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Fas Signal Promotes Lung Cancer Growth by Recruiting Myeloid-Derived Suppressor Cells via Cancer Cell-Derived PGE\textsubscript{2}\textsuperscript{1}

Yongliang Zhang,\textsuperscript{2,3}† Qiuyan Liu,\textsuperscript{2}† Minggang Zhang,\textsuperscript{*} Yizhi Yu,\textsuperscript{†} Xia Liu,\textsuperscript{†} and Xuetao Cao\textsuperscript{3}†\textsuperscript{*}

Fas/FasL system has been extensively investigated with respect to its capacity to induce cellular apoptosis. However, accumulated evidences show that Fas signaling also exhibits nonapoptotic functions, such as induction of cell proliferation and differentiation. Lung cancer is one of cancer’s refractory to the immunotherapy, however, the underlying mechanisms remain to be fully understood. In this study, we show that Fas overexpression does not affect in vitro growth of 3LL cells, but promotes lung cancer growth in vivo. However, such tumor-promoting effect is not observed in FasL-deficient (gld) mice, and also not observed in the immune competent mice once inoculation with domain-negative Fas-overexpressing 3LL cells, suggesting the critical role of Fas signal in the promotion of lung cancer growth in vivo. More accumulation of myeloid-derived suppressor cells (MDSC) and Foxp3\textsuperscript{+} regulatory T cells is found in tumors formed by inoculation with Fas-overexpressing 3LL cells, but not domain-negative Fas-overexpressing 3LL cells. Accordingly, Fas-ligated 3LL lung cancer cells can chemoattract more MDSC but not regulatory T cells in vitro. Furthermore, Fas ligation induces 3LL lung cancer cells to produce proinflammatory factor PGE\textsubscript{2} by activating p38 pathway, and in turn, 3LL cells-derived PGE\textsubscript{2} contribute to the Fas ligation-induced MDSC chemoattraction. Furthermore, in vivo administration of cyclooxygenase-2 inhibitor can significantly reduce MDSC accumulation in the Fas-overexpressing tumor. Therefore, our results demonstrate that Fas signal can promote lung cancer growth by recruiting MDSC via cancer cell-derived PGE\textsubscript{2}, thus providing new mechanistic explanation for the role of inflammation in cancer progression and immune escape. 


\textbf{T}umor cells consistently release many kinds of immunosuppressive and proinflammatory factors such as TGF-β, IL-10, PGE\textsubscript{2}, and vascular endothelial growth factor (VEGF),\textsuperscript{4} which facilitate tumor immune escape and tumor growth. Soluble mediators produced by cancer cells can recruit and activate immune suppressive cells, which further stimulate tumor progression (1). In recent years, more attention has been paid for immune suppressive cells infiltrating in the tumor tissue, they facilitate the constitution of immune suppressive network that inhibits the host immunity and supports the tumor growth (2). Of the immune suppressive cells, myeloid-derived suppressor cells (MDSC) and regulatory T cells (Treg) attach more attraction in this field and have been found to play important roles in promoting tumor immune escape (3–5).

MDSC represent a heterogeneous population of myeloid cells comprising immature macrophages, granulocytes, dendritic cells (DCs), and other myeloid cells at earlier stages of differentiation that can be identified in mice by expression of CD11b and Gr-1. Tumor-derived factors mobilize MDSC from bone marrow into peripheral blood, and then MDSC accumulate and expand in tumor tissues, where MDSC exert their tumor-promoting effect through inhibiting T and NK cell proliferation and activation (6–8), facilitate tumor metastasis, and even contribute to the tumor angiogenesis by directly incorporating into the tumor endothelium (9). Treg cells represents a small fraction of the overall CD4\textsuperscript{+} T cell population, expressing a high level of GITR and Foxp3 molecules, and mediating immune suppression through a cell-cell contact mechanism (10). In tumor-bearing mice, Treg cells are recruited and predominantly accumulated at tumor sites. Removal of CD4\textsuperscript{+}CD25\textsuperscript{+} T cells by injecting anti-CD25 Ab into tumor-bearing mice can enhance antitumor responses (4). Therefore, accumulation of MDSC and Treg in tumor tissue is crucial for tumor immune escape and supposed to be the target of immunotherapy (11). However, little is known about the natural signals in the tumor microenvironment responsible for inducing accumulation and maintaining residence of MDSC and Treg cells in the tumor tissues.

