Review

The Dual Effects of Interleukin-18 in Tumor Progression

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Interleukin-18 (IL-18) was discovered as an interferon-γ-inducing factor and had a critical role in inflammatory and immune response. It stimulates natural killer (NK) and T cells and enhances Th1 immune response. These activated immune cells eliminate cancer cells and virus-infected cells effectively. However, IL-18 has also been found to promote tumor progression. Higher expression or secretion level of IL-18 is detected in various cancer cells in comparison with normal control, and IL-18 is able to induce angiogenesis, migration/metastasis, proliferation and immune escape. These dual effects and the mechanism of IL-18 need to be investigated further as it relates to cancer. *Cellular & Molecular Immunology.* **2007;4(5):329-335.**

Introduction

Interleukin-18 (IL-18) was first discovered as an interferon-γ (IFN-γ) inducing factor (IGIF) (1). It was believed that IL-18 induced T helper type-1 (Th1) immune response because of its characterization as an IGIF (1). In fact, IL-18 is a member of IL-1 family and its receptor also belongs to IL-1 receptor (IL-1R) family. IL-18 receptor (IL-18R) consists of a ligand-binding domain α-chain and a signal-transducing domain β-chain (2). When IL-18 binds to IL-18Rα, IL-18Rβ transduces its signal to stimulate the MAPK pathway involved in producing IFN-γ (3). Interestingly, IL-18 is expressed in a biologically inactive form, pro-IL-18, with 24 kDa molecular weight, and it confers functional activity by proteolytic cleavage of IL-1β converting enzyme (ICE) resulting in an 18 kDa final molecule, which can then act as a biological signal transducer (4).

The expression and secretion of IL-18 is shown in various kinds of cells from immune cells to cancer cells. Macrophages like Kupffer cells secrete IL-18 after activation by stimuli such as LPS (1). Recently, some cancer cells have been reported to secrete IL-18 (5, 6), so the role of IL-18 needs to be investigated in cancers.

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The role of IL-18 in immune activation

Since IL-18 was discovered, the study of its role has been outstanding in scientific achievements. Not to mention its role in the induction of IFN-γ, IL-18 can activate immune cells with or without help of IL-12. IL-18 directly regulates IFN-γ promoter activity by binding the AP-1 site in the IFN-γ promoter region, while IL-12 induces its production only after the activation of co-stimulatory signals by CD28 in T cells (7). Moreover, IL-18 synergizes with IL-12 for the production of IFN-γ and the proliferation in T cells (8, 9). Similar to T cells, NK cells are also activated and proliferated by IL-18 (10). Specifically, IL-18 up-regulates the perforin-mediated cytotoxicity in NK cells (11), while macrophages produce IFN-γ under the presence of IL-18 and IL-12 (12).

Other than IFN-γ production, IL-18 has an important role in the host defense system. IL-18-mediated immune responses overcome not only viral, bacterial and fungal infection but also pathogenic infection by activated immune cells (13, 14). Furthermore, transformed cells and tumors are eliminated effectively by IL-18-mediated activation of immune cells such as cytotoxic T cells and NK cells (15-17). Here, its anti-tumor effects and mechanism will be discussed more specifically.
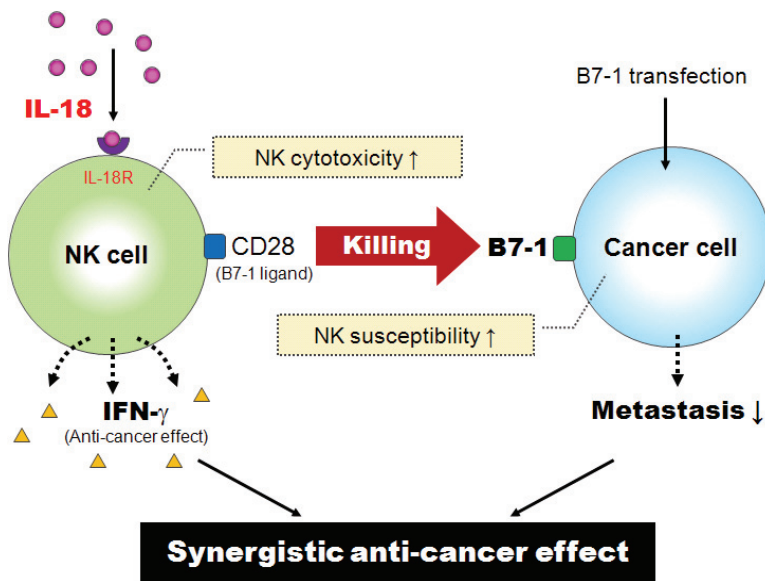
IL-18 and T cells
T cell-mediated immune response is a critical event in adaptive immunity. Through interaction with antigen presenting cells and helper T cells, the complicated sequential events of the immune response are activated. Helper T cell (Th) activation generated two different types of immune responses: helper type-1 (Th1) and helper type-2 (Th2) response, depending on the type of cytokines involved. Th1 immune response is activated primarily by IL-2, IL-12, IFN-γ and TNF-β, which are secreted during cell-mediated immune response like killing activity by either cytotoxic T

