



Surfactin exerts an anti-cancer effect through inducing allergic reactions in melanoma skin cancer

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ABSTRACT

Surfactin is a mast cell degranulator, that increases the immune response via the degranulation of mast cells. Recently, numerous studies reported that allergic reactions play an important role in the reduction of melanoma development. So, this study aimed to investigate the anti-cancer effects of surfactin in a melanoma skin cancer *in vivo* model and a melanoma cell line, B16F10. Oral administration of surfactin significantly increased survival rate and reduced tumor growth and tumor weight on melanoma skin cancer *in vivo* model. Surfactin significantly increased infiltration of mast cells and levels of histamine. Surfactin significantly enhanced levels of IgE and immune-enhancing mediators, such as interferon- γ , interleukin (IL)-2, IL-6, IL-12, and tumor necrosis factor- α in serum and melanoma tissues. Activities of caspase-3, 8, and 9 were significantly enhanced by oral administration of surfactin. *In vitro* model, surfactin significantly increased B16F10 cell death via activation of caspase-3, 8, and 9 in a dose-dependent manner. Overall, our results indicate that surfactin has a significant anti-cancer effect on melanoma skin cancer through indirectly or directly inducing apoptosis of B16F10 melanoma cells. Also, these findings suggest that it will contribute to a novel perception into the role of allergic reactions in melanoma.

1. Introduction

Melanoma is a severe public health problem throughout the world due to its disadvantageous prognosis [1]. According to a histological study of melanoma, melanoma cases are classified into four major subtypes, including superficial spread (approximately 60%), acral lentiginous, nodular, and lentigo maligna [2]. Most drugs for chemotherapy are highly cytotoxicity and target highly proliferated cells using only non-specific methods, leading to adverse effects on a patient [3]. Therefore, new safer and more effective anti-cancer agents need to be developed to overcome the limitations of current therapeutic agents. Epidemiologic studies of associations between allergic conditions and cancer risk have reported that allergic diseases decrease the risk of brain tumor, glioma, meningioma, and pancreatic cancer [4–7].

Mast cells are the main effector cells of IgE antibodies [8]. Mast cells presence around a tumor has an important role in oncology [8]. Mast cells presence in several tumors has been related to favorable prognostic, and it has been indicated that mast cells presence contributes to immunomodulation of the tumor microenvironment [9]. Mast cell-

mediated allergic reactions manipulate tumors and have characteristics of the systemic immune response in Th2 tendency immunity, leading to a potential effect on development of tumor microenvironment [10,11]. AllergoOncology gives new insights into the role of IgE-mediated allergy in malignancies [10]. The immune response associated with IgE is applied for cancer treatment and IgE levels are increased by anti-cancer therapies such as chemotherapy [11,12]. Histamine produced by mast cell reduced the proliferation of cancer cells and increased apoptosis of cancer cells via inducing cell cycle arrest [13]. Collectively, these studies indicate that allergic reaction can regulate the development of cancer.

Most of the anti-cancer drugs in current use induce apoptosis of cancer [14]. Apoptosis is a typical mechanism by which compounds induce cell death [15,16]. Activation of caspases is associated with apoptosis and some anti-cancer drugs induce the activation of caspase-3, 8, and 9 [17].

Compounds of the surfactin family have potent mucosal adjuvanticity [18]. Surfactin is well known as a high potency biosurfactant and is produced by *Bacillus subtilis* strains [19]. In addition, surfactin

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induces mast cell degranulation [20]. Allergic reactions are important role in reduction of melanoma development. However, there are no studies available to explore the effect of mast cell degranulator, surfactin on melanoma development. Thus, this study aim is to investigate the anti-cancer effects of surfactin in melanoma skin cancer *in vivo* model and melanoma cell line, B16F10 cells.

2. Materials and methods

2.1. Reagents

Surfactin, avidin peroxidase, cisplatin, and other reagents were purchased from Sigma (St. Louis, MO, USA). Anti-mouse IFN- γ /IgE/IL-2/IL-6/IL-12/TNF- α antibody (Ab), biotinylated anti-mouse IFN- γ /IgE/IL-2/IL-6/IL-12/TNF- α Ab, and recombinant mouse IFN- γ /IgE/IL-2/IL-6/IL-12/TNF- α Ab were purchased from BD Pharmingen (San Diego, CA, USA).

2.2. Cell culture

The melanoma cell line, B16F10 cells were maintained in Dulbecco's Modified Eagle's medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin at 37 °C in a 5% CO₂ air atmosphere.

2.3. Established tumor growth experiments

Male C57BL/6N mice, aged 6 weeks old, were purchased from the Dae-Han Experimental animal center (Eumsung, Republic of Korea). Five mice were housed together in sterilized cages and maintained under standard laboratory conditions. The animals were placed in a 12/12 h light/dark cycle at 25 $\pm$ 2 °C and 50% humidity. The mice were randomly divided (n = 5/cage), fed with a regular diet and housed and monitored in a pathogen-free environment. Mouse care and experimental procedures were performed with the approval of the animal care committee of Kyung Hee University [KHUASP (SE)-19-238].