Death receptor Fas (CD95/APO-1) transmits death signals in susceptible cells once triggered by its natural ligand FasL, or agonist Ab (Jo2). Fas/FasL system has been demonstrated to play important roles in various biological processes, including cytolytic T cell-mediated cytotoxicity against virally infected or transformed cells, immune privilege of tumor and the mediation of some therapeutic drugs for cancer (2, 12, 13). However, other than inducing cell death, Fas also transmits proliferation and activation signals (14). For example, in the presence of certain level of FasL, Fas signal in tumor is proposed to convert from tumor suppressor to tumor promoter (15), and this phenomenon has been proved in various human cancer cells and tumor-bearing mice (16–20). Tumor-promoting effect of Fas ligation has been reported to be mediated by inducing tumor cell proliferation (17, 18).
advancing the cell cycle (19), increasing tumor motility, and invasiveness (20). These studies focused on the direct effect of Fas signaling on the tumor cells, and most of conclusions have been made on the basis of the in vitro experiments, however, the influence of Fas signal in tumor cells on tumor-bearing host was ignored.

In this study, we demonstrate that mouse Lewis lung cancer 3LL cells are resistant to Fas-induced apoptosis. Fas signal in 3LL lung cancer cells can promote lung cancer growth in vivo and increase the MDSC and Treg accumulation in tumor. Furthermore, Fas ligation can promote in vitro chemoattraction of MDSC by 3LL cells via inducing PGE2 production. Thus, our results demonstrate that Fas signal can promote lung cancer growth in vivo by recruiting immunosuppressive cells MDSC and Treg cells into tumor microenvironment, therefore providing new mechanistic explanation for the tumor promoting effect of Fas signaling in tumor microenvironment, and the role of inflammation in tumor progression and immune escape.

Materials and Methods

Mice, cell line, and reagents

Female C57BL/6J mice (6–8 wk) were obtained from Joint Ventures Sipper BK Experimental Animal. Tnfsf6gld mice on a C57BL/6 background (henceforth termed, gld) were obtained from The Jackson Laboratory and bred under specific pathogen-free conditions, used at age of 6–8 wk. The 3LL Lewis lung
FIGURE 2. Fas signal promotes the infiltration of MDSC and Foxp3+ Treg cells in the tumor tissue. A and C, 3LL/Fas-WT, 3LL/Fas-DN and parental 3LL cells (1 × 10⁶) were s.c. inoculated into C57BL/6 (B) and gld (D) mice 14 days after tumor inoculation. The ratio of MDSC and Foxp3+ cells in gated CD4+ cells were analyzed by FACS, and the results represent mean value ± SEM of three independent experiments with similar results. Original magnification, ×200. **p < 0.01; *p < 0.05; †p < 0.1. E, F, and G, One × 10⁶ 3LL/Fas-WT cells were s.c. inoculated into C57BL/6 mice (day 0), 250 μg RB6–8C5 (anti-Gr1), and control Isootype (rat IgG) were i.p. injected on day 1, and repeated on the following days 3, 6, 9, 12, 15, 18, and 21 fordepleting MDSC. E, On day 14, TIL from the tumor-bearing mice were analyzed for the ratio of MDSC in TIL and Foxp3+ cells in gated CD4+ cells by FACS. The results represent mean value ± SEM of three independent experiments with similar results, **p < 0.01; †p < 0.1. The tumor size (F) and survival of tumor-bearing mice (G) were monitored. Data points represented the mean value ± SEM from eight mice per group. The values of tumor size were compared at various time points and p values were denoted, *p < 0.05; **p < 0.01.

Detection of cell apoptosis

3LL lung cancer cells were stimulated with Jo-2 or isotype Ab in the indicated concentrations for 12 h, then the apoptotic cells were stained with FITC-Annexin V (BD Pharmingen) and propidium iodide (Sigma-Aldrich) and analyzed by FACS as described previously (22).

Assay for cytokines

MCP-1, VEGF, PGE2, IL-1β, TGF-β, and IL-10 in the supernatants of 3LL cells stimulated with or without Jo-2 Ab were assayed using ELISA kits according to the manufacturer’s instructions (R&D Systems).

Assay for cell chemoattraction

Cell chemoattraction was performed in 3.0-µm pore size polyethylene terephthalate track-etched membrane cell-culture inserts (BD Labware). The supernatants of 3LL cells stimulated with or without 1 µg/ml Jo2 or isotype Ab for 12 h (600 µl) were five-fold diluted with RPMI 1640 medium. In the experiment to confirm the role of PGE2 in the cell chemoattraction, anti-PGE2, Ab (5, 10, 20, or 50 µg/ml) was added into the supernatants of 3LL cells stimulated with 1 µg/ml Jo2. Then CD11b+Gr1+ MDSC (2 × 10⁶) and CD4+CD25+ Treg cells (2 × 10⁶) purified from spleen of tumor-bearing mice using FACSdiVa sorting system (Becton Dickinson) and Miltenyi regulatory T cell isolation kit, respectively, were placed into transwell inserts. Four hours after incubation at 37°C, the cells that had migrated into low chambers were harvested, suspended in 200 µl PBS, and counted by FACSCalibur in low rate within 90 s and analyzed by CellQuest software (BD Biosciences).

