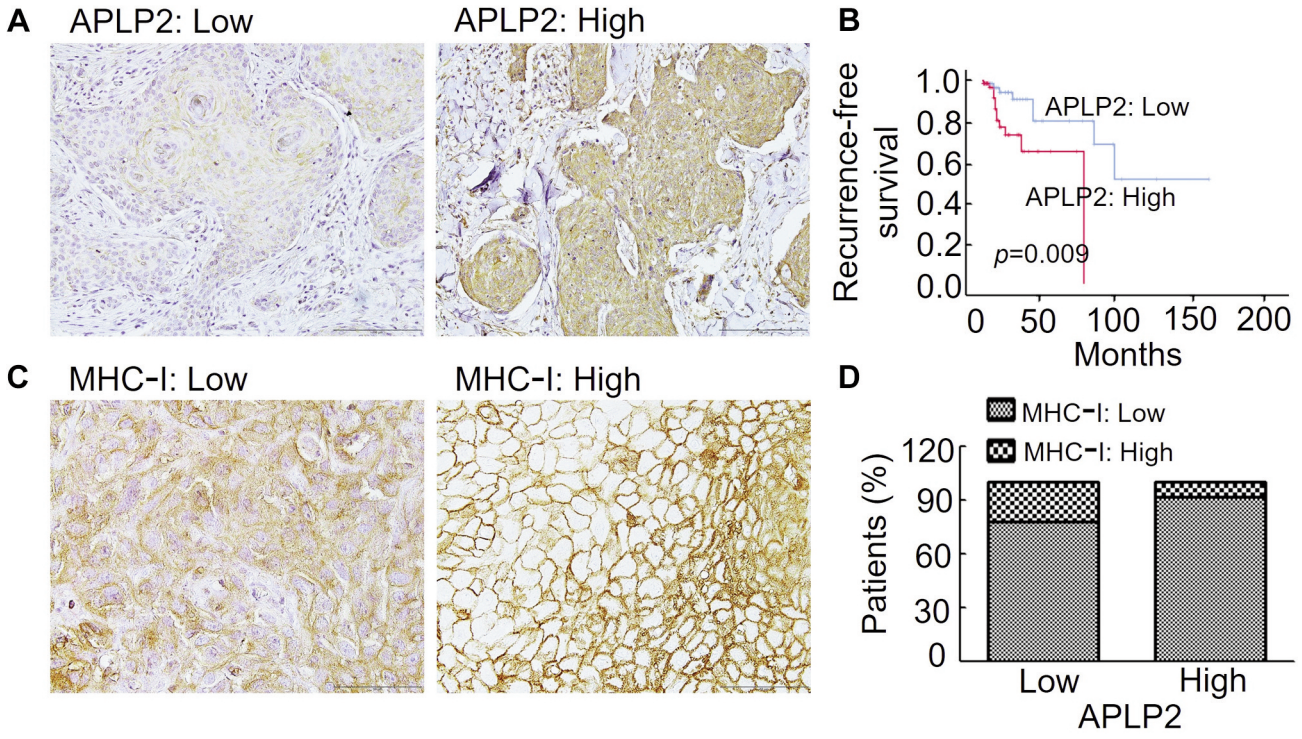

Figure 1. *Amyloid precursor-like protein 2 (APLP2) and major histocompatibility complex class I (MHC-I) expression in patients with cutaneous squamous cell carcinoma (CSCC). A: Representative patterns of low and high expression of APLP2 in CSCC tissues (original magnification: 200×; Scale bar: 100 μm). B: Prognostic significance of APLP2 expression in CSCC cohort. Cases in the CSCC cohort with high APLP2 expression had worse recurrence-free survival (p=0.009). C: Representative patterns of low and high membranous expression of MHC-I in CSCC tissues (Original magnification: 200×; Scale bar: 100 μm). D: An inverse association between APLP2 expression and membrane expression of MHC-I was found in CSCC tissues (p=0.032).*

MHC-I expression was observed in 102 (72.3%) CSCC tissue samples. Immunoreactivity for membranous MHC-I expression was high in 21 (14.9%) CSCC tissue samples and low in 120 (85.1%) samples. Representative patterns of low and high membranous MHC-I expression in CSCC tissue samples are shown in Figure 1C. High membranous MHC-I expression was detected more often in patients with a low expression of APLP2 (55.8%) than in those with a high expression of APLP2 (28.6%) (p=0.032) (Figure 1D).

