Original Articles

Insulin-like growth factors inhibit dendritic cell-mediated anti-tumor immunity through regulating ERK1/2 phosphorylation and p38 dephosphorylation

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ABSTRACT

Insulin-like growth factors (IGFs) can promote tumorigenesis via inhibiting the apoptosis of cancer cells. The relationship between IGFs and dendritic cell (DC)-mediated immunity were investigated. Advanced-stage ovarian carcinoma patients were first evaluated to show higher IGF-1 and IGF-2 concentrations in their ascites than early-stage patients. IGFs could suppress DCs' maturation, antigen presenting abilities, and the ability to activate antigen-specific CD8+ T cell. IGF-treated DCs also secreted higher concentrations of IL-10 and TNF-α. IGF-treated DCs showed decreased ERK1/2 phosphorylation and reduced p38 dephosphorylation. The percentages of matured DCs in the ascites were significantly lower in the IGF-1 or IGF-2 highly-expressing WF-3 tumor-bearing mice. The IGF1R inhibitor – NVP-AEW541, could block the effects of IGFs to rescue DCs' maturation and to restore DC-mediated antigen-specific immunity through enhancing ERK1/2 phosphorylation and p38 dephosphorylation. IGFs can inhibit DC-mediated anti-tumor immunity through suppressing maturation and function and the IGF1R inhibitor could restore the DC-mediated anti-tumor immunity. Blockade of IGFs could be a potential strategy for cancer immunotherapy.

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Introduction

Obese subjects have been reported to be at a higher risk of infection and to be more prone to cancer [1,2]. Several hormones and growth factors such as insulin-like growth factor (IGF) [3], insulin [4], adiponectin [5] and leptin [6] have been implicated in both energy balance and carcinogenesis. The involvement of the IGF family of proteins in cancer was first suspected because IGF-1 was found to enhance the growth of a variety of cancers including colon, lung, and breast cancers [7–9]. The IGF system consists of two ligands, IGF-1 and IGF-2, three cell-membrane receptors, and six high-affinity IGF binding proteins [10]. The IGF pathway involves elements controlling the endocrine, paracrine, and autocrine systems when regulating fetal development, growth, and metabolism [11]. The IGF family of proteins act either directly on cells via the IGF-1 receptor (IGF1R), which is over-expressed in many tumors, or indirectly through its action with other cancer-related molecules such as the p53 tumor suppressor [12].

DCs have been shown to play a role in both the priming of adaptive immune responses and the induction of self-tolerance [13]. DCs can process antigen and present it on their surface molecules to other immune cells. Once activated, DCs migrate to the lymphoid nodes where they interact with T cells and B cells to initiate adaptive immune responses [14]. DCs can exist in two functional states, immature and mature, but only mature DCs have the ability to prime an immune response [14]. However, mature DCs only demonstrate an antigen rather than a processing status. Mature DCs have been shown to express high cell-surface levels of major histocompatibility complex (MHC) molecules, CD40, CD80, CD83 and CD86, and also to be functionally immunogenic [14] and to correlate with T-cell-priming ability. Immature DCs lack these cell-surface expressions and antigen presenting activity, and thus are characterized by high endocytic activity and low T-cell activation potential [14].

The effects of IGFs such as IGF-1 and IGF-2 on immunity are still unclear. Many immune cells and bone marrow stromal cells have been shown to express IGF-1 [15], and nearly all immune cells such as...
as T lymphocytes and B lymphocytes [16], mononuclear cells [17] and NK-cells [18] have been shown to express IGF1R and to be susceptible to the effects of IGF. Alterations of IGF-1 levels in the course of immune and inflammatory reactions can serve or compromise immunity. The differentiation and proliferation of T cells have been reported to be impaired in fetal thymic organ cultures treated with anti-IGF-2, anti-IGF1R, or anti-IGF2R [19]. However, only a few studies have investigated the function of IGFs in the development and physiology of immunity, the majority of which have only focused on IGF and chronic inflammation [20,21]. The exact function of IGFs on host immunity, and especially with respect to immune cells such as dendritic cells (DCs) remains unclear. In this study, we investigated whether IGFs could regulate the maturation of DCs, DC-mediated immunity, and the mechanisms.

