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Antiproliferative Activity of IL-27 on Melanoma

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IL-27 is a member of the IL-6/IL-12 family and activates both STAT1 and STAT3 through its receptor, which consists of WSX-1 and gp130. We previously demonstrated that IL-27 has potent antitumor activities, which are mediated through CD8+ T cells, NK cells, or its own antiangiogenic activity. In this study, we demonstrate that IL-27 also possesses a direct antiproliferative activity on melanoma. Although WSX-1 expression was hardly detected in parental mouse melanoma B16F10 cells, IL-27 activated STAT1 and STAT3 and up-regulated MHC class I in B16F10 transfectants expressing wild-type WSX-1. In contrast, IL-27 failed to activate STAT1 and up-regulate MHC class I in those expressing mutant WSX-1, in which the putative STAT1-binding Tyr-609 of the cytoplasmic region was replaced by Phe. IL-27 inhibited the tumor growth of transfectants expressing wild-type WSX-1 in a dose-dependent manner. IL-27 augmented the expression of IFN regulatory factor (IRF)-1 and IRF-8, which possess tumor suppressor activities, in B16F10 transfectants expressing wild-type WSX-1. Down-regulation of IRF-1 but not IRF-8 with small interfering RNA partially blocked the IL-27-induced growth inhibition. A small, but significant, direct antiproliferative effect of IL-27 was also observed in vivo. Moreover, several human melanoma cells were revealed to express both IL-27 receptor subunits, and activation of STAT1 and STAT3 and growth inhibition by IL-27 were detected. These results suggest that IL-27 has an antiproliferative activity on melanomas through WSX-1/STAT1 signaling. Thus, IL-27 may be an attractive candidate as an antitumor agent applicable to cancer immunotherapy.


We, and other groups, previously reported that IL-27 has a potent ability to induce tumor-specific antitumor and protective immunity using colon carcinoma colon 26 (9, 10) and TBJ neuroblastoma (11) through CTL and NK cells. Recently, we further demonstrated that IL-27 exerts antitumor activity against poorly immunogenic B16F10 melanoma, which is mediated through NK cells but not CTL (12). We also showed that IL-27 has a potent antiangiogenic activity by inducing antiangiogenic chemokines, IFN-γ-inducing protein (IP-10, CXCL10) (13, 14) and monokine induced by IFN-γ (MIG, CXCL9) (15), as does IFN-γ, but in an IFN-γ-independent manner (16). Thus, IL-27 exerts antitumor activities through multiple mechanisms including CTL, NK cells, and antiangiogenic activity.

IFNs have been investigated as a potential therapy for many types of tumors (17–20). IFN-γ production is central to the antitumor effect of IL-12 (21, 22), and tumor cell sensitivity to IFN-γ is necessary for the antiangiogenic and antitumor effect of combination therapy with IL-12 and IL-18 (23, 24). The antitumor activities of IFN-γ are attributed to not only indirect mechanisms, such as immunomodulation, but also antiangiogenesis and direct actions on tumor cells (17–20). IFN-γ is capable of potently inhibiting cell growth in a number of tumor models. The antiproliferative mechanisms by which IFN-γ exerts its effects seem to be complicated. IFN-γ induces cell cycle arrest and apoptosis, resulting in growth suppression (25–27). IFN-γ-induced apoptosis is mediated through regulating the expression of a number of apoptosis-related proteins, including TNF receptor Fas and other cell death receptors and their respective ligands, several Bcl-2 family members, and caspases (28–32).

Because IL-27 has similar properties to IFN-γ including STAT1 activation and induction of T-bet, IP-10, and MIG (3, 6, 16), we investigated the direct role of IL-27 on tumor cells using mouse and human melanoma cell lines. Although mouse B16F10 melanoma does not express one of IL-27R subunits, WSX-1, we found B16F10 transduced with WSX-1 cDNA becomes responsive to...

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3 Abbreviations used in this paper: TCCR, T cell cytokine receptor; IRF, IFN regulatory factor; Tg, transgenic; m, mouse; h, human; pY, phosphotyrosine; PL, proplidium iodide; siRNA, small interfering RNA; PKC, protein kinase C; IP, IFN-γ-inducing protein; MIG, monokine induced by IFN-γ.

