Overexpression of Indoleamine 2,3-Dioxygenase in Human Endometrial Carcinoma Cells Induces Rapid Tumor Growth in a Mouse Xenograft Model

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Abstract

Purpose: Indoleamine 2,3-dioxygenase (IDO) is a tryptophan-catabolizing enzyme that induces immune tolerance in mice. Our prior study showed that high tumoral IDO expression in endometrial cancer tissues correlates with disease progression and impaired patient survival. The purpose of the present study was to clarify the functional role of IDO in human endometrial cancer cells and to investigate the therapeutic potential of IDO inhibitors.

Experimental Design: IDO cDNA was transfected into the human endometrial carcinoma cell line AMEC, resulting in the establishment of stable clones of IDO-overexpressing AMEC cells (AMEC-IDO). AMEC-IDO cells were characterized in vitro as well as in vivo using a mouse xenograft model.

Results: There was no significant difference in in vitro cell proliferation, migration, or chemosensitivity to paclitaxel between AMEC-IDO and control vector-transfected cells (AMEC-pcDNA). However, in vivo tumor growth was markedly enhanced in AMEC-IDO—xenografted nude mice when compared with AMEC-pcDNA—xenografted mice. Splenic natural killer (NK) cell counts in AMEC-IDO—xenografted mice were significantly decreased when compared with control mice. Furthermore, conditioned medium obtained from AMEC-IDO cell cultures markedly reduced the NK lysis activity of nude mice. Finally, oral administration of the IDO inhibitor 1-methyl-D-tryptophan in combination with paclitaxel in AMEC-IDO—xenografted mice strongly potentiated the antitumor effect of paclitaxel, resulting in significantly prolonged survival.

Conclusions: This is the first evidence showing that IDO overexpression in human cancer cells contributes to tumor progression in vivo with suppression of NK cells. Our data suggest that targeting IDO may be a novel therapeutic strategy for endometrial cancer.

Immune escape and acquisition of “tolerance” by tumor cells are essential to cancer growth and progression. Therefore, much attention has been paid to elucidating the mechanism of tumor-induced immunosuppression and also to overcoming the immune resistance of tumors as a novel strategy for cancer therapy (1–3). Recent studies suggest that the immunoregulatory enzyme indoleamine 2,3-dioxygenase (IDO) may contribute to tumor-induced tolerance (4, 5). IDO (EC 1.13.11.42) is a heme-containing intracellular enzyme that catalyzes the initial and rate-limiting step in the metabolism of the essential amino acid tryptophan along the kynurenine pathway (6).

Immunosuppression by IDO was first documented in the mouse placenta, where IDO prevents rejection of the allogeneic fetus and induces fetal-maternal tolerance during pregnancy (7). Subsequent studies clarified the mechanisms of IDO immunosuppression to be local depletion of tryptophan and production of toxic tryptophan catabolites, causing growth arrest and apoptosis of alloreactive T cells or natural killer (NK) cells that are extremely sensitive to tryptophan shortage conditions (8, 9). The tryptophan-derived catabolite kynurenine also inhibits the expression of specific triggering receptors on NK cells and suppresses NK-cell function (10).

In cancer, IDO is expressed in various tumor cells, and tumors expressing IDO can resist immune rejection by tumor-associated antigen-specific host cytotoxic T cells in mouse models (11). IDO is also expressed by dendritic cells in tumor-draining lymph nodes in mice, which induce tolerance to tumor-derived antigens and activate immunosuppressive...
regulatory T cells (12, 13). More recently, it was shown that treatment with IDO inhibitors or IDO small interfering RNA could recover host antitumor immunity and reduce tumor formation in tumor-bearing mice (14–16). IDO inhibitors also potentiated the antitumor activity of chemotherapeutic agents in mice, suggesting involvement of IDO in tumor chemoresistance (14).