C57BL/6N mice were inoculated with B16F10 cells by subcutaneous (s.c) injection of 3 $\times$ 10⁵ cells to the right back flank. The mice (n = 10 per group) were orally administrated with phosphate-buffered saline (PBS), surfactin (0.05, 0.5, and 5 mg/kg), or cisplatin (1 mg/kg, anti-cancer drug) every two days for 22 days. Tumor growth was monitored by measuring the length (L) and width (W) of tumors. The volume (V) of the tumor was calculated by the formula $V = (L \times W^2)/2$. At the end of the study (day 22), animals were euthanized and tumor sizes were measured. Tumor samples were cut into pieces and immediately frozen in liquid nitrogen for protein extraction. The remaining tumor portions were fixed in 10% formalin for histological analysis.

2.4. Alcian blue and safranin staining

Alcian blue and safranin (A&S) were used to stain mast cells [21]. Sections were stained for 2 min. The staining procedure was modified from method of Kalesnikoff and Galli. Intact or degranulated-mast cell is defined according to previous study [22].

2.5. Histamine assay

Histamine contents in serum and tissue were measured according to a previous report [23]. Fluorescent intensities were measured at 440 nm (excitation at 360 nm) with a spectrofluorometer.

2.6. Enzyme-linked immunosorbent assay (ELISA)

After anesthetization, blood was withdrawn from the heart and then, the serum was prepared by centrifugation at 3000g at 4 °C for 10 min. Cytokines levels in serum and melanoma tissue samples were measured according to the manufacturer's specification (BD Pharmingen, San

Diego, CA, USA). Cytokine levels of melanoma tissues were normalized by the total protein amounts, which were calculated using a bicinchoninic acid (Sigma, St. Louis, MO, USA).

2.7. Caspase assay

Enzymatic activities of caspase-3, 8, and 9 were measured using caspase colorimetric assay kits (R&D system Inc., Minneapolis, MN, USA). Briefly, total protein (150 μ g) extracted from melanoma tissues and B16F10 cells was used to analyze caspase activity, which was followed by incubation with caspase-3 (Ac-DEVD-pNA), 8 (Ac-IETD-pNA), or 9 (Ac-LEHD-pNA) substrate for 4 h at 37 °C. Cleavage of the substrate was analyzed at 405 nm using an ELISA reader.

2.8. Blood biochemical parameters

After 22 days of the experiment, blood samples were collected from the animals in all groups and the serum was prepared by centrifugation at 3000 $\times$ g at 4 °C for 10 min. A DRI CHEM NX500 analyzer (Fujifilm, Tokyo, Japan) determined the lactate dehydrogenase (LDH), blood urea nitrogen (BUN), alanine transaminase (ALT), aspartate aminotransferase (AST), and creatine kinase (CK) levels.

2.9. 3-(4,5-dimethylthiazolyl)-2,5-diphenyltetrazolium bromide (MTT) assay

MTT assay was generally used to evaluate cell death as well as cell viability [24]. Briefly, B16F10 suspension (3 $\times$ 10⁵ cells/well) was cultured for 72 h after treatment with surfactin (0.05, 0.5, and 5 μ g/ml). The MTT solution (5 mg/ml) was added and further incubated for 4 h at 37 °C. Next, the medium was discarded, and the formazan was dissolved in dimethyl sulfoxide. Absorbance at 540 nm was then measured by using an ELISA reader.

2.10. Statistical analysis of data

In vivo data were expressed as mean $\pm$ standard errors of means (SEMs). Data obtained with B16F10 cells are presented as the mean $\pm$ SEMs from three independent experiments performed at least in duplicate. An independent *t*-test was used for comparisons of two treatment conditions. In the comparisons of three or more treatment conditions, an ANOVA with Tukey's post-hoc test was used. Kaplan–Meier survival curves were calculated using overall survival data. Statistical significance was accepted for *p* values < 0.05.

3. Results

3.1. Surfactin increased the survival rate in melanoma *in vivo* model

To confirm the anti-cancer efficacy of surfactin *in vivo*, murine melanoma models were established in C57BL/6N mice using the B16F10 melanoma cell line. We evaluated the mortality of each group of treated mice. As shown in Fig. 1A, on day 22, the survival rate of mice in the surfactin-administered group was significantly higher than that of the B16F10 group. Tumor size was measured every 2 days using Vernier calipers. As shown in Fig. 1B, melanoma tumor size in the surfactin-administrated group was smaller than that in the B16F10 group (*p* < 0.05). In addition, oral administration of surfactin significantly reduced the tumor weight compared to the B16F10 group (Fig. 1C, *p* < 0.05). The body weight of the B16F10 group was significantly increased compared to the normal group, whereas surfactin reduced the body weight compared to the B16F10 group but not significantly (Fig. 1D). Cisplatin (positive control) significantly increased the survival rate and reduced the tumor size (Fig. 1, *p* < 0.05).