Figure 1. The synergistic effect of IL-18 and B7-1 in the immune system. IL-18 and B7-1 expressed on tumor cells synergistically activate NK cell cytolytic activity by stimulating production of IFN-γ and increase the infiltration of NK cells to the tumor site resulting in reducing cancer cell metastasis.

cells (CTLs) or NK cells (18). Th1 response is prominent in autoimmune disorders, graft rejection, and chronic inflammatory disorders. Otherwise, Th2 immune response generally plays a role in humoral immunity such as antibody-mediated immune response related to B cells and is generally activated by IL-4, IL-5, IL-6 and IL-10 (19). These Th responses regulate each other antagonistically.

According to the conventional view of IL-18 as a pro-inflammatory cytokine, IL-18 is primarily in charge of Th1 immune response. Kim et al. reported the efficient induction of antigen-specific Th1 responses by ovalbumin (OVA)/IL-18 fusion DNA (20). They suggested that the direct linkage of IL-18 to OVA as a representative antigen model could effectively lead to the Th1 response, not only resulting in IFN-γ and OVA-specific IgG2a production but also inhibition of IL-4 production. Furthermore, the direct linkage of IL-18 to anti-CD3 single-chain Fv also induced efficient Th1 immune responses (21). Anti-CD-3sFv/IL-18 fusion DNA stimulated the production of OVA-specific IgG2a and IFN-γ as well as reduction of IL-4. The synergistic effect of Th1 response caused by both OVA/IL-18 and anti-CD3sFv/ IL-18 fusion DNA is more prominent than that of single DNA injection or simple DNA mixture in each case because the half-life of the fusion protein is longer than intact protein in the case of IL-18 (22). Altogether, this evidence shows that IL-18 is a critical molecule in the activation of Th1 immune response, and its effect is more powerful when fused with a stimulator of immune response such as OVA antigen or an activator of T cells such as anti-CD3sFv and, therefore, fused DNA vaccine instead of expensive cytokine therapy is suggested as a beneficial and effective treatment of certain infections and allergic response. Moreover, Chang et al. (23) explained recently that intratumoral injection of IL-18 DNA enhances IFN-γ production and caused regression of liver tumor. It is suggested that intratumoral injection could be used for cancer treatment without

side-effects. Besides DNA vaccines, IL-18 has potential efficacy as a therapeutic adjuvant. When IL-18 DNA is treated with prostate-specific antigen (PSA) DNA, tumors are almost completely rejected after 30 days of injection, resulting in T helper cell proliferation, IFN-γ production and enhanced splenocyte activity (24). IL-18 therefore represents a powerful and effective candidate for gene therapy.

In relation to infection, *Mycobacterium avium* is the most common cause of bacterial infection in AIDS patients, resulting in severely shortened life. Therefore, to enhance Th immune response, IL-18 would be beneficial in the treatment of AIDS patients, especially in patients with bacterial infection. Injection of DNA-encoding IL-18 and anti- CD3sFv/ IL-18 fusion DNA both show dramatic resistance to *Mycobacterium avium* complex (MAC) through the enhancement of IFN-γ (25, 26). Furthermore, it has been found that bacterial loads in lung are dramatically reduced by either IL-18 DNA or anti-CD3sFv/IL-18 fusion DNA injection and cytotoxicity of lung cells significantly increased in both cases. These results provided the basis of outstanding usage of IL-18 DNA and IL-18 fusion DNA as a vaccination to patients with certain bacterial infection.