Materials and methods

Patients and specimens

Eighty-two women with ovarian carcinoma treated in National Taiwan University Hospital were enrolled. The Institutional Review Board of the hospital reviewed and approved the study protocol. Informed consent was obtained before collecting the ascites samples, which were obtained during surgery and immediately frozen at −20°C until analysis.

Concentrations of cytokines in the ascites of the ovarian cancer patients by cytokine bead array and ELISA

To evaluate the concentrations of various cytokines in the ascites, a customized cytokine bead array (Procarta cytokine profiling kit, Affimmun) was used. The data were further analyzed by Luminex software. The IGF-1 and IGF-2 concentrations were also detected by ELISA kits (Immunodiagnostic Systems; Mediadnost).

Cell lines

The generation and maintenance of the murine WF-3 tumor cell line [22], TC-1 tumor cell line [23] and E7-specific CDB+ T cell line [24] were performed as described previously. IGF-1 or IGF-2 highly-expressing WF-3 tumor cell lines were generated using pcDNA3 vector (Invitrogen) as follows. To obtain mIGF-1, the primer sets: 5′-ccggctcgagtggatttctttttcgcctca-3′ and 5′-ccgggaattcgggaggctcctcctacattc-3′ were used. To obtain mIGF-2, the primer sets: 5′-ccgcctgctcggtcgggtgctcgcctctcca-3′ and 5′-ccgcctgctcggtcgggtgctcgcctctcca-3′ were used. The transfection of mIGF-1 or mIGF-2 into the WF-3 cell line was performed using Lipofectamine™ reagent (Invitrogen). G418 (Geneticin; Invitrogen) was added to select the stable clones. The efficacy of cell line transfection was around 50%. Each single clone was amplified (Invitrogen). G418 (Geneticin; Invitrogen) was added to select the stable clones. The culture medium was replaced with fresh medium every 2 days. Because the median fluorescence intensities (MFI) of CD80 between 50 ng/ml of IGF-1 and/or IGF-2-treated BMM-derived DCs were not different (Supplement Fig. S1), so the concentration of 100 ng/ml IGF-1 and/or IGF-2 was used for the following experiments of BMM-derived DCs. For the IGF experiments, 100 ng/ml IGF-1 or IGF-2 (R&D Systems) was added on the first day of culture and the IGF-containing medium every 2 days. The PBS-treated group was used as the control. Lipopolysaccharide (50 ng/ml) (LPS, Sigma-Aldrich Chemie GmbH) was used as exogenous stimuli to activate DCs at day 6, and the cells were collected 24 h later for further studies and analysis.

For the IGF blocking experiments, 0.3 μM of IGF-1 receptor kinase inhibitor-NVP-AEW541 (kindly provided by Novartis Pharma AG) was added 20 minutes before adding IGF-1 and/or IGF-2 [27]. The DCs were cultured, treated with LPS, and collected for further studies as described earlier.

Analysis of the maturation status of the BMM-derived DCs treated with IGFs and/or NVP-AEW541 by flow cytometric analysis

The DCs were stained with FITC-conjugated anti-CD11c (eBioscience), PE-conjugated anti-MHC class I (eBioscience), PE-Cy5-conjugated anti-CD80 (BioLegend), anti-CD86 (BioLegend) or anti-MHC class II (eBioscience) Ab. Flow cytometric analysis was performed using a BD Biosciences FACSCalibur flow cytometer (Becton Dickinson), with CELLQuest software.

Antigen presenting and processing abilities of the BMM-derived DCs treated with IGFs and/or NVP-AEW541 by flow cytometric analysis

To evaluate the antigen presenting activities of the DCs, the DCs were cultured with 2 μg/ml MHC I Kb compatible short peptide (FITC-conjugated OVA257-264 (SIINFEKL)) (Invitrogen) for 7 days [26,28]. On day 6, 50 ng/ml LPS was added and the cells were collected 24 h later. The DCs were harvested and stained with PE-conjugated anti-CD11c (BD Biosciences) and assessed by flow cytometry at the indicated time points.

To evaluate the antigen processing activities of the BMM-derived DCs, the cells were cultured with 50 μg/ml MHC II Kb compatible long peptide (FITC-conjugated OVA253-265 (ISQAVHAAHAEINEAGR)) (Invitrogen) for 6 days. The PBS-treated group was used as the control. Lipopolysaccharide (50 ng/ml) (LPS, Sigma-Aldrich Chemie GmbH) was used as exogenous stimuli to activate DCs at day 6, and the cells were collected 24 h later for further studies and analysis.