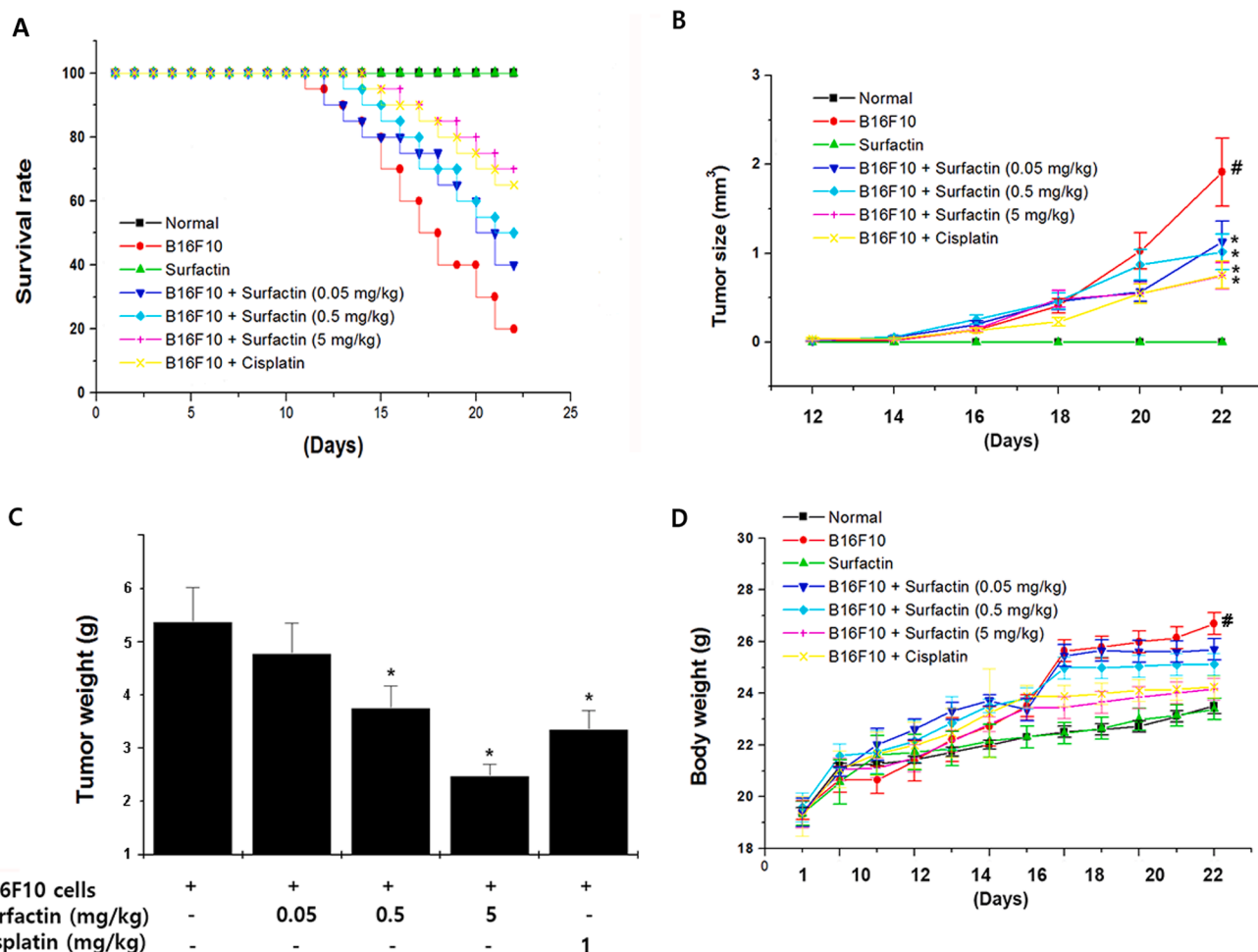


Fig. 1. Surfactin increased survival rate in melanoma *in vivo* model. C57BL/6 mice were inoculated with B16F10 cells by s.c injection of 3×10^5 cells to the right flank. The mice ($n = 10$ per group) were orally administrated with PBS, surfactin (0.05, 0.5, and 5 mg/kg), or cisplatin (1 mg/kg) every two days for 22 days. (A) Plot of Kaplan–Meier survival rate (B) Tumor size was monitored daily. (C) Tumor weight. (D) Body weight. * $p < 0.05$, significantly different from the normal group. * $p < 0.05$, significantly different from the B16F10 group.

3.2. Surfactin increased mast cells infiltration and histamine levels in melanoma *in vivo* model

Mast cell-mediated allergic reactions can exert pro-tumor effects by influencing the microenvironment [25]. Thus, we assessed whether the anti-cancer effect of surfactin is related to mast cell activation by counting the number of intact and degranulated mast cells as described in a previous study [22]. As exhibited in Fig. 2A and B, infiltration of mast cells was significantly enhanced by oral administration of surfactin compared to that in the B16F10 group. Also, as exhibited in Fig. 2, not only was infiltration of mast cells significantly enhanced by oral administration of cisplatin compared to the B16F10 group, but also the cisplatin-administered group exhibited higher levels of histamine in serum and melanoma tissues compared to that of the B16F10 group. Mast cells are major producer of histamine and histamine has a critical role in allergic reaction [26]. The surfactin-administered group exhibited higher levels of histamine in serum and melanoma tissues compared to those of the B16F10 group (Fig. 2C and D, $p < 0.05$).

3.3. Surfactin increased levels of immune-enhancing-related cytokines in melanoma *in vivo* model

IgE has an important role in AllergoOncology and have a latent ability to lead death of tumor cell [27]. As shown in Fig. 3A, surfactin-treated groups exhibited higher levels of serum IgE compared to that

of the B16F10 group ($p < 0.05$). IFN- γ is an immune-enhancing cytokine and is being explored as a potential anti-tumor immune responses factors [28]. The levels of serum IFN- γ in the surfactin groups were higher than that of the B16F10 group (Fig. 3B, $p < 0.05$). Cytokines play an important role in the modulation of immune responses, which may be an effective factor in cancer therapy [29]. IL-2, IL-6, IL-12, and TNF- α increase the immune reaction and have an anti-cancer effect [30]. Thus, we investigated whether surfactin could increase these cytokine levels. The effects of the surfactin on the levels of serum IL-2, IL-6, IL-12, and TNF- α are shown in Fig. 3. Surfactin-administered groups significantly higher levels of serum IL-2, IL-6, IL-12, and TNF- α compared to those of the B16F10 group (Fig. 3B–F, $p < 0.05$). In melanoma tissues, the protein levels of IgE, IFN- γ , IL-2, IL-6, IL-12, and TNF- α were significantly higher in the surfactin-administered groups than in the B16F10 group (Fig. 4, $p < 0.05$).