IL-18 and NK cells
In the immune system, CTLs and NK cells function to clear transformed cells and tumors from the body. In addition to using anti-CD3sFv as an activator of cell-mediated immune response, B7-1 costimulatory molecule in combination with IL-18 was used by Cho et al. against malignant skin tumors like melanoma (27). The immunization with [IL-18 + B7-1] enhanced IFN-γ production *in vivo* and reduced tumor burden as well as prolonged patients survival. Furthermore, tumor metastasis was also inhibited by [IL-18 + B7-1]. Immuno-histochemistry showed that NK infiltration at the site of tumor tissue was increased and its cytolytic ability was significantly enhanced by [IL-18 + B7-1] compared to [IL-12

+ B7-1]. It suggested that the anti-tumor effect of IL-18 is correlated with NK cells rather than CTL because the cytotoxicity was not as disturbed by T cell depletion as it was by NK depletion. The relationship between IL-18 and B7-1 in the activation of the cell-mediated immune response against tumors is summarized in Figure 1. Recently, IL-18-B7.2 gene therapy with irradiation given to melanoma-bearing mice successfully showed anti-tumor effects (28).

The balance of activation and inhibition signals is responsible for the natural cytolytic events in NK cells. For example, NKG2D, which is a C-type lectin-like protein that is expressed on NK cells and CTL, is one of the representative activating receptors. It can deliver activating signals that are strong enough to overcome inhibition signals due to the help of transmembrane adapter protein DAP10 which contains a long cytoplasmic signal transducing domain. However, tumors are able to avoid attack by NK cells and CTL because they express NKG2D ligand on their surface, which re-activates immune cells. This ability of tumor cells to evade immune attack can fortunately be defeated by treatment with cytokines. Smyth et al. (29) proposed that IL-2/IL-18 combination activates NK cells to kill tumors by NKG2D-mediated killing and suppresses tumors through perforin- and Fas/FasL-mediated cytolytic activity. A recent study reported that IL-2/IL-18 combination induced the recovery of NKG2D expression, which is reduced by transforming growth factor (TGF)-β (17). This NKG2D recovery serves as a model for improving NK cell ability to inhibit tumor cell-derived TGF-β, which prohibits the anti-tumor immune responses and acts directly as a tumor progression factor and down-regulates NKG2D expression (30, 31). As it is well known as an activator of NK cells, IL-18 is a popular candidate for cytokine therapy.

The role of IL-18 in cancer

As previously stated, IL-18 is known as an immune activator. Activated NK or T cells by IL-18 eliminate spontaneous cancer or pathogen infected cells. Contrary to the anti-cancer effect of IL-18, its pro-cancerous effect has been recently suggested. The first evidence of the pro-cancer effects of IL-18 is the elevated expression or secretion levels of IL-18 in cancer patients. IL-18 was expressed and secreted in common skin tumors including squamous cell carcinoma (SCC), melanoma and skin cancer cell lines (5). Additionally, increase IL-18 expression in skin tumors progressed inferred that IL-18 has a positive correlation with malignancy of skin tumors (5). Similarly, in gastric cancer patients, the analysis of the expression pattern of IL-18 and tumor progression revealed that IL-18 was expressed highly in the tumor region in comparison with non-tumor region and related with distant metastasis (32). Eissa et al. (33) and Merendino et al. (34) have reported that IL-18 is also an important marker of breast cancer progression because higher IL-18 levels were detected in serum from breast cancer patients with metastasis compared to healthy volunteers and breast cancer patients without metastasis.

Here, we summarize the pro-cancerous effects of IL-18 as it relates to angiogenesis, metastasis and immune escape.