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IL-27. Stimulation with IL-27 induced not only up-regulation of MHC class I expression, but also antiproliferative responses in these transfectants, which were mediated through WSX-1/STAT1 and partially through IRF-1. Moreover, several human melanoma cell lines were shown to express IL-27 receptor and stimulation with IL-27 induced antiproliferative effect on these human cell lines as well. Thus, IL-27 can exert antitumor activity through direct antiproliferative activity as well as CD8+ T cells, NK cells, and antiangiogenic activity.

Materials and Methods

Cell culture and mice

Mouse B16F10 melanoma cell line was cultured in RPMI 1640 medium supplemented with 10% FBS. Human melanoma cell lines, SK-MEL-13, -28, and -37, were provided by Drs. L. J. Old and G. Ritter (Ludwig Institute for Cancer Research, New York Branch, Memorial Sloan-Kettering Cancer Center, New York, NY) and Dr. T. Takahashi (Aichi Cancer Center, Nagoya, Japan), and cultured in IMDM supplemented with 10% FBS. Other human melanoma cell lines, SBC12, A101D, p22, MeWo, G361, and p39, were cultured in MEM supplemented with 10% FBS. Transgenic (Tg) mice expressing IL-27 (IL-27 Tg) under the control of a liver-specific promoter were established as described (33). All animal experiments were performed in accordance with our Institutional Guidelines.

Preparation of B16F10 transfectants

Mouse WSX-1 cDNA was isolated by RT-PCR using total RNA prepared from Con A-activated spleen cells and confirmed by sequencing. Mutant WSX-1 cDNA, in which the putative STAT1-binding Tyr^{609} of the cytoplasmic region was replaced by phenylalanine, was generated using standard PCR methods. hWSX-1 cDNA was isolated from HEK293T cells. Mouse wild-type WSX-1, its mutant, and hWSX-1 cDNAs were subcloned into p3xFLAG-CMV-14 vector (Sigma-Aldrich). B16F10 cells were transfected with these expression vectors and the empty vector as control using FuGene 6 (Roche) and selected with geneticin (G418).

Western blotting

Cells were lysed in a lysis buffer containing protease inhibitors, and resultant cell lysates were separated by SDS-PAGE under reducing conditions and transferred to polyvinylidene difluoride membrane (Millipore) as described previously (35). The membrane was then blocked, probed with a primary Ab and then with an appropriate secondary Ab conjugated to HRP, and visualized with the ECL detection system (Amersham Biosciences) according to the manufacturer’s instructions.

Proliferation assay

Cells were stimulated with IL-27 for 48–96 h and pulsed with [H]thymidine for the last 18–24 h. [H]Thymidine incorporation was counted using TopCount (PerkinElmer).

Apoptosis assay

Apoptosis was assessed by flow cytometric analysis of cells stained with Annexin V-FLTC and propidium iodide (PI) according to the manufacturer’s instruction (BD Biosciences). Cell cycle analysis was performed using PI. B16F10 transfectants were stimulated with IL-27 (10 ng/ml) for 20 min. Total cell lysates were then prepared and analyzed for phosphorylation of STAT1 and STAT3 and FLAG-tagged WSX-1 expression by Western blotting using anti-pY-STATs, anti-total STATs, anti-FLAG, and anti-actin.

Small interfering RNA (siRNA) transfection

Mouse IRF-1 and IRF-8 siRNAs (ON-TARGETplus SMARTpool) and negative control siRNA (siGLO RISC-free) were purchased from Thermo Scientific. B16F10 transfectants were plated in 12-well plates and transfected with these siRNAs using HiPerFect Transfection reagent (Qiagen) according to the manufacturer’s protocol.