Evidence from these animal studies has prompted further examination of IDO in human cancers. IDO expression has been associated with poor clinical outcome in malignant melanoma (12), ovarian cancer (17), and colorectal cancer (18). We have recently shown that IDO is expressed in tumor cells in endometrial cancer tissues, and high IDO expression is strongly correlated with disease progression and impaired patient survival, suggesting that IDO is a novel prognostic indicator for endometrial cancer (19). Furthermore, our subsequent studies showed that tumor IDO expression was correlated with a reduced number of tumor-infiltrating lymphocytes and NK cells in endometrial cancer tissues (20). However, the functional significance of IDO in human cancer, especially cellular and molecular mechanisms contributing to human tumor progression, remains to be determined.

In the present study, we established a stable clone of IDO-overexpressing human endometrial cancer cells and characterized it in vitro and in vivo. Our data first show that IDO overexpression leads to rapid growth of human tumors in nude mouse models. We also show efficacious antitumor activity of IDO inhibitors in combination with chemotherapeutic agents in IDO-overexpressing human endometrial cancer–xenografted mice.

Materials and Methods

**Cell lines and reagents.** We used two human endometrial adenocarcinoma cell lines: AMEC and RL95. AMEC was generously donated by Aichi Medical University (Aichi, Japan). RL95 was purchased from the American Type Culture Collection. Both of cell lines were maintained in RPMI 1640 (Sigma-Aldrich) supplemented with 10% FCS (Sigma-Aldrich) and penicillin-streptomycin at 37°C in a humidified atmosphere of 5% CO2. Recombinant human IFN-γ with a specific activity of 1 × 10^7 units/mg was purchased from R&D Systems. 1-Methyl-D-tryptophan (1-MT) was purchased from Sigma-Aldrich. Paclitaxel and carboptatin were purchased from Bristol-Myers Squibb.

**Plasmid construction and transfection.** The mammalian expression vector for human IDO (pcDNA3.1-IDO) was constructed by inserting full-size human IDO cDNA (21) between the KpnI and XbaI sites of pcDNA3.1 (Invitrogen). Transfection into AMEC cells was carried out using Lipofectamine 2000 according to the manufacturer’s instructions (Invitrogen). Stable transfectants were selected by growth in medium supplemented with 400 μg/mL G418 (Sigma-Aldrich), and more than 20 clones resistant to G418 were obtained as IDO-overexpressing clones (AMEC-IDO). For negative control experiments, the pcDNA3.1 vector without inserted IDO cDNA was transfected into AMEC cells (AMEC-pcDNA).

**Western blot analysis.** Cells were lysed in buffer consisting of 1% Triton X-100 and the protease inhibitor mixture in PBS. After centrifugation at 15,000 × g for 20 min, a supernatant was obtained as the protein extract. Protein extract (30 μg) was separated by SDS/12.5% PAGE, transferred onto a nitrocellulose membrane, and immunoblotted with an anti-IDO monoclonal antibody that was prepared as described previously (22). Immunoreactive proteins were stained using a chemiluminescence detection system (Amersham).

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**High-performance liquid chromatography.** IDO enzyme activity was evaluated by measuring the concentrations of tryptophan and kynurenine in the conditioned medium (CM) of AMEC cells using a Shimadzu Prominance high-performance liquid chromatography (HPLC) system with an Inertsil ODS column (GL Sciences). The mobile phase was 10 mmol/L ammonium acetate containing 10% methanol.

**Reverse transcription-PCR.** Total RNA was extracted from mouse tumor tissues and reverse transcribed using ReverTra Dash according to the manufacturer’s instructions. Reverse transcription products were applied to PCR using specific IDO primers (23): 5′-GCAAATGGCAAGAACCAGCACAC-3′ (upstream) and 5′-CTACGGAGACAGCAGCCTTCACAC-3′ (downstream). The glyceraldehyde-3-phosphate dehydrogenase control primers were 5′-ATGTTGGATCCGTTGCTAGC-3′ (upstream) and 5′-GCAATCGAAGTGGAAAGTGTAATCAAGCTGG-3′ (downstream). Amplification conditions were described previously (23). PCR products were visualized under UV light after gel electrophoresis in 1% agarose containing ethidium bromide.