3.4. Surfactin increased caspase-3, 8, and 9 activities in melanoma *in vivo* model

Inactivation or decreases of caspase family brings about damaging apoptosis activation during tumorigenesis [31]. It leads to an impressive imbalance in growth dynamics, eventually leading to the aberrant growth of a tumor [31]. Thus, we investigated whether surfactin could affect caspase activation. As seen in Fig. 5, the activations of caspase 3, 8, and 9 in melanoma tissues were significantly increased by oral

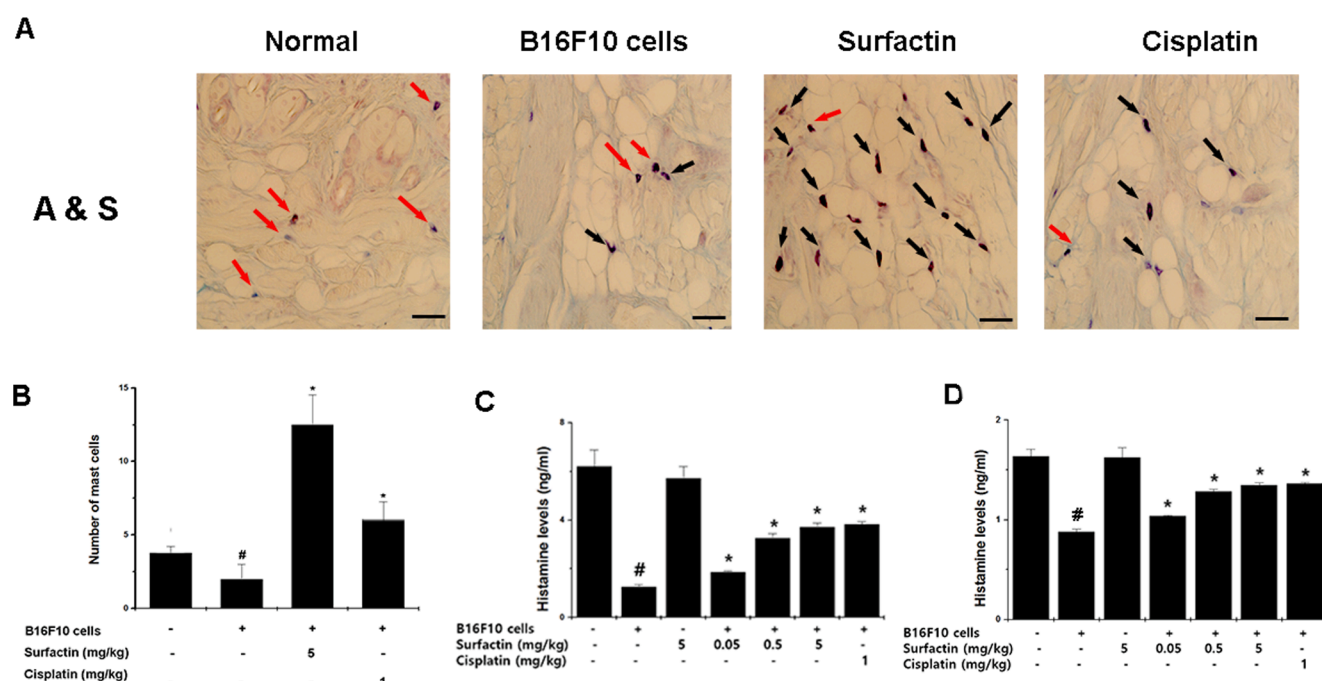


Fig. 2. Surfactin increased mast cells infiltration and histamine levels in melanoma *in vivo* model. (A) Melanoma tissues stained with A & S (Original magnification $\times 400$, scale bar = 100 μm , intact mast cells, indicated by the red arrows; degranulated mast cells, indicated by the black arrows). (B) The numbers of mast cells were counted by two blinded individuals. Five randomly selected tissue sections per mouse were included. The absolute number of cells counted is presented as mean $\pm$ S.E.M. Levels of (C) serum histamine and (D) melanoma tissue histamine were measured by the ELISA method. # $P < 0.05$, significantly different from the normal group. * $P < 0.05$, significantly different from the B16F10 group. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