RT-PCR

Total RNA was extracted using a guanidinium thiocyanate procedure, cDNA was prepared using oligo(dT) primer and SuperScript RT (Invitrogen), and RT-PCR was performed using TaqDNA polymerase as described (36). Cycle conditions and primers used for mouse and human WSX-1, gp130, and hypoxanthine phosphoribosyltransferase were as previously described (16, 37).
Statistical analysis was performed by Student’s t test. A value of \( p < 0.05 \) was considered to indicate a statistically significant difference.

Results

**B16F10 transfectants expressing wild-type WSX-1 become responsive to IL-27**

We previously noticed that B16F10 cells do not express one of the IL-27R subunits, WSX-1, and are not responsive to IL-27 (16). Therefore, we thought that these cells might be useful to investigate signaling through WSX-1, if B16F10 cells could become responsive to IL-27 by transfection with wild-type WSX-1. We prepared two independent clones of B16F10 cells transfected with vector alone and wild-type WSX-1-expression vector. As anticipated, IL-27 induced phosphorylation of both STAT1 and STAT3 in B16F10 transfectants expressing wild-type WSX-1 but not vector alone (Fig. 1, A and B). To confirm that the forced expression of WSX-1 is enough to mediate downstream signaling in response to IL-27, we next examined whether IL-27 could up-regulate MHC class I expression on these transfectants, because we previously demonstrated that IL-27 up-regulates MHC class I expression on activated naïve CD4\(^+\) T cells in a STAT1-dependent manner (6). IL-27 greatly up-regulated MHC class I expression on B16F10 cells expressing wild-type WSX-1 but not vector alone, as did IFN-\(\gamma\) but irrespective of WSX-1 expression (Fig. 1C). These results suggest that B16F10 transfectants expressing wild-type WSX-1 become responsive to IL-27 and, therefore, that these cells are suitable for investigating the signaling through WSX-1.
experiments. With IL-27 (10 ng/ml) for a further 24 h, and total cell lysate was prepared and subjected to Western blot using anti-IRF-1, anti-IRF-8, and anti-actin. siRNA-transfected cells were also stimulated with IL-27 (10 ng/ml) for a further 48 h and pulsed with [3H]thymidine for the last 24 h in triplicate. [3H]Thymidine incorporation was measured, and relative proliferation (percent) to that of unstimulated cells was calculated. Data are shown as means ± SD. * and **, p < 0.05, compared with no cytokine and control Ab, respectively. Similar results were obtained in two independent experiments. B, B16F10 cells expressing wild-type WSX-1 were transfected with siRNA specific to IRF-1 and/or IRF-8 for 24 h. These cells were then stimulated with IL-27 (10 ng/ml) for a further 24 h, and total cell lysate was prepared and subjected to Western blot using anti-IRF-1, anti-IRF-8, and anti-actin. The siRNA-transfected cells were also stimulated with IL-27 (10 ng/ml) for a further 48 h and pulsed with [3H]thymidine for the last 24 h in triplicate. [3H]Thymidine incorporation was measured, and relative proliferation (percent) to that of unstimulated cells was calculated. Data are shown as means ± SD. *, p < 0.05 compared with control siRNA. Similar results were obtained in three independent experiments.

WSX-1 signaling is mediated by STAT1 independently of STAT3 and important for IL-27-induced up-regulation of MHC class I

It was previously demonstrated that STAT1 binds to tyrosine residue at amino acid 609 in the cytoplasmic region of WSX-1 by an in vitro pull-down assay (3). Therefore, we further prepared B16F10 transfectants expressing mutant WSX-1, in which the putative STAT1-binding Tyr609 of the cytoplasmic region was replaced by Phe, and examined whether IL-27 can activate STATs and induce MHC class I expression. Consistent with the previous report (3), IL-27 could not activate STAT1 in B16F10 transfectants expressing mutant WSX-1, but activated STAT3 similarly to those expressing wild-type WSX-1 (Fig. 1, A and B). In addition, IL-27 failed to up-regulate MHC class I expression on B16F10 transfectants expressing mutant WSX-1 (Fig. 1C). These results suggest that WSX-1 signaling is mediated by STAT1 independently of STAT3 and is important for IL-27-induced up-regulation of MHC class I.