Pro-angiogenic function of IL-18

Angiogenesis is the process of new vessel formation and is critical in inflammatory response, wound healing and tumor progression (35). Solid tumors require oxygen and nutrients for their growth, and therefore typically need new blood vessels. Angiogenesis is regulated by the balance between angiogenic activators and inhibitors (36). A lot of angiogenic stimulators such as vascular endothelial growth factor (VEGF) (37), platelet-derived endothelial growth factor (PDEGF), fibroblast growth factor (FGF) (38), TGF-β (39), and angiogenin (40) are known to mediate angiogenesis. In gastric cancer, high expression of IL-18 is detected in invasive tumor tissues and such tissues have higher microvessel density (32). This indicates the possibility that high expression of IL-18 is associated with new blood vessel formation. Cho et al. (41) demonstrated that IL-18 stimulates production of VEGF mRNA and protein level in rheumatoid arthritis (RA), which supports the theory that IL-18 directly stimulates angiogenic activity through the enhancement of migration of human microvascular endothelial cells (HMVEC) in RA (42). In addition, when fibroblast-like synoviocytes (FLS) were pre-treated by IL-18 with curcumin, an AP-1 inhibitor, IL-18-enhanced VEGF production was reduced to basal level. Furthermore, IL-18 stimulates the binding of AP-1 to VEGF promoter, resulting in VEGF production. Amin et al. (43) has reported that IL-18 increases the production of various angiogenic factors such as stromal cell-derived factor 1α (SDF-1α)/CXCL12, MCP-1/CCL2, and VEGF in RA synovial tissue (ST) through the phosphorylation of JNK-1/2, PI3K, p38MAPK, PKC and NF-κB.

Although most of the pro-angiogenic effects of IL-18 were identified in RA, pro-angiogenic function of IL-18 in cancer was verified recently. IL-18 stimulates thrombo-spondin-1 (TSP-1) production which is a pro-angiogenic factor in gastric cancer (44). Both TSP-1 mRNA and protein levels were increased by IL-18 in a time- and dose-dependent manner. When gastric cancer cells were pretreated by JNK inhibitor, the enhanced secretion of TSP-1 by IL-18 was reduced, suggesting that activated JNK by IL-18 affects TSP-1 production. In addition, serum level of IL-18 is increased by advanced stage of disease and has a positive relationship with VEGF level in myeloma patients (45), showing that IL-18 regulates VEGF level not only in RA but also in various cancers.

Additionally, the effect of angiogenic factors in IL-18 production has been shown in gastric cancer cells. Kim et al. has identified that pro-angiognic factor, VEGF, regulates IL-18 production and its mRNA and protein level is increased by VEGF in time- and dose-dependent manners (46). IL-18 is increased by VEGF *via* the regulation of reactive oxygen species (ROI) and the ERK1/2 pathway. This means that pro-angiogenic factors can also induce the maturation of IL-18, suggesting a positive relationship between IL-18 and VEGF production in cancer. As VEGF
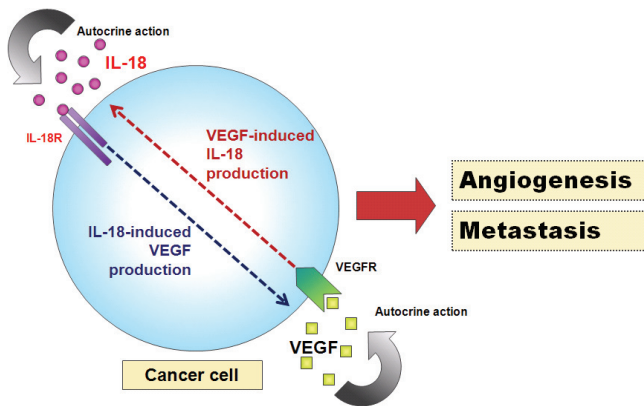
Figure 2. The positive-feedback regulation of IL-18 and VEGF in cancer. IL-18 produced by cancer cell stimulates pro-angiogenic factor, VEGF, by autocrine action. IL-18-induced VEGF also activates IL-18 secretion through VEGF receptor. Through this hypothetical positive-feedback mechanism between IL-18 and VEGF, the sequential events of angiogenesis and migration in cancer cells are able to be stimulated and the tumor progression might be accelerated.

stimulates IL-18 production and secreted IL-18 induces VEGF production, cancer progression is able to increase by this auto-regulating mechanism, and we suggest the auto-regulating hypothesis in cancer depicted in Figure 2.