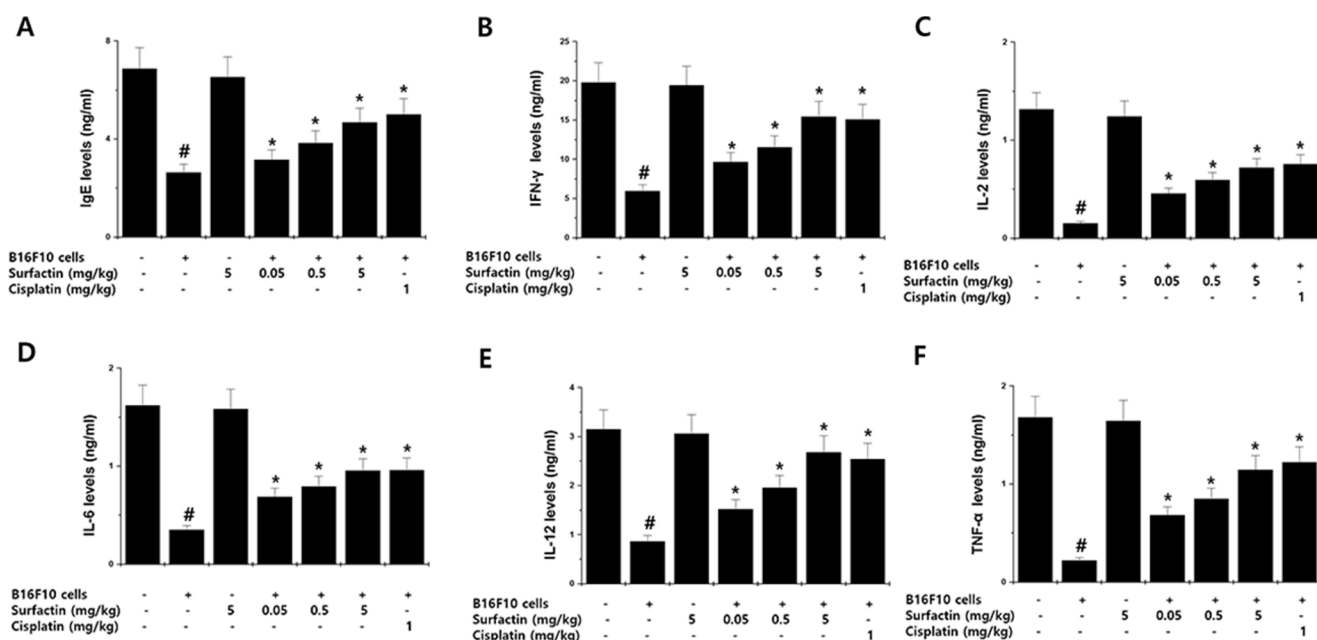


Fig. 3. Surfactin increased levels of immune-enhancing-related cytokines in serum. Levels of (A) IgE, (B) IFN- γ , (C) IL-2, (D) IL-6, (E) IL-12, and (F) TNF- α in serum were measured by the ELISA method. # $P < 0.05$, significantly different from the normal group. * $P < 0.05$, significantly different from the B16F10 group.

administration of surfactin compared to the B16F10 group ($p < 0.05$).

3.5. Effects of surfactin on blood biochemical parameters in melanoma *in vivo* model

To specify the side effects of surfactin, levels of several blood biochemical parameters were determined by using a DRI CHEM NX500

analyzer. As shown in Table 1, levels of serum LDH, BUN, ALT, AST, and CK in the B16F10 group were significantly higher than those in the normal group, whereas surfactin or cisplatin-administered group significantly reduced the serum LDH, BUN, ALT, AST, and CK levels from those in the B16F10 group.