IL-27 induces antiproliferative effect on B16F10 transfectants expressing wild-type WSX-1 through STAT1 activation

Because IFN-γ is known to possess direct antiproliferative activity and proapoptotic activity (17–20), we next examined whether IL-27, which activates STAT1 as does IFN-γ, can exert similar effects using B16F10 transfectants. Incubation of B16F10 expressing wild-type WSX-1 but not vector alone with IL-27 dose-dependently decreased the number of bright, mitotic and adherent cells, and increased cells with round shape (Fig. 2A). Consistent with the morphological change, cell growth of B16F10 expressing wild-type WSX-1, but not vector alone, was greatly suppressed by IL-27 in a dose-dependent manner (Fig. 2B). In marked contrast, similar morphological change and cell growth inhibition by IL-27 was not observed in B16F10 expressing mutant WSX-1, which lacks the ability to activate STAT1 (Fig. 2, A and B). Annexin V binding to cell surface phosphatidyl serine is one hallmark of apoptotic cell death, and PI, which stains DNA, is a marker for permeabilized, necrotic cells. Flow cytometric analysis after the Annexin V–PI staining revealed that IL-27 increased Annexin V-positive and PI-positive population in B16F10 expressing wild-type WSX-1, but not vector alone (Fig. 2C). The cell cycle phase distribution was also evaluated, and the results revealed that IL-27 increased the number of cells in subG1 phase in B16F10 expressing wild-type WSX-1, but not vector alone (Fig. 2D). These results suggest that IL-27 induces antiproliferative effect on B16F10.
transfectants expressing wild-type WSX-1 together with apoptosis through STAT1 activation.

**IL-27 induces expression of IFN-γ and IFN-α/β-inducible molecules through WSX-1/STAT1 signaling, and IRF-1 is partially, but significantly, involved in IL-27-induced antiproliferative effect**

To further examine the molecular mechanism by which IL-27 induces antiproliferative effect, we performed a microarray analysis using RNA prepared form IL-27-stimulated B16F10 transfectant expressing wild-type WSX-1 and expressing mutant WSX-1, and tried to identify molecules, which are preferentially augmented in the former. Most greatly enhanced genes are IFN-γ and IFN-α/β-inducible molecules including IRF-1, IRF-8 (IFN consensus sequence binding protein), IP IFI204 (p204), CXCL9 (MIG), CXCL10 (IP-10), CXCL11 (IFN-inducible T cell α-chemoattractant), myxovirus resistance 1 (Mx1), 2′,5′-oligoadenylate synthetases (OAS), and STAT1 (data not shown). To next confirm the enhancement, B16F10 transfectants were stimulated with various concentrations of IL-27 for 24 h, and total cell lysate was prepared and subjected to Western blot analysis. Among these enhanced molecules, we focused on two IRF members, IRF-1 and IRF-8, because they were reported to possess a tumor suppressor activity (38, 39). Consistent with the microarray data, IL-27 greatly enhanced the expression of both IRF-1 and IRF-8 at protein levels in a dose-dependent manner in B16F10 transfectant expressing wild-type WSX-1, but not in that expressing vector alone or mutant WSX-1 (Fig. 3A). Because several genes among enhanced genes are known to be involved in the antiproliferative activity mediated by IFN-γ and IFN-α/β, we next evaluated whether the IL-27-induced expression of IRF-1 and IRF-8 and antiproliferative activity are not an indirect effect through IFN-α, IFN-β, or IFN-γ using neutralizing mAbs against them. Neither IL-27-induced expression of IRF-1 and IRF-8 (Fig. 3B) nor antiproliferative activity (Fig. 3C) was inhibited by neutralizing mAbs against IFN-α, IFN-β, or IFN-γ, although IFN-γ-mediated effects were completely inhibited by neutralizing mAbs against IFN-γ.

