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# Therapeutic potential of interleukin-27 against cancers in preclinical mouse models

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**Keywords:** antitumor, IL-27, poly(I:C), TLR3, TRAIL

**Abbreviations:** ADCC, antibody-dependent cell-mediated cytotoxicity; COX-2, cyclooxygenase-2; DC, dendritic cell; EBI3, Epstein–Barr virus-induced gene 3; EMT, epithelial–mesenchymal transition; gp, glycoprotein; IL, interleukin; IRF, interferon regulatory factor; MHC, major histocompatibility complex; MMP, matrix metalloproteinase; NK, natural killer; PD-L1, programmed death-ligand 1; $PGE_2$, prostaglandin $E_2$; poly(I:C), polyinosinic-polycytidylic acid; STAT, signal transducer and activator of transcription; Tim-3, T-cell immunoglobulin and mucin domain-3; TLR, Toll-like receptor; Tr1, IL-10-producing regulatory T; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; Treg, regulatory T; VEGF, vascular endothelial growth factor.

Since we first reported the antitumor efficacy of IL-27 in 2004, accumulating evidence obtained by several groups using a variety of preclinical mouse models indicates that IL-27 possesses potent antitumor activity against various types of tumors through multiple mechanisms depending on the characteristics of individual tumors without apparent adverse effects.

Cancer immunotherapy has become one of the most promising new treatments that harnesses powers of the immune system to control cancer and achieve long-term cure. This strategy includes therapeutic vaccines, T-cell checkpoint inhibitors, adoptive therapy of genetically modified immune cells, elimination of immunosuppressive cells such as regulatory T (Treg) cells, and new cytokines. Interleukin (IL)-27 is one of the IL-12 family cytokines, which play critical roles in the regulation of differentiation into respective helper T (Th) cells and their functions.[1] IL-27 consists of p28, which is also called IL-30, and Epstein–Barr virus-induced gene 3 (EBI3), and its receptor (R) is composed of IL-27Rα (WSX-1) and glycoprotein (gp)130, a common receptor subunit for the IL-6 family cytokines.[1] IL-27 possesses both pro-inflammatory and anti-inflammatory properties; it promotes the early induction of Th1 differentiation and generation of cytotoxic T lymphocytes (CTLs), but it inhibits the differentiation of naive $CD4^+$ T cells into Th2, Th9, and Th17 cells and suppresses the production of pro-inflammatory cytokines through activation of both signal transducer and activator of transcription (STAT)1 and STAT3.[1]

Antitumor effect of IL-27 was first demonstrated in 2004 using mouse colon carcinoma Colon 26 transfected with IL-27 expression vector, which greatly reduced tumor growth through mainly $CD8^+$ T cells.[2] Since then accumulating evidence by several groups has revealed that IL-27 possesses potent antitumor activity against a variety of tumor models, ranging from a transplanted mouse tumor genetically engineered to secret IL-27 before transplantation to a human therapeutic model by injection of IL-27 protein into immunodeficient mice after transplantation of human tumor as preclinical tumor models.[2-7] Molecular mechanisms of the antitumor activity were demonstrated to be mediated in multiple ways by $CD8^+$ T cells,[2] natural killer (NK) cells,[3] and macrophages,[4] antibody-dependent cell-mediated cytotoxicity (ADCC), anti-angiogenesis,[5,6] direct antiproliferative effects,[7] inhibition of expression of cyclooxygenase-2 (COX-2) and prostaglandin $E_2$ ($PGE_2$), and suppression of epithelial–mesenchymal transition (EMT),[4] depending on the characteristics of individual tumors (**Fig. 1**). Since several tumors were revealed to express both IL-27R subunits on the cell surface, IL-27 elicited direct antiproliferative effects via WSX-1/STAT1 signaling, presumably, by its cytostatic effects rather than by cytotoxic effects.[1] Recently, we have further elucidated a novel antitumor mechanism that IL-27 augments the expression of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and Toll-like receptor 3 (TLR3) in human melanoma cell lines, SK-MEL-13, 28, and 37, and that IL-27 inhibits the tumor growth in cooperation with a TRL3 agonist, polyinosinic-polycytidylic acid [poly(I:C)], partly in a TRAIL-dependent manner.[7] Moreover, repeated injections of IL-27 protein and poly(I:C) cooperatively suppressed *in vivo* tumor growth of human melanoma in immunodeficient non-obese diabetic/severe combined mice.[7] Similar therapeutic effects by repeated injections of IL-27 protein were also demonstrated against various types of human tumors transplanted in immunodeficient mice.[4,6] The human tumors so far revealed to be susceptible to the treatment by IL-27 are not only melanoma[7] but also multiple