Antigen presentation abilities of the BMM-derived DCs treated with IGFs and/or NVP-AEW541 for the activation of antigen-specific cytotoxic CD8+ T lymphocytes analyzed by flow cytometric analysis

The BMM-derived DCs (1 × 106 cells/well) were pulsed with 1 μg/ml Db-compatible MHC I E7 peptide [aa 49–57] (Kelowna International Scientific Inc.) at day 7 and then co-cultured with the E7-specific CD8+ T cell line (1:10 ratio) overnight. Protein transport inhibitor BD GolgiPlug™ (BDBiosciences) was added 6 h before collecting the cells. The co-cultured cells were then stained with PE-conjugated anti-CD8 Ab (BioLegend) and FITC-conjugated anti-IFN-γ Ab (BioLegend), and analyzed by flow cytometry.

In vitro tumor killing activity of antigen-specific cytotoxic CD8+ T lymphocytes activated by BMM-derived DCs treated with IGFs

The BMM-derived DCs (1 × 105 cells/well) were pulsed with 1 μg/ml Db-compatible MHC I E7 peptide [aa 49–57] at day 7 and then co-cultured with the E7-specific CD8+ T cell line (1:5 ratio) overnight. The co-cultured cells were then co-cultured with the irradiated TC-1-LG cell line (1:8 ratio) in a 96-well plate (1 × 105 cells/well) for 24 h. Luciferin (Promega) was added and total flux (p/s) from each well was measured using IVIS® Imaging Systems.

Cytokine secretion of the BMM-derived DCs treated with IGFs analyzed by bead-based immunoassays

The supernatants of the DCs were collected at day 7 and stored at −20°C until analysis. The concentrations of various cytokines in the supernatants were detected by BD™ CBA Flex Sets (BD Biosciences).
Western blot analysis of the BMM-derived DCs treated with IGFs and/or NVP-AEW541

The DCs were lysed in PhosphoSafe™ Extraction Reagent (Novagen, Merck KGaA) with a proteinase inhibitor (Sigma). The protein extracts were quantified using a Pierce™ BCA Protein Assay Kit (Thermo).

The protein extract of each group was resolved by SDS/PAGE (10% gel), transferred onto an NC membrane (Millipore, Merck KGaA), and probed with antibodies specific to ERK, phospho-ERK, Akt, JNK, or phospho-JNK (Cell Signaling), phospho-Akt (Santa Cruz), p38 (BioLegend), phospho-p38 (Millipore) and α-tubulin (Abcam). Horseradish peroxidase-conjugated goat anti-rabbit secondary antibodies (Hyclon Biotech) were then probed, and Western Lightning™ Plus-ECL (PerkinElmer) was used to visualize the specific bands.

The ratios of phospho-p38 to total p38 (p/p38), phospho-ERK to total ERK (p/ERK/ERK), phospho-AKT to total AKT (pAKT/AKT) and phospho-JNK to total JNK (pJNK/JNK) were further quantified by densitometer.

In vivo tumor challenge of mock, IGF-1, or IGF-2 highly-expressing WF-3 tumor cells

The WF-3 mock, mIGF-1 highly-expressing and mIGF-2 highly-expressing WF-3 tumor cells (2 × 10^5/mouse) were injected intraperitoneally (6 mice per group). The mice were sacrificed on days 14 and 35 post-injection to collect the intraperitoneal fluid. The ascites were separated into supernatant and cellular components. The supernatant was stored at −20 °C, and the cells which were defined as ascites-associated cells (AACS) were stored at −135 °C until analysis. The AACS were then stained with various surface markers of DCs for flow cytometric analysis as described earlier. The concentrations of IL-6 and IL-10 in the supernatant component of the ascites were also detected by ELISA kits (BD Biosciences; R&D Systems).

Statistical analysis

Statistical analyses were performed using the Statistical Package of Social Studies software version 13.0 (SPSS; IBM). The results were confirmed by conducting at least three independent experiments in all of the in vitro and in vivo experiments. All of the data were expressed as mean ± SE (standard error). One-way analysis of variance (ANOVA) and the Mann–Whitney U test and Spearman’s rank correlation (R value higher than 0.6 was defined as strong correlation) were applied.