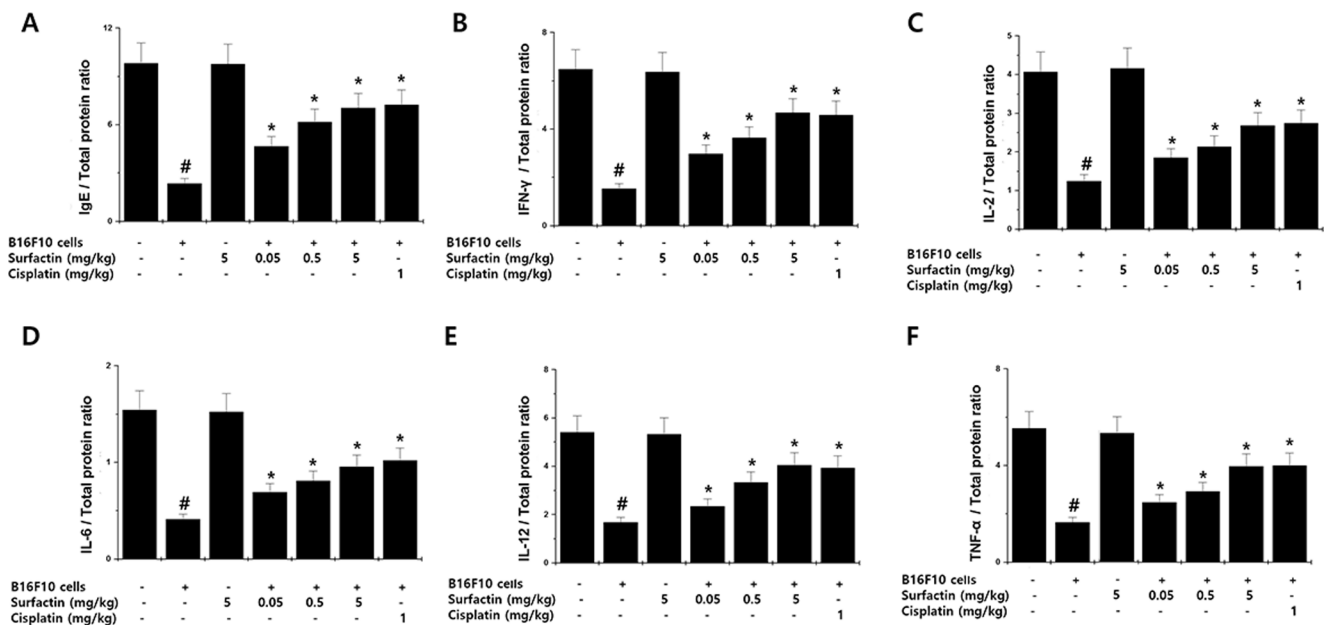


Fig. 4. Surfactin increased levels of immune-enhancing-related cytokines in melanoma tissue. Levels of (A) IgE, (B) IFN- γ , (C) IL-2, (D) IL-6, (E) IL-12, and (F) TNF- α in melanoma tissue were measured by the ELISA method. All parameters measured in melanoma tissue homogenate were presented as a ratio to the total protein levels of the intranasal tissue. # $P < 0.05$, significantly different from the normal group. * $P < 0.05$, significantly different from the B16F10 group.

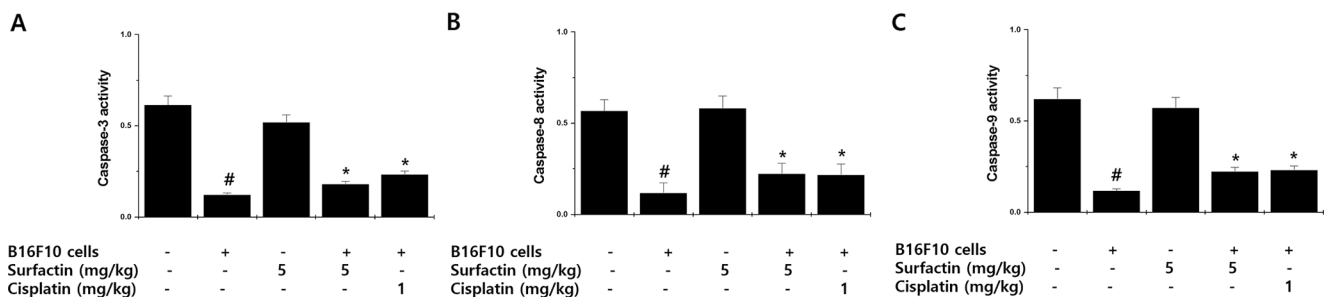


Fig. 5. Surfactin increased caspase-3, 8, and 9 activities in melanoma *in vivo* model. (A-C) Caspase-3, 8, and 9 activities in the melanoma tissues were measured by caspase assay kits. # $P < 0.05$; significantly different from the normal group. * $P < 0.05$, significantly different from the B16F10 group.

Table 1
Effects of surfactin on the blood biochemical parameters.

	LDH (U/L)	BUN (mg/dL)	ALT (U/L)	AST (U/L)	CK (mg/dL)
Normal	1620.00 $\pm$ 141.42	18.64 $\pm$ 0.99	43.6 $\pm$ 4.50	364.4 $\pm$ 23.33	0.14 $\pm$ 0.01
B16F10	23633.33 $\pm$ 707.11 [#]	130.88 $\pm$ 4.13 [#]	139.67 $\pm$ 20.51 [#]	835.75 $\pm$ 31.53 [#]	2.17 $\pm$ 0.03 [#]
B16F10 + Surfactin	13133.33 $\pm$ 1814.75*	23.7 $\pm$ 0.17*	62.67 $\pm$ 0.58*	538.00 $\pm$ 20.00*	0.29 $\pm$ 0.01*
B16F10 + Cisplatin	4052.65 $\pm$ 1234.58*	28.52 $\pm$ 1.35*	65.32 $\pm$ 5.21*	585.15 $\pm$ 3.25*	0.51 $\pm$ 0.03*

Values are means $\pm$ SEM. # $p < 0.05$ vs. normal group. * $p < 0.05$ vs. B16F10 group. B16F10, B16F10-inoculated group; B16F10 + Surfactin, Surfactin (5 mg/kg)-administered group; B16F10 + Cisplatin, Cisplatin (1 mg/kg)-administered group.

3.6. Surfactin induces cell death via activation of caspase-3, 8, and 9 in B16F10 cells

Several studies have shown that surfactin directly increases cell death in cancer cells by inducing apoptosis [32,33]. Therefore, to

investigate whether surfactin could directly induce apoptosis in B16F10 melanoma cells, MTT and caspase assays were performed. The results showed that surfactin had a marked dose-dependent effect on cell death in B16F10 cells (Fig. 6A). Finally, to determine whether surfactin could induce caspase activation, caspase assay was performed for caspase-3, 8, and 9. As exhibited in Fig. 6B-D, activities of caspase-3, caspase-8, and caspase-9 were significantly enhanced by treatment with surfactin ($p < 0.05$).

4. Discussion

Our results revealed that surfactin suppressed tumor development and increased survival rate. We showed that surfactin increased the accumulation of mast cells, levels of histamine, IgE, immune enhancing-related cytokines, and the activation of caspase-3, 8, and 9 without side effects. Our results of the present study revealed that treatment of surfactin may strengthen the negative effect on melanoma cell viability and indicated that surfactin-induced apoptosis may prevent melanoma. Additionally, results may indicate dependence between activation of allergic response and a reduced prevalence of melanoma.