Then, to examine the role of these IRFs in IL-27-induced antiproliferative activity, we transfected B16F10 cells expressing wild-type WSX-1 with siRNAs specific to respective IRFs or negative control siRNA. The expression levels of IRF-1 and IRF-8 were largely reduced by the transfection with respective siRNAs but not control (Fig. 3D). Densitometric analyses revealed that the
expression levels in cells transfected with respective siRNAs (100 nM) were <5% of those in cells transfected with control siRNA (data not shown). Concomitant with the reduced expression of IRF-1, the ability of IL-27 to inhibit cell growth was partially, but significantly, blocked (Fig. 3E). In contrast, the reduced expression of IRF-8 hardly affected the antiproliferative ability of IL-27 when 10, 30, and 100 nM IRF-8 siRNA were used (Fig. 3D and data not shown). These results suggest that IL-27 induces expression of several IFN-γ- and IFN-αβ-inducible molecules through WSX-1/STAT1 signaling, and that IRF-1 is partially, but significantly, involved in the IL-27-induced antiproliferative effect.

**Direct antiproliferative activity of IL-27 on B16F10 transfectant expressing WSX-1 in vivo**

To investigate the direct antiproliferative activity of IL-27 in vivo, we first examined the effect of pretreatment with IL-27 in vitro on the tumor growth in vivo. B16F10 transfectants expressing WSX-1 or vector alone were cultured in the presence or absence of IL-27 for 24 h and then washed with PBS. Resultant cells were s.c. injected into wild-type mice. However, almost no significant difference in the tumor growth was observed among these groups (Fig. 4A). To explore the reason, B16F10 transfectants expressing WSX-1 or vector alone were either stimulated with varied concentrations of IL-27 for 48 h or stimulated with IL-27 for only 24 h, then washed with PBS and cultured in the absence of IL-27 for more 24 h. The proliferative activity was determined by incorporation of [3H]thymidine for the last 18 h. The effect of washing IL-27-treated cells with PBS greatly reduced the antiproliferative activity of IL-27 even in vitro (Fig. 4B). Similar result was obtained when IFN-γ was used instead of IL-27. These results suggest that continuous exposure to IL-27 is necessary for efficient inhibition of tumor growth.

Therefore, we next used IL-27 Tg mice, which constitutively express high levels of IL-27 (approximately >1 ng/ml in serum) (33), because the dose or route of administration affect the effectiveness of in vivo treatment with rIL-27. B16F10 transfectants (2 × 10^5, 4 × 10^5, and 8 × 10^5 cells/mouse) expressing WSX-1 or vector alone were s.c. injected into IL-27 Tg mice and control wild-type mice. Tumor growth of both transfectants was greatly reduced in IL-27 Tg mice compared with that in wild-type mice (Fig. 5), presumably due to NK cell- and anti-angiogenesis-dependent mechanisms as reported previously (12, 16). Of note is that the tumor growth of B16F10 transfectant expressing WSX-1 in IL-27 Tg mice, but not in control

**FIGURE 6.** Several human melanoma cells express IL-27R subunits and are responsive to IL-27. A, Total RNA was prepared from various human melanoma cell lines and analyzed for mRNA expression of IL-27R subunits, WSX-1 and gp130, by RT-PCR. B, The intensity of each band in A was measured, and relative intensity was calculated and shown. C, These human melanoma cell lines were also analyzed for cell surface expression of IL-27R subunits, WSX-1 and gp130, by FACS using biotinylated anti-TCCR/WSX-1 (solid line), anti-gp130 (solid line), and their respective control Abs (plain line with shading). As positive control, B16F10 transfectants overexpressing hWSX-1 or vector alone were analyzed using biotinylated anti-TCCR/WSX-1. D, Human melanoma cell lines (1 × 10^6 cells/ml) were stimulated with IL-27 (10 ng/ml) for 20 min. Total cell lysates were then prepared and analyzed for phosphorylation of STAT1 and STAT3 by Western blot using anti-pY-STATs and anti-total STATs. E, The intensity of each band in D was measured, and relative intensity was calculated and shown. Similar results were obtained in two independent experiments.
To finally explore whether IL-27 can induce antiproliferative effect on these human melanoma cells as mouse B16F10 transfectants expressing wild-type WSX-1, we first examined whether human melanoma cells also express IL-27R subunits, WSX-1 and gp130. To test these possibilities, total RNA was extracted from various human melanoma cells and RT-PCR was performed. Although expression levels of WSX-1 and gp130 varied among each cell line, most of these cells expressed both subunits at the mRNA level (Fig. 6, A and B). Similar cell surface expression of both subunits was detected at protein level by FACS analysis using respective specific Abs (Fig. 6C). Consistent with the IL-27R expression, IL-27 induced phosphorylation of STAT1 and STAT3 in most of these cells (Fig. 6, D and E). These results suggest that several human melanoma cells express IL-27R subunits and are responsive to IL-27.