Concentrations of IGF-1 correlated with IL-2, IL-6, IL-10, IL-12 and TNF-α cytokines in early-stage ovarian cancer patients

Patients of early stages had lower median concentrations of IGF-1 (4.4 ng/ml (Fig. 1A) vs. 54.3 ng/ml (Fig. 1B), p = 0.007, Mann–Whitney U test) and IGF-2 (2962 pg/ml (Fig. 1C) vs. 3696 pg/ml (Fig. 1D), p = 0.041, Mann–Whitney U test) as compared with patients of early stages. Besides, patients of advanced stages had higher frequencies of high IGF-1 and IGF-2 as compared with those of early stages (Fig. 1A–D). Correlations between the other cytokines and IGFs were further evaluated. The IGF-1 showed strong correlations (R ≥ 0.6 by Spearman’s correlation) with IL-2, IL-6, IL-10, IL-12, and TNF-α in the early-stage patients (Fig. 1E). However, there was no strong correlation between IGF-2 and the cytokines in the early-stage patients (Fig. 1E). There were no strong correlations between IGF-1 or IGF-2 and the other cytokines in advanced-stage patients (Fig. 1F).

IGF-1 receptor kinase inhibitor-NVP-AEW541 could reverse the suppressive effect of the IGFs on the maturation of BMM-derived DCs

The MFIs of CD80 of IGF-treated groups were lower than those of the PBS-treated group (IGF-1 group 183.5 ± 202.0, IGF-2 group 193.2 ± 2.9, PBS-treated group 202.0 ± 2.9, p = 0.025, one-way ANOVA; Fig. 2A). And the MFIs of CD86 also showed similar phenomena as the MFI of CD80 (p = 0.025, one-way ANOVA; Fig. 2B) co-stimulating markers of the BMM-derived DCs were significantly lower than those in the PBS-treated group when treated with IGF-1 and/or IGF-2. However, the expressions of MHC class I (p = 0.99, one-way ANOVA; Fig. 2C) and class
II (p = 0.98, one-way ANOVA; Fig. 2D) of the BMM-derived DCs were not significantly different whether or not they were treated with IGFs.

We then examined whether NVP-AEW541 could reverse the suppressive effect of IGFs on the expression of the surface markers of the BMM-derived DCs. The percentages of CD80 (p = 0.95, one-way ANOVA; Fig. 2A) and CD86 (p = 0.58, one-way ANOVA; Fig. 2B) expressions of the BMM-derived DCs treated with NVP-AEW541 and IGFs were not significantly different compared with those treated with NVP-AEW541 alone. The MFIs of MHC class I (p = 0.97, one-way ANOVA; Fig. 2C) and class II (p = 0.93, one-way ANOVA; Fig. 2D) expressions of the BMM-derived DCs treated with NVP-AEW541 and/or IGFs were not significantly different, either.

NVP-AEW541 could inhibit the suppressive effect of IGFs on the antigen presenting abilities of the BMM-derived DCs

The representative figures of the percentages of FITC-OVA_{257-264} short peptide-loaded DCs in flow cytometric analysis are shown in Fig. 3A. The percentages of FITC-OVA_{257-264} short peptide-loaded DCs treated with IGF-1 and/or IGF-2 were significantly lower than those treated with PBS alone (p < 0.001, one-way ANOVA; Fig. 3B).

We then examined whether NVP-AEW541 could reverse the suppressive effect of the IGFs on the antigen presenting ability of the BMM-derived DCs. The representative figures of the percentages of FITC-conjugated OVA_{257-264} short peptide-loaded DCs in flow cytometric analysis treated with NVP-AEW541 and IGFs are shown in Fig. 3A. When treated with NVP-AEW541 and IGF-1 and/or IGF-2, the percentages of FITC-OVA_{257-264} short peptide-loaded DCs were not statistically different compared with those treated with NVP-AEW541 alone (p = 0.95, one-way ANOVA; Fig. 3B).

We further evaluated whether IGFs could influence the antigen processing ability of the BMM-derived DCs. The percentages of FITC-conjugated OVA_{323-339} long peptide loaded DCs treated with IGF-1 and/or IGF-2 were not statistically different compared to those treated with PBS alone (p = 0.46, one-way ANOVA; Fig. 3C).