The influence of APLP2 knockdown on biological behavior of CSCC cells. APLP2 knockdown was confirmed by western blot analysis, and APLP2 expression was predominantly reduced in the *APLP2*$^{\Delta}$ HSC-1 and A431 cells compared with mock-transfected controls (Figure 2A). Compared to mock-transfected controls, the number of cells decreased 1.40-fold and 1.47-fold in *APLP2*$^{\Delta}$ A431 cells at 36 and 72 h after seeding, respectively (Figure 2B). Similar results were observed in HSC-1 cells. Moreover, the number of invading cells was also significantly reduced in *APLP2*$^{\Delta}$ cells compared with mock-transfected control A431 and HSC-1 cells (p=0.026 and p=0.009, respectively) (Figure 2C).

In the mouse xenograft models, we found that tumor volumes were significantly higher in the group which were induced with mock-transfected control than those induced with *APLP2*$^{\Delta}$ cells (Figure 2D). Moreover, the labeling index for Ki67 was also significantly higher in the control group than in the *APLP2*$^{\Delta}$ group (p=0.008). In addition, extensive invasive growth patterns were observed in the control group. By contrast, well-circumscribed pushing tumor borders were found in the *APLP2*$^{\Delta}$ group. Interestingly, membranous expression of MHC-I was significantly lower in the tumor cells of the control group than those of the *APLP2*$^{\Delta}$ group (p=0.008) (Figure 2E).

Discussion

In this study, we investigated the functions and mechanisms of action of APLP2 in the pathogenesis of CSCC. Although aberrant APLP2 expression is frequently observed in various types of cancer, its functions in cancer progression remain largely unknown (4, 15, 32-35). Currently, controversy exists as to whether APLP2 is an oncogene or a tumor-suppressor gene involved in tumor progression. Gao *et al*. indicated that

Table II. *Clinicopathological characteristics of study patients with cutaneous squamous cell carcinoma (n=141) according to amyloid precursor-like protein 2 expression.*

Variable			APLP2		*p*-Value
			Low	High	
Age	Median, years	75	74	77	
Distribution, n (%)	≤75 Years	71 (50.4)	38 (53.5)	33 (46.5)	0.24
	>75 Years	70 (49.6)	30 (42.9)	40 (57.1)	
Sex, n (%)	Male	67 (47.5)	30 (44.8)	37 (55.2)	0.501
	Female	74 (52.5)	38 (51.4)	36 (48.6)	
Location, n (%)	Head and neck	119 (84.4)	54 (45.4)	65 (54.6)	0.394
	Trunk	18 (12.8)	12 (66.7)	6 (33.3)	
	Extremities	2 (1.4)	1 (50.0)	1 (50.0)	
	Acral	2 (1.4)	1 (50.0)	1 (50.0)	
Tumor size	Mean±SD, cm	1.91±0.96	1.84±0.86	1.98±1.06	
Distribution, n (%)	≤2 cm	88 (62.4)	44 (50)	44 (50)	0.606
	>2 cm	53 (37.6)	24 (45.3)	29 (54.7)	
Differentiation, n (%)	WD	66 (46.8)	29 (43.9)	37 (56.1)	0.345
	MD	64 (45.4)	35 (54.7)	29 (45.3)	
	PD	11 (7.8)	4 (36.4)	7 (63.6)	
Invasion of subcutaneous fat, n (%)	No	122 (86.5)	63 (51.6)	59 (48.4)	0.049
	Yes	19 (13.5)	5 (26.3)	14 (73.7)	
Median histoscore (range)	APLP2	105 (0-210)	30 (0-95)	130 (100-210)	
	MHC-I	0 (0-240)	0 (0-210)	0 (0-240)	

MD: Moderately differentiated; PD: poorly differentiated; SD: standard deviation; WD: well differentiated.