Then, we examined whether IL-27 can induce antiproliferative effect on these human melanoma cells. As hIFN-γ greatly inhibited the growth of human melanoma cells, SK-MEL-13, -28, and -37, both mouse and hIL-27 significantly inhibited the growth of these human cells in a dose-dependent manner (Fig. 7A). Moreover, IL-27 inhibited the growth of several other human melanoma cells including SBc12, A101D, p22, and MeWo, but not G361 and p39 (Fig. 7B). These results suggest that IL-27 induces antiproliferative effect on several human melanoma cells as well as mouse melanoma B16F10 transfectants.

Discussion

In the present study, we have demonstrated that IL-27 has a direct antiproliferative activity on m and h melanomas, in addition to its previously demonstrated antitumor activities mediated through CD8+ T cells, NK cells, and its antiangiogenic effect (9–12, 16). IL-27 was revealed to induce various IFN-γ- and IFN-α/β-inducible genes, including STAT1, MHC class I, IRF-1, and IRF-8, through WSX-1/STAT1 signaling. IL-27-induced antiproliferative activity was highly dependent on STAT1 and partially on IRF-1. IRF-1 is a transcriptional factor originally identified as a regulator of the IFN-β gene and initiates the transcription of specific genes including IFN-α and β, MHC class I and II expression, inducible NO synthase, IL-12, and IL-15 (40). In addition, several studies demonstrated that IRF-1 and IRF-8 function as a tumor suppressor (38, 39). For instance, ectopic expression of IRF-1 can revert oncogene-transformed culture cells to a normal phenotype (41), whereas the loss of IRF-1 contributes to tumor development in conjunction with c-Ha-ras in vivo (42). Moreover, IRF-1 was shown to play a critical role in DNA damage-induced apoptosis in mature T lymphocytes (43). Thus, IL-27 may use similar mechanisms to those of IFN-γ to induce antiproliferative activity through WSX-1/STAT1 signaling.

Although IL-27 greatly induced the expression of both IRF-1 and IRF-8, only IRF-1 appears to be partially involved in IL-27-induced antiproliferative activity. Therefore, to further explore the molecular mechanisms, we examined the effect of various inhibitors of p38 MAPK (SB203580), ERK1/2 (PD98059) and protein kinase C (PKC) δ (rottlerin). Among these inhibitors, rottlerin partially, but significantly, blocked the IL-27-induced antiproliferative activity in B16F10 transfectants expressing wild-type WSX-1, suggesting a role for PKCδ in IL-27-induced antiproliferative activity (unpublished data). PKCδ is a heterogenous family of serine/threonine kinases mediating important intracellular signaling pathways (44). PKCδ, one of the PK family members, functions early in the apoptotic pathway, and inhibition of PKCδ suppresses the release of cytochrome c and caspase activation (45). It was previously demonstrated that IFN-γ activates PKCδ (46), which plays an important role in IFN-γ signaling by mediating Ser phosphorylation of STAT1, leading to facilitation of the transcription of IFN-γ-stimulated genes and induction of apoptosis (47). However, we failed to clearly detect phosphorylation of PKCδ in response to IL-27 (unpublished data). This may be due to the lower expression of PKCδ in the B16F10 cells or the lower sensitivity of Ab, which we used. Thus, although IRF-1 and PKCδ appear to be involved in the IL-27-induced antiproliferative activity, further studies are necessary to clarify the precise molecular mechanism by which IL-27 induces antiproliferative activity.