Mast cells exist in proximity to blood, nerves, and fibroblasts [34]. Remarkably, mast cells play anti- or pro-tumorigenic roles, but it is not specific to model, type, and stage of tumor [8]. Accumulation of mast

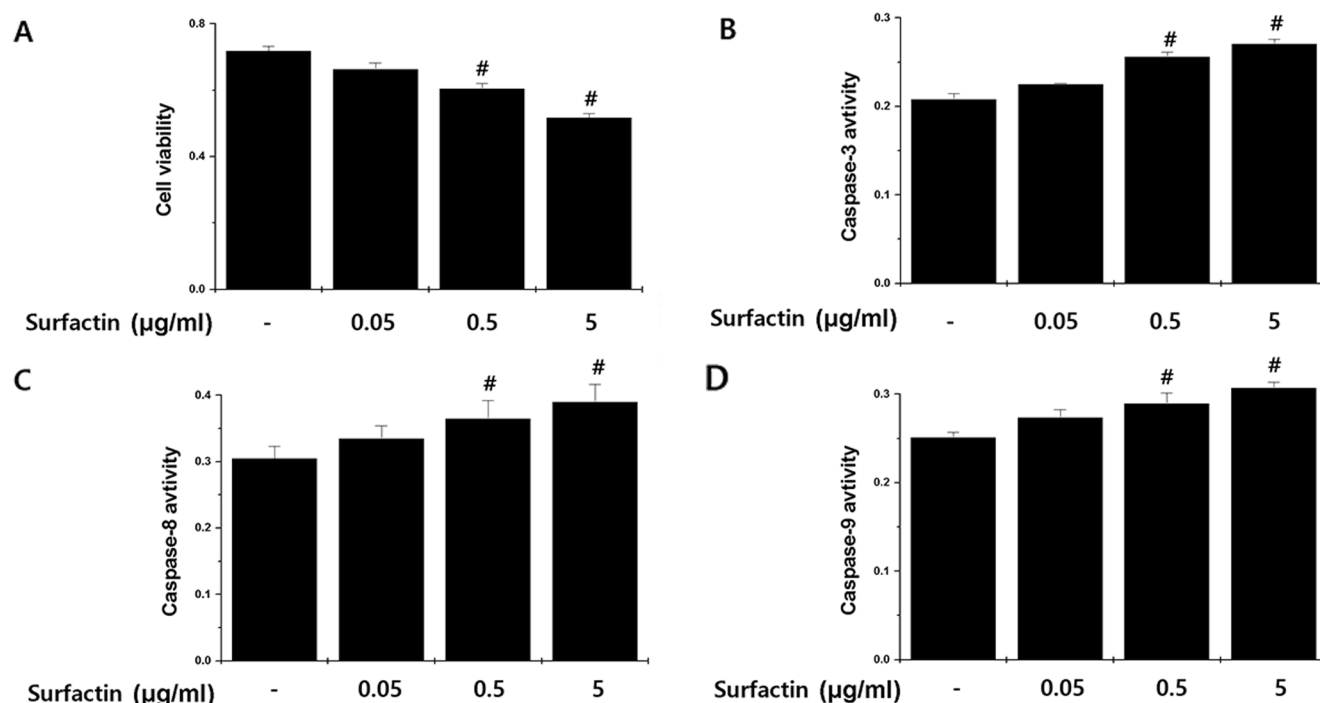


Fig. 6. Surfactin induced cell death via activation of caspase-3, 8, and 9 in B16F10 cells. (A) B16F10 cells (3×10^5) were treated with surfactin (0.05, 0.5, and 5 μg/ml) for 72 h. Cell viability was determined by MTT assay. (B–D) B16F10 cells (3×10^6) were treated with surfactin (0.05, 0.5, and 5 μg/ml) for 72 h. Caspase activities were measured using caspase colorimetric assay kits. Each datum represents the mean $\pm$ S.E.M. of three independent experiments. [#] $p < 0.05$; significantly different from the unstimulated cells.

cells has been observed near the perimeter of cutaneous tumor [25]. Current evidence suggests that accumulated mast cells have a vast array of mediators and inhibitory effects on malignancies [35]. Schitteck et al., proven that mast cell knock-out mice progress melanoma metastasis more promptly than normal mice [36]. Histamine can have different effects on cancerous cells depending on the tumor cell features [26]. In melanoma cases, histamine could be used in a combination cancer treatment to up-regulate the effectiveness and down-regulate the toxicity of melanoma therapy [37]. IgE has an important role in allergic responses and is characterized as advantageous for cancer therapy [27]. Also, IgE be concerned with natural tumor surveillance as well as take advantage of tumor control in immunotherapy of action and passion [10]. In the present study, we verified that surfactin increased the accumulation of mast cells and the levels of histamine and IgE in a concentration-dependent manner. These results indicate that surfactin has an anti-cancer effect on melanoma through its capacity to increase allergic reactions.

Mast cells play a dual role in cancer development and growth [38,39]. Previous studies suggest that the role of mast cells depends on the type of tumor [38,39]. In some tumors, mast cells promote growth, angiogenesis, and metastasis of tumor, whereas in other tumor types, mast cells reduce tumor growth [38–41]. Chromolin sodium, a chemical known as a mast cell stabilizer and inhibitor, decrease survival rate through increasing tumor growth and hypoxia in patients with breast, prostate, and ovarian cancers [38–41]. However, the role of a mast cell stabilizer has not been elucidated in melanoma until now. Therefore, further research is needed to identify the role of mast cell stabilizing drugs in melanoma.