NVP-AEW541 could restore the activation ability of the BMM-derived DCs to stimulate IFN-γ secretion of antigen-specific CD8^+ cytotoxic T lymphocytes suppressed by the IGFs

We then evaluated whether IGF-induced immature BMM-derived DCs had a reduced ability to activate the IFN-γ secretion of antigen-specific CD8^+ cytotoxic T lymphocytes. The percentages of
IFN-γ-secreting E7-specific CD8+ T lymphocytes were significantly lower in those treated with IGF-1 and/or IGF-2 compared to those treated with PBS alone (p = 0.003, one-way ANOVA; Fig. 3D).

We further evaluated whether NVP-AEW541 could restore the suppressive effect of the IGFs on the BMM-derived DCs for the ability to activate CD8+ T lymphocytes. The percentages of IFN-γ-secreting E7-specific CD8+ T lymphocytes were not statistically different in those treated with NVP-AEW541 and IGFs compared to those treated with NVP-AEW541 alone (p = 0.18, one-way ANOVA; Fig. 3D).

Tumor killing activities of antigen-specific cytotoxic CD8+ T lymphocytes were lower when activated by BMM-derived DCs treated with IGFs

We then evaluated whether the tumor killing activities of antigen-specific CD8+ cytotoxic T lymphocytes will be reduced when activated by IGF-treated BMM-derived DCs. The representative figures of IFN-γ-secreting E7-specific CD8+ T lymphocytes were significantly lower in those treated with IGF-1 and/or IGF-2 compared to those treated with PBS alone (p = 0.003, one-way ANOVA; Fig. 3D).

IGFs regulated the cytokine secretion of the BMM-derived DCs

The concentrations of IL-6 (Fig. 5A), IL-10 (Fig. 5B), and TNF-α (Fig. 5C) in the BMM-derived DCs treated with IGF-1 and/or IGF-2 were significantly higher when stimulated with LPS compared to those without LPS stimulation. The concentrations of IL-2, IL-4, or IL-12 were too low to be detected with or without LPS stimulation (data not shown).
The concentrations of IL-6, IL-10, and TNF-α were not statistically different between the groups without LPS stimulation (Fig. 5). When stimulated with LPS, the IL-6 concentrations of the PBS group were significantly lower in the IGF-1 and/or IGF-2-treated groups (p = 0.02, one-way ANOVA; Fig. 5A), whereas the concentrations of IL-10 (p = 0.04, one-way ANOVA; Fig. 6B) and TNF-α (p = 0.049, one-way ANOVA; Fig. 6C) were significantly higher in the IGF-treated groups compared with those in the PBS-treated group.

IGFs inhibited the maturation of BMM-derived DCs via suppressing ERK1/2 phosphorylation and p38 de-phosphorylation

We further investigated the signaling pathway involved in the IGF inhibition of the maturation of BMM-derived DCs. The representative figures of Western blotting of various molecules are shown in Fig. 6A. IGF-1 and/or IGF-2-treated BMM-derived DCs significantly inhibited ERK1/2 phosphorylation after LPS stimulation compared with the PBS-treated group (Fig. 6B). However, the suppression of ERK1/2 phosphorylation of the BMM-derived DCs by the IGFs could be rescued by NVP-AEW541.

The de-phosphorylation of p38 in the LPS-stimulated PBS-treated BMM-derived DCs was not observed in the LPS-stimulated IGF-1 and/or IGF-2-treated groups (Fig. 6B). However, de-phosphorylation of p38 was again noted in the BMM-derived DCs pre-treated by NVP-AEW541 compared to those without pretreatment with NVP-AEW541 (Fig. 6B). There were no differences in Akt phosphorylation and JNK phosphorylation between the PBS and IGF-treated groups with LPS stimulation (Fig. 6B).