**In vitro cell proliferation assay.** Cells were plated at a density of 2,000/200 mL of culture medium containing 10% FCS in 96-well plates and cultured for 24 to 72 h. Cell viability was assayed every 24 h using a modified 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium salt (MTS) assay with the CellTiter 96 Aqueous One Solution Cell Proliferation Assay kit (Promega). Absorbance was measured at 492 nm with a microplate reader. In vitro sensitivity to chemotherapeutic agents was similarly evaluated by MTS assay after incubation of cells in the presence of various concentrations of paclitaxel or carboptatin for 72 h.
In vitro cell migration and invasion assay. Cell migratory potential was evaluated using 24-well Transwell chambers with 8.0-μm pore membranes (Corning, Inc.). Cells were suspended in the upper chamber at a density of \(5 \times 10^5\) in 200 μL of serum-free medium. The lower chamber contained 800 μL of medium supplemented with 10% FCS as a chemoattractant. After incubation for 16 h, the remaining cells on the upper surface of the filters were removed by wiping with cotton swabs, and migrating cells on the lower surface were stained using May-Grünwald-Giemsa. The numbers of cells on the lower surface of the filters was counted under a microscope. Data were obtained from three independent experiments in triplicate. Cell invasion was similarly evaluated using 24-well Matrigel invasion chambers (Becton Dickinson).

In vivo studies using a mouse xenograft model. Female nude mice (BALB/c nu/nu) at 6 wk of age were obtained from Japan SLC. AMEC-IDO or AMEC-pcDNA cells (\(5 \times 10^6\) per mouse) were s.c. inoculated into the lower lateral flank of each mouse (\(n = 10\) per group). Tumor volume was measured with calipers and calculated using the following formula: \(\pi/6 \times (\text{longest diameter}) \times (\text{shortest diameter})^2\). In some experiments, mice were sacrificed 28 d after tumor inoculation for the use of spleen cells. In addition, tumor tissues were fixed in 10% formalin, embedded in paraffin, and stained with H&E for histologic examination. To examine the effect of the IDO inhibitor 1-MT on tumor growth and survival, AMEC-IDO–inoculated mice were orally given 1-MT at 0, 0.5, or 5 mg/mL (\(n = 5\) per group) in the drinking water. The administration of 1-MT was initiated on day 14 when the growth of inoculated tumor was macroscopically confirmed (the diameters were \(5\) mm) and lasted until mice died. In some experiments, mice were treated with i.p. paclitaxel (20 mg/kg/wk) on days 14 and 21 in combination with the daily oral 1-MT treatment (1 mg/mL) or treated with i.p. paclitaxel alone or vehicle alone (\(n = 5\) per group). The dose of paclitaxel used in this study showed significant antitumor effects without severe or lethal toxicity in tumor-bearing nude mouse model, as described in our previous report (24). All procedures were done in accordance with the Animal Experimentation Guide of Institute for Laboratory Animal Research, Nagoya University Graduate School of Medicine.

Flow cytometric analysis. Fluorescence-activated cell sorting (FACS) was done using FACS Calibur (Becton Dickinson). Spleen cells were obtained from nude mice. After removal of RBCs, the cells were double stained with FITC-labeled anti-mouse panNK (CD49b, clone DX5; eBioscience) and phycoerythrin-labeled anti-mouse CD122 (interleukin-2 receptor β, clone TM-b1; Becton Dickinson). FACS data were analyzed using CellQuest software (Becton Dickinson).