Cisplatin is an important therapeutic drug for ovarian, lung, and head and neck cancers. Cisplatin exhibits anti-cancer effects through various mechanisms [42]. Previous study showed that mast-cell degranulation was significantly increased by the administration of cisplatin [43]. In the present study, cisplatin increased the infiltration of mast cells and the level of histamine compared to those in the control group. Therefore, we assume that the anti-cancer effect of cisplatin may

be partially related to mast cell activation.

Cytokine-based chemotherapy is one type of cancer immunotherapies [44]. These strategies include immuno-modulatory cytokines, with IFN- γ , IL-2, IL-6, IL-12, and TNF- α shown to be among the most potent inducers of anti-tumor activity in a variety of pre-clinical studies [45]. IFN- γ immunotherapy is effective in a small subset of cancer patients when used as a single agent [46]. In a few cases, treatment of systemic IL-2 has significant clinical advantages in patients with metastatic melanoma, resulting in long-term survival [47]. Minichsdorfer et al. (2015) reported that administration of IL-6 prevents the growth of early-stage metastatic melanoma cells [48]. Anti-cancer activation by local or systemic treatment of IL-12 has been demonstrated in preclinical studies in numerous tumor cell lines [49]. TNF- α is an intrinsic crucial player in the inhibition of melanoma growth [50]. In the present study, we observed that surfactin increased the levels of these cytokines in a concentration-dependent manner. These results indicate that surfactin has an anti-cancer effect on melanoma through increasing the levels of immune-enhancing-related cytokines.

Apoptosis has been established as modulating tissue development and cell growth [51]. Since suppression of apoptosis results in progression, initiation, and growth of tumors, it can result to effectually impair tumor progress [52]. Apoptosis is characterized by activation of the caspase cascade [53]. Actually, several caspases have an important role in apoptosis, several caspases are not, and several caspases have capacities other than participating in executing apoptosis [52,53]. Caspase-3 protein and its mRNA expression in cancer patients are lower than in morphologically normal samples [49]. Caspase-3 is cleaved by caspase-8 activation, ultimately the cleavage of caspase-3 results in the apoptosis [54]. Caspase-9 is a critical apoptosis marker and is an apoptotic initiator protease in the intrinsic apoptosis pathway [55]. In this regard, we demonstrated that surfactin markedly increased the activities of caspase-3, 8, and 9 in a melanoma mouse model. Furthermore, our results proved that surfactin induced the activation of caspase-3, 8, and 9 in B16F10 cells. Based on these findings, our results suggest that surfactin suppressed the tumor development via activation of caspase-

mediated apoptosis signalling pathway.

Various serum enzymes, including LDH, BUN, ALT, AST, and CK are extensively known as tissue damage indicators [54]. The normality of various serum enzymes is low in healthy samples [56]. On the other hand, these enzymes are up-regulated remarkably after damage to organs or tissues, for instance damage to muscle or hepatic [55]. Our results proved that the levels of LDH, BUN, ALT, AST, and CK in the serum of surfactin-administered mice were significantly down-regulated compared to those of B16F10 mice. It means that surfactin did not result in tissue damage, hepatotoxicity, and nephrotoxicity. Weight loss is usually associated with increasing tumor burden and diminished health status [57]. However, previous studies reported that tumor size in tumor-bearing mice has an effect on body weight gain [58,59]. Other researchers reported that melanoma-bearing mice lose body weight by day 21 but it increases thereafter, regardless, body weights were not significantly different between drug-treated and control groups [60]. In this study, we showed that the body weight of the B16F10 group significantly increased compared to the normal group, whereas the body weight of the surfactin-administered group was recovered to that of the normal group without associated tissue damage, hepatotoxicity, and nephrotoxicity. Therefore, we suggest that surfactin could be useful as an anti-cancer agent.

Targeted therapy and immune therapy are effective for melanoma patients. However, most patients treated with targeted therapy ultimately relapse, whereas most patients treated with immune therapy are unresponsive. In addition, a patient's immunotherapy treatment cost is 100 million Korean won per year. Anti-cancer agents that utilize allergic reactions are not expected to be as costly. Although its efficacy in clinical study has not been identified, surfactin has been shown to have a significant effect on melanoma development. Therefore, we assume that the development of anti-cancer drugs that utilize allergic reactions can economically and effectively overcome the disadvantages of currently used cancer therapies.

In summary, we show that surfactin suppress melanoma development and improves survival rate. Moreover, surfactin promotes immune response enhancement related mediators such as mast cells, histamine, IgE, cytokines and induces the activation of apoptosis-related enzyme such as caspase 3, 8, and 9. In addition, surfactin treatment did not result in tissue damage, hepatotoxicity, and nephrotoxicity. Therefore, these results suggest that surfactin might be useful in the treatment of melanoma skin cancer. However, further studies into metastasis effects, histological toxicity, safety, and pharmacokinetics are required before the clinical application of surfactin.

CRedit authorship contribution statement

Hee-Yun Kim: Methodology, Software, Writing - original draft, Writing - review & editing, Formal analysis. **Hanchul Jung:** Data curation, Investigation. **Hyung-Min Kim:** Funding acquisition, Conceptualization. **Hyun-Ja Jeong:** Supervision, Project administration, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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