The IGF-1 and IGF-2 highly-expressing WF-3 tumor cells had higher expressions of IGF-1 or IGF-2 compared to the WF-3 mock tumor cells, respectively (Fig. 7A). The levels of IGF-1 in the cell culture media from the IGF-1 highly-expressing WF-3 tumor cells after 72 h of incubation were significantly higher than from the mock and IGF-2 highly-expressing WF-3 tumor cells (WF-3 mock 0.15 ± 0.00 ng/ml, IGF-1 highly-expressing WF-3 123.82 ± 7.00 ng/ml, IGF-2 highly-expressing WF-3 0.07 ± 0.00 ng/ml, p = 0.001, one-way ANOVA). In contrast, the levels of IGF-2 in the cell culture media from IGF-2 highly-expressing WF-3 tumor cells were significantly higher than those from the mock and IGF-1 highly-expressing WF-3 cells (p = 0.004, one-way ANOVA). IGF-1 and IGF-2 highly-expressing WF-3 tumor cells exhibited similar cell proliferative rates compared with the WF-3 mock tumor cells (for 72h, p = 0.06, one-way ANOVA) (Fig. 7B).

The concentrations of IL-6 and IL-10 in the ascites of the tumor-bearing mice were further evaluated. These concentrations of IL-6 and IL-10 showed higher concentrations of IL-6 and IL-10

Fig. 4. In vitro tumor killing activities of antigen-specific cytotoxic CD8+ T lymphocytes activated by BMM-derived DCs treated with or without IGFs. (A) Representative figures of the tumor killing abilities of antigen-specific cytotoxic CD8+ T lymphocytes activated by BMM-derived DCs treated with IGFs by IVIS® Imaging Systems. (B) The bar figure of average luminescence of TC-1-LG cells (*: p = 0.047, one-way ANOVA).

Fig. 5. The effects of IGFs on the cytokine secretion of BMM-derived DCs. (A) The concentrations of IL-6 in the supernatants of cultured DCs (filled: supernatant from the culture medium of BMM-derived DCs without LPS stimulation; open: supernatant from the culture medium of LPS-stimulated BMM-derived DCs) (*: p = 0.02, one-way ANOVA). (B) The concentrations of IL-10 in the supernatants of the cultured DCs (*: p = 0.04, one-way ANOVA). (C) The concentrations of TNF-α in the supernatants of cultured DCs (*: p = 0.049, one-way ANOVA). All experiments were performed in at least three separate experiments.
and IL-10 (\(p = 0.83\), one-way ANOVA; \(Fig. 7D\)) were not statistically different between the WF-3 mock-tumor bearing mice, IGF-1 and IGF-2 highly-expressing WF-3 groups after 14 days of tumor challenge. However, both the concentrations of IL-6 (\(p = 0.046\), one-way ANOVA; \(Fig. 7C\)) and IL-10 (\(p = 0.04\), one-way ANOVA; \(Fig. 7D\)) were significantly increased in the IGF-1 and IGF-2 highly-expressing WF-3 bearing mice compared with those in the WF-3 mock group after 35 days of tumor challenge.

Percentage of immature DCs increased in the ascites-associated cells (AACs) of IGF-1 or IGF-2 highly-expressing WF-3 bearing mice

The expressions of the various surface markers on the CD11c+ ascites-associated cells (AACs) were not statistically different between the WF-3 mock-tumor bearing mice, IGF-1 and IGF-2 highly-expressing WF-3 groups after 14 days of tumor challenge (\(Fig. 7E\)) (for CD80, \(p = 0.58\); for CD86, \(p = 0.71\); for MHC class I, \(p = 0.95\); for MHC class II, \(p = 0.73\), all by one-way ANOVA).

However, the percentages of CD80+ and CD86+ AACs were significantly reduced in the IGF-1 and IGF-2 highly-expressing WF-3 bearing mice compared with those in the WF-3 mock group after 35 days of tumor challenge (\(Fig. 7F\)). The percentages of MHC class I or II surface markers were not different between these three groups (\(Fig. 7F\)).

Discussion

Increased IGF-1 and IGF-2 expressions have been detected in various cancers, including colon, pancreatic, breast and ovarian cancers [9]. The advanced-stage patients with ovarian carcinoma had higher IGF-1 and IGF-2 concentrations in their ascites than the early-stage patients (\(Fig. 1\)). The same pattern has also been reported in other types of cancer. Circulating IGF-1 has been positively associated with all-cause mortality and prostate cancer mortality in patients with advanced prostate cancer [29]. In addition, higher levels of IGF-2 have also been reported in hepatocellular carcinoma patients with an advanced stage resulting in poor outcomes [30].