NK cytotoxicity assay. NK cytotoxic activity was tested against a NK-sensitive lymphoma cell line, YAC-1 (Riken Cell Bank), in a 6-h 51Cr release assay. Spleen cells were obtained from nude mice and used as effector cells. YAC-1 target cells were labeled with 50 mCi of 51Cr for 30 min at 37°C and washed thrice. Cells were added into a 96-well round-bottomed microplate to the final target cell concentration of \(2 \times 10^4\) per well and incubated with effector cells for 6 h at E:T ratio of 50:1, 10:1, and 5:1. Samples of supernatant were harvested and radioactivity was counted in a gamma counter. Maximum release and spontaneous release were determined by incubating the target cells with 1% Triton X-100 or medium alone. All experiments were made in triplicate. The percent specific cytotoxicity was calculated according to the following formula: % lysis = (experimental release - spontaneous release)/(maximum release - spontaneous release) × 100.

Statistical analysis. Statistical comparisons among groups were done using ANOVA with Bonferroni corrections. Kaplan-Meier method was used to generate survival curves, and the comparisons were done using log-rank tests. \(P < 0.05\) was considered significant.

Results

Establishment of stable clones of IDO-overexpressing human cancer cells. First, we tested whether IDO expression could be...
induced by IFN-γ, which is an inducer of IDO (22), in two human endometrial carcinoma cell lines. As shown in Fig. 1A, IDO protein expression was strongly induced by treatment with IFN-γ for 48 hours in RL95 cells, but not in AMEC cells. Thus, we transfected human IDO cDNA into AMEC cells to establish stable clones of IDO-overexpressing endometrial cancer cells. More than 20 clones were obtained and their IDO expression levels were evaluated by Western blotting. Two clones with especially high IDO expression, AMEC-IDO2 and AMEC-IDO9, were selected (Fig. 1B) and used in the following experiments. There were no morphologic changes among the IDO-overexpressing cells (AMEC-IDO2 and AMEC-IDO9), control vector–transfected cells (AMEC-pcDNA), and wild-type AMEC cells (Fig. 1C). Immunofluorescence staining showed diffuse cytoplasmic expression of IDO in AMEC-IDO2 cells, but not in AMEC-pcDNA cells (Fig. 1C). To confirm IDO enzymatic activity, we measured the concentrations of tryptophan and its main catabolite kynurenine in CM of cultured cells using HPLC. As shown in Fig. 1D, the concentration of tryptophan was completely depleted in the CM of AMEC-IDO2 cells after 48 hours, whereas ~50% was still left in the CM of AMEC-pcDNA cells. Additionally, the concentration of the tryptophan catabolite kynurenine was markedly increased in the CM of AMEC-IDO2 cells after 48 hours, whereas there was no significant increase in the kynurenine concentration of the CM of AMEC-pcDNA cells when compared with the control medium without cell culture.

**Effect of IDO overexpression on in vitro cell proliferation, migration, and chemosensitivity.** There was no significant difference in in vitro cell proliferation among AMEC-IDO2, AMEC-IDO9, and AMEC-pcDNA cells (Fig. 2A), although the cell viability of AMEC-IDO2 and AMEC-IDO9 cells was slightly decreased by more than 72-hour cell culture without medium change (data not shown); this may have been due to consumption of the essential amino acid tryptophan in the culture medium. Similarly, IDO overexpression did not affect the in vitro cell migratory potential (Fig. 2B) or cell invasion activity (data not shown). Previous studies suggested that IDO might be involved in chemoresistance to anticancer agents (14, 17). Thus, we next tested the chemosensitivity of IDO-overexpressing AMEC cells in vitro. We observed no significant effect of IDO overexpression on chemosensitivity to paclitaxel (Fig. 2C) or carboplatin (data not shown). Similarly, IDO induction by pretreatment of RL95 cells with IFN-γ for 48 hours did not affect in vitro chemosensitivity to paclitaxel (Fig. 2D) or carboplatin (data not shown).