Table III. *Multivariable Cox-regression analysis for recurrence-free survival of 141 patients with cutaneous squamous cell carcinoma.*

Variables		Hazard ratio (95% CI)	*p*-Value
Age	Increasing	0.979 (0.938-1.022)	0.331
Sex	Male	1	
	Female	0.962 (0.322-2.869)	0.944
Site	Head and neck	1	
	Trunk	0.597 (0.145-2.450)	0.474
	Extremities	0.000 (0.000-0.000)	0.997
	Acral	0.000 (0.000-0.000)	0.986
Size	≤2.0 cm	1	
	>2.0 cm	2.611 (0.989-6.891)	0.053
Differentiation	WD	1	
	MD	1.642 (0.501-5.380)	0.413
	PD	7.683 (1.714-34.439)	0.008
Invasion of subcutaneous fat	No	1	
	Yes	1.035 (0.273-3.918)	0.959
APLP2 expression	Low	1	
	High	4.282 (1.188-15.432)	0.026
MHC-I expression	Low	1	0.001
	High	6.247 (2.166-18.012)	

APLP2: Amyloid precursor-like protein 2; MD: moderately differentiated; MHC-I: major histocompatibility complex class I; PD: poorly differentiated; WD: well differentiated.

APLP2 has tumor-suppressive functions in renal cell carcinoma (33). In renal cell carcinoma, APLP2 expression was significantly reduced in tumor tissues compared to corresponding normal tissues, and a low expression of APLP2 was found to be a poor prognostic indicator in patients (33). *APLP2* down-regulation has also been observed in lung cancer; however, this study did not evaluate the function of APLP2 down-regulation in cancer progression (36). Some investigators