Due to the increasing body of evidence, IGFs are regarded to be involved in the progression of human cancers and may be a target for therapeutic interventions. The signaling pathways of the IGF family vary between cancer cells and DCs. IGFs have been identified as a progressive factor to stimulate cancer progression through the cell cycle by activating the PI3K/Akt signal transduction pathway and modulating cyclin-dependent kinases [31,32]. In addition, IGFs could activate the Raf-1/MEK/ERK pathway and downstream nuclear factors, resulting in the induction of cellular proliferation [9]. In the current study, IGFs could induce the activation of the Raf-1/MEK/ERK pathway resulting in delayed maturation of the DCs (\(Fig. 6\)). It indicates that different pathways can be induced by IGFs resulting in different effects on different cells.

Short-term culture of DCs with IGF can initiate the maturation of DCs and inhibit the apoptosis of DCs. Liu et al. showed that DCs with short-term culture of IGF-1 for 2 days resulted in maturation of DCs and delayed apoptosis of DCs [33]. Our purpose was to evaluate the effects of IGFs on DCs in ovarian cancer patients. So we treated the DCs with IGF-1 and/or IGF-2 for 6 days to mimic the tumor micro-environment. Our results showed inhibition of the maturation of BMM-DCs and suppression of the DC-mediated immunity. Different concentrations and culture periods could generate different effects on DCs.

The IGF family can stimulate DCs to secrete different cytokine profiles. IGFs could enhance the secretion of IL-10 in BMM-derived DCs in this survey (\(Fig. 5B\)). Previous studies have indicated that IL-10 can enhance the immunosuppressive status of the tumor environment through stimulation of macrophage B7-H4 expression, and cause the suppression of tumor-associated antigen-specific T cell immunity [34,35]. In addition, elevated IL-6 and IL-10 levels have been reported to favor a Th2 inhibitory immune...
response [36]. IGFs may therefore play a role in the suppression of anti-tumor immunity in the tumor microenvironment.

IGFs can be immunosuppressive growth factors that can inhibit the maturation of DCs, which are the important cells that generate anti-tumor immunity. DCs bridge innate and adaptive immune response and serve as key regulators of T and B cell immunity due to the superior ability of DCs to uptake, process and present antigens compared to other antigen-presenting cells. However, DCs are unable to undergo an efficient maturation process in an immunosuppressive tumor microenvironment. The secretion of immunosuppressive factors in tumors and the lack of danger signals required for DC activation have been reported to lead to immature or tolerogenic DCs [37,38]. Naïve T cells that react with immature or tolerogenic DCs are either deleted in secondary lymphoid organs or become Tregs due to T-cell anergy. We discovered that the IGFs could suppress DC maturation. However, the IGF-treated DCs did not exhibit a stronger antigen processing ability, indicating that the IGF-treated DCs acted as tolerogenic DCs but not functional immature DCs.

The IGF1R kinase inhibitor-NVP-AEW541 could block the suppressive effects of the IGFs on the DCs. Previous studies have indicated that a variety of human cancers are correlated with
aberrant expression and activation of IGF1R. Therefore, blockade of the IGF-IGF1R signaling pathway has been used to target IGF1R-expressing tumors. IGF1R kinase inhibitor [39,40], monoclonal anti-IGF1R antibodies [41] and short hairpin RNA [42] have been used in various types of cancer cells, including pancreatic cancer, non-small cell lung cancer, digestive/gastrointestinal cancers, lung cancer and colon cancer. Both IGF-1 and IGF-2 can bind to IGF1R, and therefore NVP-AEW541 can efficiently block the signal transduction of both IGF-1 and IGF-2 [43]. The blockade of the IGF1R signaling pathway by the IGF1R inhibitor provides a new target to generate potent anti-tumor immunity by rescuing the impaired function of DCs. IGF1R inhibitors have recently been used in cancer therapy. The inhibition of IGF1R signaling has been shown to inhibit the growth of Ewing’s sarcoma cell lines [44]. IGF1R-directed antibodies have yielded single agent clinical efficacy in Ewing’s sarcoma. Patients with advanced solid malignancies or non-Hodgkin’s lymphoma have been given AMG 479, a fully human monoclonal antibody to IGF1R [45]. In the current study, NVP-AEW541 was used to rescue the function of IGF-treated DCs. Therefore, the IGF1R inhibitor can be potentially used as an immunotherapeutic drug.

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Conflict of interest

The authors declare that they have no conflicts of interest.

Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.canlet.2015.01.007.

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