**Effect of IDO overexpression on tumor growth in vivo.** To determine the role of IDO in human tumor progression,

![Graphs showing the effects of IDO overexpression on cell proliferation and chemosensitivity](https://example.com/graphs.png)
in vivo tumor growth of IDO-overexpressing AMEC cells was evaluated in a nude mouse model. As shown in Fig. 3A, the growth rate of tumors in AMEC-IDO2–xenografted mice was significantly enhanced from day 21 through day 28 after inoculation when compared with AMEC-pcDNA–xenografted mice. The macroscopic appearance at day 28 after inoculation showed large s.c. tumor masses in AMEC-IDO2–xenografted mice (Fig. 3B). Histologic findings confirmed that both xenografted tumors consisted of viable cancer cells with solid proliferation (Fig. 3C). It seemed that the number of host stromal cells, including immune cells, was increased at the tumor-stromal interface in AMEC-pcDNA tumors, whereas aggressive tumor infiltration into the host stroma and vessels was seen in AMEC-IDO2 tumors. Reverse transcription-PCR analysis confirmed high IDO mRNA expression in AMEC-IDO2 tumors and no IDO expression in AMEC-pcDNA tumors (Fig. 3D).

Effect of IDO overexpression on NK cell population and function. To determine the mechanism by which AMEC-IDO2 tumors rapidly grow in nude mice, we tested the effect of IDO overexpression on NK cells, which are important antitumor effector cells in nude mice. FACS analysis showed that the percentage of panNK+/CD122+ mouse NK cells in spleens taken from AMEC-IDO2 tumor-bearing nude mice on day 28 after inoculation was significantly lower than in those taken from AMEC-pcDNA tumor-bearing mice (Fig. 4A and B). Similarly, the absolute number of splenic NK cells in AMEC-IDO2 tumor-bearing mice was markedly reduced compared with those in AMEC-pcDNA tumor-bearing mice (Fig. 4C). In these experiments, the number of splenic NK cells in AMEC-pcDNA tumor-bearing mice was increased when compared with non–tumor-bearing control nude mice (Fig. 4B and C). This host antitumor NK cell response observed in AMEC-pcDNA tumor-bearing mice was strongly suppressed in AMEC-IDO2 tumor-bearing mice. To further examine the effect of IDO overexpression on NK cell function, NK lysis activity was evaluated using a 51Cr release assay. There were no significant differences in splenic NK cytotoxicity among AMEC-IDO2–bearing mice, AMEC-pcDNA–bearing mice, and non–tumor-bearing control mice (Fig. 4D). Next, we investigated the effect of CM obtained from cultures of IDO-overexpressing cells on the NK function of nude mice. As shown in Fig. 5, NK lysis activity was markedly reduced when effector and target cells were incubated in the presence of AMEC-IDO2–derived CM but not AMEC-pcDNA–derived CM.

Antitumor effect of 1-MT against IDO-overexpressing tumors in vivo. Finally, we investigated the antitumor effects of the IDO inhibitor 1-MT and its therapeutic potential in IDO-overexpressing endometrial cancer–xenografted nude mice. Because the d-stereoisomer of 1-MT was shown to be more effective in reversing T-cell suppression and more efficacious as an anticancer agent when compared with the l-isomer in immunocompetent mice (15), we used 1-MT in this study. Daily oral administration of 1-MT in the drinking water at the concentrations of 0.5 to 5 mg/mL did not affect the tumor growth rate, although the tumor volume was slightly (but not significantly) decreased by 1-MT at 5 mg/mL on day 42 after inoculation (Fig. 6A). In contrast, the tumor burden was markedly reduced when mice were treated with daily oral 1-MT at 1 mg/mL in combination with i.p. administration of paclitaxel (20 mg/kg) on days 14 and 21 when compared with untreated mice or those treated with paclitaxel alone (Fig. 6B). Furthermore, mice treated with 1-MT combined
with paclitaxel showed significantly prolonged survival ($P = 0.0059$, log-rank test) when compared with mice that were untreated or treated with paclitaxel alone.