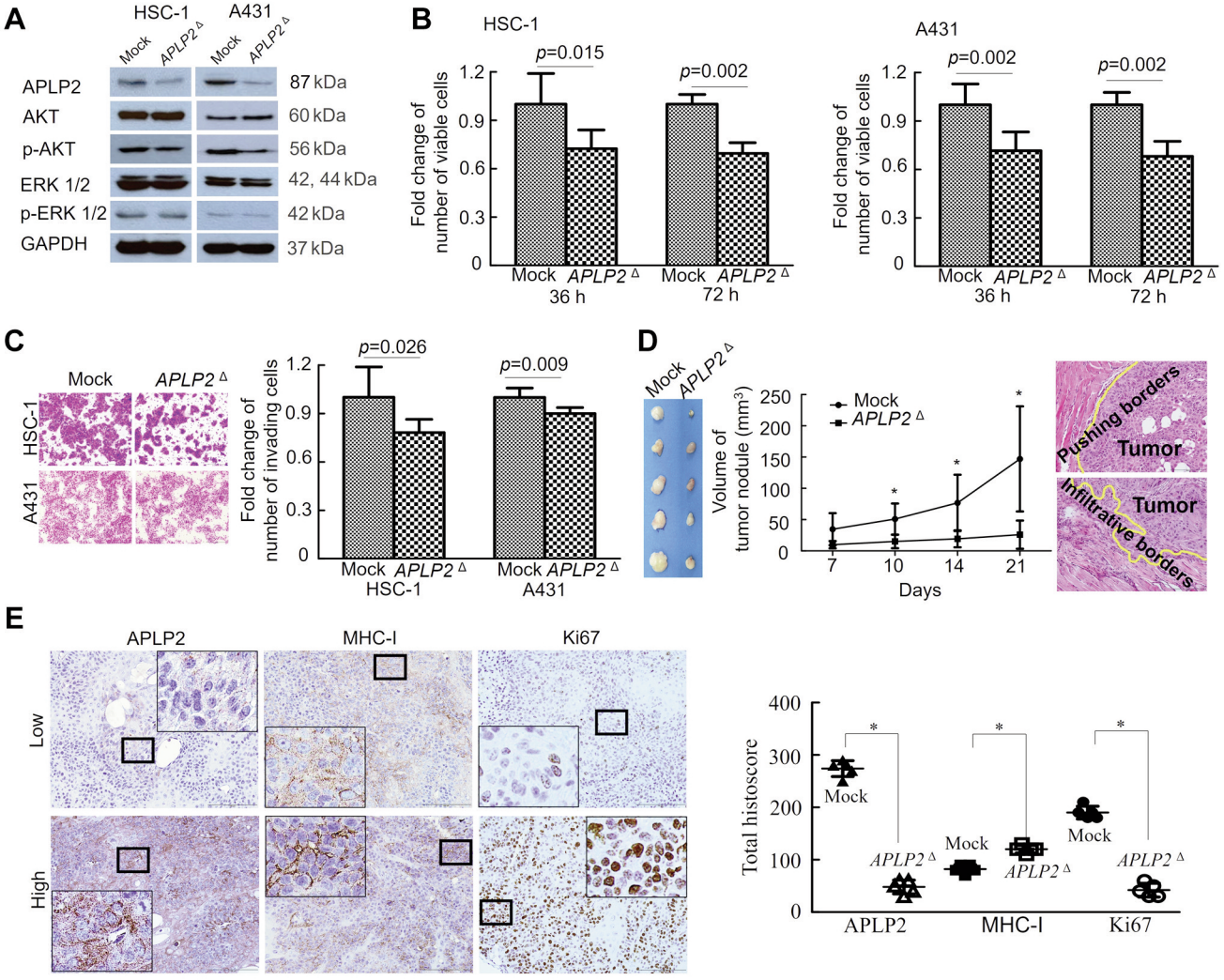

Figure 2. *The impact of amyloid precursor-like protein 2 (APLP2) expression on the biological behavior of cutaneous squamous cell carcinoma (CSCC) cells. A: Expression of both APLP2 and phospho-AKT (p-AKT) was predominantly reduced in APLP2-knockdown cells compared with mock control cells. B: APLP2 knockdown significantly reduced the viability of HSC-1 and A431 cells compared with mock controls at each indicated time point. C: Representative patterns of invading cells in each group. Invasion ability was significantly reduced in APLP2-knockdown cells. D: Knockdown of APLP2 expression in A431 cells attenuated tumorigenic activities in vivo. The volume of tumor nodules was significantly smaller in the APLP2-knockdown group compared with the control group (*p<0.05). Representative pushing or infiltrative borders of tumor nodules in xenograft mouse models. Extensive invasive growth patterns were observed in the control group. By contrast, well-circumscribed pushing borders were found in the APLP2Δ group. E: Representative patterns of APLP2, major histocompatibility complex class I (MHC-I), and Ki67 expression in tumor nodules. Quantification using the histoscore showed membranous MHC-I expression was significantly higher in the group with APLP2 knockdown than in the control group; in contrast, Ki67 expression was significantly reduced (*p=0.008).*

have demonstrated the oncogenic functions of APLP2 in various types of cancer (4, 15, 32, 34, 35). *APLP2* down-regulation attenuated the proliferation of cancer cells in colon and pancreatic cancer (32, 34). Moreover, APLP2 down-regulation was shown to inhibit both migration and invasion by pancreatic cancer cells by regulating the actin cytoskeleton (35). In ovarian cancer, *APLP2* down-regulation was related to reduced cancer cell survival (15), and prolonged survival and attenuated metastasis were observed after both heterozygous and homozygous knockout of pancreas-specific *APLP2* in a mouse model of spontaneous pancreatic cancer (4). Consistent with these findings, we discovered *APLP2* down-regulation attenuated the proliferation and invasion of CSCC cell lines, both *in vitro* and *in vivo*. Increased invasive ability of cancer cells facilitates their invasion into surrounding tissues as well as distant metastasis, and it can result in recurrence and poor

prognosis of patients with cancer (37, 38). In this study, we found that high APLP2 expression was detected more often in patients with subcutaneous fat invasion and was significantly associated with poorer RFS. Thus, APLP2 expression may play a critical role in CSCC pathogenesis by promoting the proliferation and invasion of CSCC cells.

AKT phosphorylation is a crucial event that can activate key signaling pathways related to cell motility, growth, survival, and metabolism in cancer cells (39-41). Some investigators showed that APP expression mediated AKT activation can accelerate cell growth, survival, and apoptosis in breast cancer (42). APP and APLP2 share a sequence similarity and functional redundancy (17, 43). In this study, we found p-AKT expression was significantly reduced after *APLP2* knockdown in CSCC cells *in vitro*. APLP2-mediated activation of AKT may be a crucial molecular mechanism of CSCC pathogenesis.