IL-18 as a metastasis stimulator in cancer
Metastasis is an important characteristic of advanced cancer. Typically, cancer in patients with metastasis is more aggressive than primary cancer, correlating with low patient survival and poor prognosis of cancer therapy (47). Metastasis is a complex process that includes the degradation of extracellular matrix (ECM) and the regulation of various adhesion molecules and chemokine receptors (48-50). After ECM degradation cancer cells migrate to another site by the regulation of adhesion molecules, which is critical in metastasis. Recently, IL-18 has been proposed as a cell migration stimulator in melanoma and gastric cancer. Jung et al. (51) confirmed the differences in migration ability in murine melanoma cell lines, B16F10 and antisense IL-18 cDNA transfected B16F10 (antisense IL-18/B16F10). In comparison with wild type B16F10 (WT), the migration ability of antisense IL-18/B16F10 was decreased and exogenous recombinant IL-18 treatment to these cells enhanced migration capacity in a time- and dose-dependent manner. The effect of IL-18 on cancer cell migration is similar in gastric cancer. Kim et al. (52) has identified the pro-metastatic function of VEGF that stimulates cell migration of gastric cancer cells through IL-18 production. Moreover, VEGF stimulates IL-18 mRNA and protein levels, which then affects the migration ability of gastric cancer cells. Blocking IL-18 with either siRNA or IL-18 binding protein (IL-18BP) in gastric cancer cells did not cause VEGF-induced migration, which is therefore mediated through IL-18 production. Indeed, the induction of IL-18 by VEGF

stimulates f-actin polymerization which is a common process shown at the site of cell movement.

In atherosclerosis, IL-18 is also able to regulate migration of smooth muscle cell (SMC). Chandrasekar et al. has confirmed that IL-18 stimulates migration of human coronary artery SMC in matrix metalloproteinase-9 (MMP-9) and MMP-2 dependent manners (53). Moreover, IL-18 induces expression of MMP-9 mRNA and protein levels *via* activated NF-κB and AP-1. As previously mentioned, the degradation of ECM is required for cell migration and MMP is well known as an important ECM-degrading enzyme. Actually, IL-18 stimulates transmigration of human myeloid leukemia cell line, HL-60 through MMP-9 (54). IL-18 significantly increased MMP-9 expression at both mRNA and protein levels in this leukemia cell line. Through MMP-9 production by IL-18, HL-60 is able to degrade ECM surrounding cells and increases invasion ability. Jiang et al. (55, 56) also demonstrated that IL-18 is an important stimulator of lung cancer metastasis. Poorly metastatic cells were transfected with constructed sense IL-18, and expression was enhanced, resulting in increased invasion ability along with reduction of E-cadherin protein level in the stable transfectant. In addition, when highly metastatic cells were transfected with antisense IL-18, the lower IL-18 expression levels corresponded with decreased invasion ability and up-regulation of E-cadherin. Therefore, IL-18 is a powerful stimulator for cancer cell metastasis.

Because cancer cells are attached to vascular endothelial cells and migrate through the blood stream, the adherence of cancer cell to vascular endothelial cells is a critical event during metastasis (57). Some reports suggest that tumor-derived pro-inflammatory cytokine induces adhesion receptor of endothelial cells for cancer cell attachment (58). Vidal-Vanaclocha et al. confirmed that hepatic metastasis was reduced in IL-1β or IL-1β converting enzyme (ICE) mutant mice after injection of murine melanoma cell line B16M (59). Furthermore, B16M cultured medium (B16M-CM) activated hepatic sinusoidal endothelium (HSE) by release of TNF-α and IL-1β, while increasing the attachment ability of B16M cells to HSE through increase in VCAM-1 expression. However, the B16M-CM-induced adhesiveness of B16M cells to HSE was abrogated by blocking of IL-18. Because ICE is an important enzyme for the production of mature IL-18, reduced metastasis was detected in IL-1β or IL-1β converting enzyme (ICE) mutant mice. Neutralization of IL-18 by using IL-18 binding protein (IL-18BP) has also been shown to decrease hepatic metastasis of melanoma cells (60). When IL-18BP was given as pretreatment before the injection of B16M cells, hepatic metastasis of B16M cells were reduced about 75% in comparison with IL-18BP non-treated mice. IL-18BP pretreatment was also shown to decrease VCAM-1 expression. Taken together, these data reconfirm the assertion of Vidal-Vanaclocha et al. that IL-18 induces metastasis of murine melanoma cells *via* increasing of VCAM-1 expression in HSE.