**Discussion**

Although prior studies showed immunosuppressive roles for IDO and its involvement in immune tolerance toward tumor-associated antigens in various tumor-bearing mouse models (5, 11–13), these data were limited to IDO function in murine tumor cells or dendritic cells. Thus, the functional role of IDO in human cancer has not yet been identified despite the fact that IDO is highly expressed by tumor cells in various types of human cancers and associated with poor clinical outcome (17–20). One reason for this may be the lack of constantly IDO-expressing human cancer cell lines available for both in vitro and in vivo experiments. In this study, we successfully established for the first time stable clones of an IDO-overexpressing human cancer cell line by transfection of human IDO cDNA into the endometrial cancer cell line AMEC and showed the functional implications of IDO in tumor progression both in vitro and in vivo.

Our HPLC data showed that the tryptophan in the culture medium was totally catabolized by tumoral IDO in AMEC-IDO cells, resulting in abundant production of the main catabolite kynurenine. These results indicate that the AMEC-IDO cells used in our study contained highly enzymatically active IDO protein. Although tryptophan is an essential amino acid not only for normal cells but also for cancer cells, AMEC-IDO cells could proliferate well in vitro for up to 72 hours without medium change. In contrast, CD8+ T cells and NK cells are easily induced to G1 cell cycle arrest or apoptosis in vitro after shorter time periods by tryptophan depletion and/or the presence of tryptophan-derived catabolites, such as kynurenine, 3-hydroxykynurenine, and 3-hydroxyanthranilic acid (8, 9, 25, 26). These findings suggest that cancer cells may be less sensitive to shortage of tryptophan or exposure to toxic catabolites than T cells or NK cells, which could in turn contribute to the tumor escape from host immune response.

Fig. 4. A, flow cytometric analysis of CD122+, panNK+ cells from spleens on day 28 after s.c. inoculation of AMEC-pcDNA and AMEC-IDO2 cells in nude mice. In non–tumor-bearing control nude mice, vehicle alone was s.c. injected instead of tumor cells. UR, upper right fraction. B and C, percentage (B) and absolute number (C) of CD122+, panNK+ cells in spleen cells taken from non–tumor-bearing control mice or tumor-bearing mice. Both NK cell percentage ($P = 0.0094$) and count ($P = 0.0250$) were significantly decreased in AMEC-IDO2 tumor-bearing mice when compared with AMEC-pcDNA tumor-bearing mice. D, NK cell cytotoxicity of spleen cells taken from non–tumor-bearing or tumor-bearing nude mice measured by $^{3}$H release assay. There was no significant difference in NK lysis activity among the three groups.
surveillance and creation of localized tolerance within the tumor microenvironment.

It is of interest that our in vivo study showed rapid growth and progression of IDO-overexpressing AMEC tumors in nude mice despite the lack of significant changes observed in the in vitro proliferation and migration studies. In nude mice, the T-cell–mediated immune response to tumors is lacking, and the main antitumor effector cells are NK cells. Recent work on innate immunity suggests that NK cell function is critical for controlling the initial tumor growth and determining chemosensitivity (27, 28). NK cells have also been shown to provide potent antitumor immunity in mice and their depletion has been linked to metastatic spread (29). Clinically, the presence of tumor-infiltrating NK cells correlates with favorable patient outcome in various human cancers (30–32). Thus, we checked NK cell count and function in IDO-overexpressing tumor-bearing nude mice. Our data show that the splenic NK cell count was reduced in AMEC-IDO–xenografted mice but lysis activity remained unchanged. However, the addition of AMEC-IDO cell-derived CM markedly suppressed NK lysis activity. These results are consistent with previous reports showing that apoptosis was induced in not only T cells but also NK cells by tryptophan catabolism (8, 9) and that NK cell killer function was suppressed via down-regulation of the specific triggering receptor NKp46 and NKG2D by the tryptophan-derived catabolite kynurenine (10). Taken together, our results suggest that IDO-overexpressing tumors in nude mice systemically reduced NK cell population and locally produced toxic tryptophan catabolites, both of which may lead to suppression of NK cell–mediated tumor killing. Although possible effects on other antitumor effector cells in nude mice, such as activated macrophages, neutrophils, or B cells, remain to be determined, impairment of NK function by tumoral IDO may have contributed, at least in part, to rapid tumor progression.