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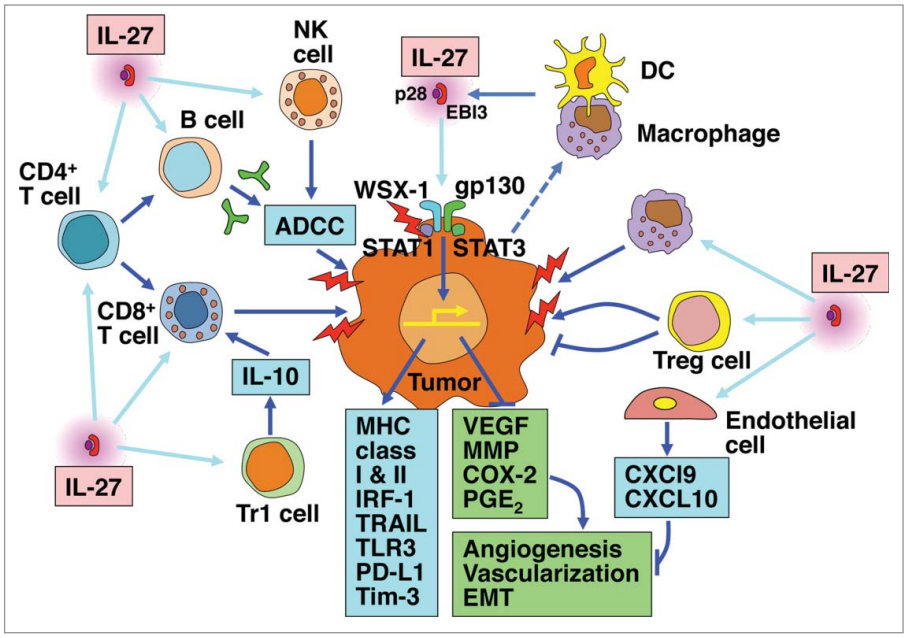


**Figure 1.** Potent antitumor activity of IL-27 through multiple mechanisms, which are mediated by $CD8^+$ T cells, NK cells, macrophages, macrophages, ADCC, anti-angiogenesis, direct anti-proliferative effect, inhibition of COX-2 and $PGE_2$ expression, and suppression of EMT, depending on the characteristics of individual tumors. Abbreviations: ADCC, antibody-dependent cell-mediated cytotoxicity; COX-2, cyclooxygenase-2; DC, dendritic cell; EBI3, Epstein–Barr virus-induced gene 3; EMT, epithelial–mesenchymal transition; gp, glycoprotein; IL, interleukin; IRF, interferon regulatory factor; MHC, major histocompatibility complex; MMP, matrix metalloproteinase; NK, natural killer; PD-L1, programmed death-ligand 1; $PGE_2$, prostaglandin $E_2$; poly(I:C), polyinosinic-polycytidylic acid; STAT, signal transducer and activator of transcription; Tim-3, T-cell immunoglobulin and mucin domain-3; TLR, Toll-like receptor; Tr1, IL-10-producing regulatory T; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; Treg, regulatory T; VEGF, vascular endothelial growth factor.

myeloma,[6] B-acute lymphoblastic leukemia, follicular lymphoma, diffuse large B-cell lymphoma, acute myeloid leukemia, prostate cancer, and non-small cell lung cancer.[4] IL-27 strongly inhibited their tumor growth *in vivo* mainly due to suppression of pro-angiogenesis-related molecules such as vascular endothelial growth factors, angiopoietins, and matrix metalloproteinases, and also due to inverse augmentation of anti-angiogenesis-related molecules such as tissue inhibitor of metalloproteinase.[5,6]

Since IL-27 possesses both pro-inflammatory and anti-inflammatory activities,[1] some of them such as IL-27-mediated augmentation of IL-10 production and up-regulation of programmed death-ligand 1 (PD-L1) may function against its antitumor effects, although the role of IL-27 in the induction of Treg cells is still controversial.[8,9] Therefore, it is reasonable to consider that the potent antitumor activity of IL-27 is the sum of these positive and negative effects on the immune responses to tumors. Although the role of IL-10 in induction of antitumor immune responses is often controversial, recent evidence supports a positive role for IL-10 in the induction of antitumor CTL responses, which were demonstrated in IL-10-deficeient mice and IL-10-transgenic mice. Indeed, IL-10 production by IL-27-induced CTLs was recently demonstrated to contribute to the generation of memory precursor-like effector CTLs to augment antitumor immunity.[10] Moreover, IL-27 was recently shown to induce the expression of PD-L1 in T cells, a major mediator of T-cell exhaustion, which hampers the induction of antitumor immune responses.[1] However, since antibody drugs have currently become very popular as one of the most promising cancer immunotherapies, IL-27 injection could augment antitumor immune responses in synergy with antibodies against immune checkpoints such as PD-L1, whose possibility remains to be clarified. On the other hand, IL-27 was initially demonstrated to inhibit the differentiation of naive $CD4^+$ T cells to Treg cells,[1] whereas recent reports oppositely revealed that IL-27 does not inhibit the generation of Treg cells but rather promotes it; IL-27 produced a distinct Treg cell population which expresses T-cell-specific T-box transcription factor T-bet and CXCR3, to control Th1-mediated immunity.[1] Similar paradoxical roles of IL-27 in the tumor microenvironment were also reported.[8,9] Thus, the effects of IL-27 on Treg cells may depend on the context. However, so far there is no report showing that injection of IL-27 protein or tumor cells transfected with IL-27 expression vector would augment the number of Treg cells in mice bearing tumors, although transgenic mice over-expressing IL-27 were previously demonstrated to decrease the number of Treg cells,[1] which could presumably result in enhancement of antitumor immune responses. Besides, since IL-27 was shown to have lower toxicity in mouse models,[2,3] collectively, IL-27 may have great potential as one of the most promising therapeutic cytokines against cancer.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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