IL-18 and immune escape of tumor
As previously mentioned, the immune system eliminates
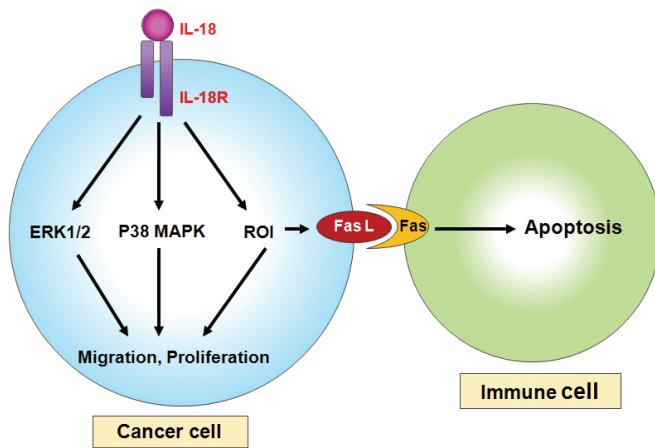
Figure 3. Pro-cancer effect of IL-18. Interaction with IL-18 and IL-18 receptor (IL-18R) activates phosphorylation ERK1/2 and p38 MAPK and increases endogenous reactive oxygen species (ROI) levels. These signaling pathways induce migration ability and promote proliferation of cancer cells. Furthermore increased-ROI level by IL-18 induces expression of Fas ligand (FasL), which interacts with Fas on immune cells. Through Fas/FasL apoptotic pathway, immune cells were died by apoptosis and cancer cells were able to escape against immune cells.

neoplasitc cells and transformed cells, however cancer cells are able to escape this mechanism. For example, cancer cells down-regulate MHC class I molecules to avoid elimination by the T cell immune system (61). In melanoma cells, IL-18 induces Fas ligand expression and gives them resistance to immune surveillance. Cho et al. tested NK susceptibility of melanoma cell line B16F10 and antisense IL-18 transfected B16F10 (antisense IL-18/B16F10) and found that antisense IL-18/B16F10 cells were more susceptible than wild type B16F10 to NK cells due to the induction of Fas ligand expression through ROI generation (6). Because cancer cells express Fas ligand, they induce the apoptosis of the immune cells which express Fas. IL-18-induced Fas ligand expression is therefore a method of tumor immune escape and survival factor of melanoma. Yoon et al. has also suggested that IL-18 is important to escape from the immune surveillance (62). They performed transfection of IL-18 receptor to the cervical carcinoma cell line C33A and saw an increase in the endogenous IL-18, Fas and ROI expression in the transfectant. Otherwise, the induction of apoptosis by using agonistic anti-Fas antibody cannot induce apoptosis of IL-18 receptor transfected C33A cells. They suggest that the resistance to Fas dependent apoptosis is due to either ROI or non-functional Fas expression by IL-18. Altogether, these data suggest that IL-18 is a key regulator of tumor immune escape.

Conclusion

According to several established concepts, IL-18 is important in inflammatory and immune response. Because IL-18

activates immune cells such as T and NK cells to kill endogenous cancer cells or virus infected cells, it has recently been used for cancer therapy. Due to its increase in use there have been some reports about the effect of IL-18 either as a vaccine or as an adjuvant for cancer therapy to improve Th1 immune response and to enhance NK cell cytotoxicity.

Nonetheless, as previously explained, IL-18 also has an important role in tumor progression. In various cancers, IL-18 expression and serum level are higher than normal regions or healthy controls. IL-18 directly promotes proliferation by regulating proliferation stimulatory factors. In angiogenesis it stimulates pro-angiogenic factors such as VEGF and TSP-1 and also regulates proliferation of vascular endothelial cells. Furthermore, it also promotes cell migration in gastric cancer and melanoma through ROI generation and regulates adhesion molecules such as VCAM-1 on endothelial cells. In addition, IL-18 up-regulates ECM degradation enzyme expression. All of these reports demonstrate the pro-cancerous effect of IL-18, which is summarized in Figure 3. Focusing on the pro-cancer effect of IL-18, IL-18 binding protein and specific anti-IL-18 antibody are useful for cancer therapy. Some reports suggest that various factors in food can down-regulate IL-18 levels. Tannic acid is able to down-regulate UVB-induced IL-18 expression by regulating MAPK signaling pathway in keratinocytes, and vitamin C can also reduce IL-18 expression levels *via* ROI generation and MAPK pathway in melanoma cell line (63, 64).

Therefore, although IL-18 stimulates host immune responses, therapy using IL-18 must be considered carefully because of its ability to also stimulate aggressiveness of cancer. Further detailed investigations about the dual effects of IL-18 are needed to be accomplished to use proper application of IL-18 therapy.

Acknowledgement

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