Fig. 5. Effect of the CM of cultured IDO-overexpressing AMEC cells on NK cytotoxicity measured by 51Cr release assay. NK lysis activity of spleen cells taken from untreated nude mice was significantly decreased when effector and target cells were incubated in the presence of AMEC-IDO2–derived CM when compared with AMEC-pcDNA–derived CM or the absence of CM at E:T ratios of 50:1 \((P = 0.0018)\), 10:1 \((P = 0.0011)\), and 5:1 \((P = 0.0213)\).

Fig. 6. Antitumor effects of 1-MT in IDO-overexpressing human cancer– xenografted models. A, AMEC-IDO2–xenografted nude mice were given 1-MT daily at 0, 0.5, or 5 mg/mL in the drinking water. There was no significant difference in tumor volume among the three groups, although the tumor growth curve was slightly decreased by treatment with 1-MT at 5 mg/mL. B, synergistic antitumor effects of 1-MT plus chemotherapy. Tumor growth in AMEC-IDO2–xenografted nude mice was significantly reduced by daily oral administration of 1-MT at 1 mg/mL in combination with i.p. paclitaxel at 20 mg/mL on day 35 \((P = 0.0253)\), day 42 \((P = 0.0237)\), and day 49 \((P = 0.0441)\) when compared with treatment with paclitaxel alone. C, survival curves for AMEC-IDO2–xenografted nude mice treated with vehicle alone, paclitaxel alone, or paclitaxel plus 1-MT. 1-MT was orally given at 1 mg/mL daily, which initiated on day 14 and lasted until mice died. Paclitaxel was i.p. given at 20 mg/mL on days 14 and 21. There was a significant difference in survival among the three groups as determined by log-rank test \((P = 0.0059)\).
Besides the immunologic actions of IDO through tryptophan catabolism, the other mechanisms by which IDO accelerates tumor progression remain obscure. A recent report suggested that IDO might be involved in tumor resistance to chemotherapeutic agents (17). In that study, IDO expression was associated with paclitaxel resistance in serous-type ovarian cancer using gene expression profiling. However, our data showed that neither IDO overexpression by transfection of the IDO gene nor IDO induction by IFN-γ affected in vitro chemosensitivity to paclitaxel in endometrial cancer cell lines. Muller et al. (14) showed that loss of the tumor suppressor gene Bin1 elevated signal transducer and activator of transcription 1 and nuclear factor-κB levels, leading to IDO expression in transformed mouse cells. Although the main role of IDO in tumors is immunosuppression of host T cells and NK cells, it is possible that tumoral IDO might directly up-regulate or down-regulate some tumor-enhancing or tumor-suppressive genes, transcriptional factors, proteins, or signaling molecules in tumor cells themselves. Further studies are needed to investigate this possibility.

Recently, several preclinical studies in mice have been done to attempt clinical application of IDO-targeted therapy. The most widely studied compound is 1-MT, a small molecular therapeutic agents against IDO-expressing human tumors possibly through inhibition of tumoral IDO activity and recovery of host NK function within the tumor microenvironment even in T-cell–deficient hosts. In addition to 1-MT, recent studies showed that plant-derived natural products with IDO-inhibiting activity (33) or IDO small interfering RNA (16) also have antitumor effects in mice. IDO inhibition may therefore be a novel and promising strategy for the treatment of IDO-expressing human tumors.

In conclusion, we showed here that overexpression of IDO by human endometrial cancer cells enhanced tumor progression in vivo. Furthermore, the present study clearly showed the therapeutic potential of the IDO inhibitor 1-MT against IDO-expressing tumors in vivo. Our previous studies showed that high level of tumoral IDO expression was found in ~50% of endometrial cancer cases, and strongly correlated with disease progression and impaired patient survival (19, 20). Taken together, our data could support the ongoing phase I clinical trials of IDO inhibitors and prompt the development of future IDO-targeted therapies for not only endometrial carcinoma but also other IDO-expressing human cancers.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

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