The characteristics of the tumor immune microenvironment (TIME) play a decisive role in the response to immune-targeted therapies (44). The TIME includes various types of immune cells, such as $CD4^+/CD8^+$ T-cells, tumor-associated macrophages, dendritic cells, tumor-associated neutrophils, myeloid-derived suppressor cells, and secretory proteins that can largely influence cancer progression, including various types of cytokines and chemokines (45). Tumor-reactive T-cells, such as $CD4^+/CD8^+$ T-cells, migrate towards tumor cells, which is a crucial step in antitumor immune responses. Based on the number and distribution of infiltrating $CD4^+/CD8^+$ T-cells in tumor tissues, the TIME is divided into three categories: Immune-inflamed, immune-excluded, and immune-desert phenotypes (46). A large number of $CD4^+/CD8^+$ T-cells infiltrate the tumor bed in the immune-inflamed phenotype; in contrast, $CD4^+/CD8^+$ T-cells are excluded from the tumor bed by other stromal components, such as cancer-associated fibroblasts, in the immune-excluded phenotype (46, 47). There are no cytotoxic lymphocytes in the tumor cell bed of the immune desert phenotype, and immune-targeted therapies rarely respond in patients with the immune desert phenotype (46, 47). In contrast, patients with an immune-inflamed phenotype usually show an excellent therapeutic response to immune-targeted therapy. Therefore, efforts are ongoing to overcome immunotherapy resistance by altering the TIME. However, the molecular mechanisms underlying the formation of different types of TIME in patients with cancer remain largely unknown.

Aberrant MHC-I expression has been observed in more than 90% of certain types of human cancer, including CSCC (48-50). Some studies have shown that the status and composition of infiltrating immune cells are significantly related to MHC-I expression in melanoma and have emphasized the crucial role of aberrant expression of MHC-I in TIME (51, 52). Aberrant MHC-I expression can be caused by reversible or irreversible structural genetic defects, and studies are ongoing to clarify the molecular mechanism underlying the reversible loss of membranous MHC-I expression in cancer cells to find a way to increase MHC-I expression on the cell surface.

Some investigators have shown that reversible defects of MHC-I may be caused by epigenetic modifications, such as methylation or histone deacetylation because membranous MHC-I expression is increased after treatment with histone deacetylase inhibitors or DNA-demethylating agents (53, 54). Moreover, some investigators found that fragile histidine triad diadenosine triphosphatase (*Fhit*) expression was significantly associated with membranous MHC-I expression in mouse fibrosarcoma cells (55). They found that membranous MHC-I expression was reduced after the knockdown of *Fhit* expression and was restored after the overexpression of *Fhit* in fibrosarcoma cells (55). A complex molecular mechanism may be involved in the reversible loss of membranous MHC-I expression in cancer cells, and APLP2-mediated intracellular trafficking of MHC-I may also be a crucial link that causes aberrant MHC-I expression. Consistent with previous studies (16, 35), an inverse correlation between APLP2 and membrane expression of MHC-I was found in the CSCC tissue samples in our study. Moreover, membranous MHC-I expression was increased after *APLP2* knockdown in the xenograft mouse model. In CSCC, APLP2 may influence MHC-I expression on the cell surface; however, the related molecular mechanisms require further evaluation.

Conclusion

APLP2 appears to act not only as an oncogene in CSCC progression but also as a possible contributor to cancer immune escape by influencing MHC-I expression on the cell surface. Clarifying the molecular mechanisms underlying the impact of the APLP2–MHC-I axis on immune escape may help overcome immunotherapy resistance in CSCC. Furthermore, APLP2 may serve as a prognostic biomarker and therapeutic target for patients with CSCC.

Conflicts of Interest

The Authors declare no conflicts of interest.

Authors' Contributions

Methodology, X.D.H. and J.H.Y.; validation, H.R.X., and M.L.Z.; formal analysis, Y.J.O. and M.L.Z.; investigation: X.D.H. and J.H.Y.; writing–original draft preparation: X.D.H. and J.H.Y; Writing–review and editing, Z.H.J. and Z.L.Z; supervision, Z.L.Z. and Z.H.J. All Authors have read and agreed to the published version of the article.

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