We previously demonstrated that IL-27 has an antitumor activity against primary melanoma and pulmonary metastasis in vivo.
using B16F10 tumor cells expressing IL-27 (16). Although parental B16F10 cells do not express WSX-1 in vitro, there was a possibility that B16F10 cells might be activated in vivo in response to factors which could result in induction of WSX-1. We therefore examined WSX-1 mRNA expression in vivo in tumors 14 days after s.c. tumor implantation by RT-PCR. However, we could not detect any induction of WSX-1 mRNA expression in B16F10 plus IL-27, B16F10 plus Neo, and B16F10 tumors at all, even in vivo (16). Thus, we concluded that the antitumor effects were not mediated through a direct effect of IL-27 on B16F10 tumor growth in this setting, but by stimulating endothelial cells to secrete antiangiogenic chemokines, such as IP-10 and MIG. Recently, we found that NK cells are also involved in the antitumor activity of IL-27 against B16F10 (12).

To evaluate the direct antiproliferative activity of IL-27 in vivo, we first examined the effect of pretreatment with IL-27 in vitro on the tumor growth in vivo. However, the pretreatment with IL-27 hardly inhibited the tumor growth in vivo (Fig. 4A). This is presumably due to the fact that antiproliferative activity of IL-27 was greatly reduced, even in vitro, if IL-27-treated tumor cells were washed with PBS (Fig. 4B). These results suggest that continuous exposure to IL-27 is necessary for efficient inhibition of tumor growth by IL-27, and imply that the effect of IL-27 on tumor growth appears to be cytostatic rather than cytotoxic. Therefore, we finally used IL-27 Tg mice, which constitutively express high levels of IL-27 (33). The tumor growth of B16F10 transfectant expressing WSX-1 in IL-27 Tg mice, but not in control mice, was slightly, but significantly, reduced compared with that expressing vector alone (Fig. 5). These results suggest that the direct antiproliferative activity of IL-27 significantly contributes to its antitumor activity in vivo, although other mechanisms, including NK cells and antiangiogenesis (12, 16), appear to contribute more than that under the present experimental conditions.

We, and others, previously demonstrated that IL-27 can augment the proliferation of naive CD4+ T cells (1, 6). Our preliminary data suggest that activation of gp130/STAT3 signaling is important for the IL-27-induced proliferation (48). In contrast, WSX-1-deficient T cells were reported to show enhanced proliferation, indicating that WSX-1 signaling has an inhibitory effect on T cell proliferation (5, 49). The later of these studies appears to be consistent with the present study showing that WSX-1/STAT1 signaling is important for the IL-27-induced inhibition of proliferation. Thus, IL-27 has opposite effects on proliferation, presumably through the different downstream signaling molecules, gp130/STAT3 for cell proliferation vs WSX-1/STAT1 for anti-proliferation. Therefore, physiological consequences in response to IL-27 could depend on their relative abundance, which may vary substantially in different cell types, under different conditions. Further studies are currently being undertaken to elucidate the molecular mechanism whereby IL-27 regulates these opposite functions.

The present study suggests that IL-27 exerts antiproliferative activity by similar mechanisms to those of IFN-γ through STAT1 activation. Moreover, IL-27 can directly activate CD4+ and CD8+ T cells, and induce their proliferation and differentiation through activation of STAT3 as well as STAT1 (6, 50, 51), resulting in establishment of antitumor immunity independently of IFN-γ (9). In contrast, IL-12 can also activate CD4+ and CD8+ T cells, and induce their proliferation and differentiation, which is similar to IL-27, but through activation of STAT4, whereas induction of the antitumor activity and establishment of protective immunity by IL-12 are highly dependent on IFN-γ (22). In addition, IL-12, but not IL-27, can markedly activate NK and NK T cells accompanied by IFN-γ production, ultimately leading to adverse effects including liver injury (9, 12, 52). Therefore, IL-27 may be an attractive candidate as an antitumor agent applicable to cancer immunotherapy through multiple mechanisms including CD8+ T cells, NK cells, antiangiogenic activity, and antiproliferative activity.

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Disclosures

The authors have no financial conflict of interest.

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