Preparation of wild-type (WT) or dominant negative Fas-overexpressing lung cancer cells

TA Cloning Expression Vectors encoding full-length of wild-type Fas (Fas-WT) or dominant negative Fas (Fas-DN) that do not contain the intracellular signaling domain were provided by Dr. J.-K. Lee (National Genome Research Institute, National Institute of Health, Seoul, Korea). The vectors Fas-WT and Fas-DN were confirmed by sequencing. The vectors Fas-WT and Fas-DN were transfected into 3LL lung cancer cells, and the transfected 3LL cells were selected in 800 µg/ml Geneticin for 14 days, then the resistant 3LL clones were selected by limiting dilution and flow cytometry for the clones that expressed high level of full-length or dominant negative Fas, and stable transfected 3LL cell clones were characterized. And, in this article, the 3LL cell clone expressing high level of Fas-WT was designated as 3LL/Fas-WT, and the clone expressing high level of Fas-DN was designated as 3LL/Fas-DN.
Preparation and observation of tumor-bearng mice

One × 10^6 3LL cells, 3LL cells expressing Fas-WT (3LL/Fas-WT), or 3LL cells expressing Fas-DN (3LL/Fas-DN) suspended in 200 µl of HBS5 were s.c. inoculated into the flank of C57BL/6 mice or gld mice, respectively. In some experiments, 250 µg RB6–5C5 (anti-Gr1 Ab) and control Isotype (rat IgG) were i.p injected on day 1, and injected repeatedly on the following day 3, 6, 9, 12, 15, 18, and 21 for depleting MDSC, or simultaneously i.p injected with COX-2 specific inhibitor SC58125 (5 mg/kg) or control DMSO once a day during 18 days. Then tumor mass were measured with a caliper after tumor inoculation, and the tumor size was calculated by multiplying vertical length by horizontal length. The survival of the tumor-bearing mice was monitored daily. Mice were sacrificed when the transplanted tumors reached 3 cm in diameter or severe ulceration developed. Experiments were performed three times and each group contained eight mice.

Phenotype analysis of tumor infiltrating lymphocytes

For analyzing the ratio of Gr1^+ MDSC and CD4^+Foxp3^+ Treg in tumor-infiltrating lymphocytes (TIL), 14 days after inoculation with parental 3LL cells, 3LL/Fas-WT, or 3LL/Fas-DN, TIL were isolated as described previously (23). Then the TIL were stained with FITC-conjugated CD4 with PE conjugated Foxp3 for Treg cells analysis, CD11b in combination with PE conjugated Gr1 for MDSC analysis, and FITC conjugated CD4 with PE conjugated Foxp3 for Treg cells analysis, respectively by flow cytometry.

RT-PCR

Total RNA of 3LL cells and MDSC isolated with anti-Gr1 Beads (MACS, Germany) was extracted with TRIzol reagent (Invitrogen). cDNA was prepared using M-MLV reverse transcriptase (Promega). cDNA was synthesized from 1 µg of the total RNA preparing with AMV reverse transcriptase (Promega) using an oligo(dT)~18~ primer. The sequences of the PCR primers synthesized by Sangon and used in this study were: 5'-CTG CTA AGA GCA TGG TTT-3 and 5'-CC ATA GGC GAT TTC TGG GAC-3' for Fas; 5'-CCA CGA TGC TTC TGCG TTG T and 5'-TCCA AAG GGT AGT CTG TTT for EP2; 5'-GGT CAT CTT ACT CAT CGC CAC CTC TC-3' and 5'-TCC CAC TAA CCT CAT CCA ACA ACA G-3', for EP4; 5'-CGT AAA GAC CTC TAT GCC AAC AC-3' and 5'-TCA GTA ACA GTC CGC CTA GAA GCA C-3' for β-actin. Cycling conditions for Fas and actin were 94°C for 30 s, annealing at 55°C for 30 s, and primer extension at 72°C for 45 s, the optimum numbers of cycles were 34 cycles for Fas, 29 cycles for β-actin. Cycling conditions for EP2/EP4 were 94°C for 30 s, annealing at 65°C for 30 s, and primer extension at 72°C for 60 s for 38 cycles.

Immunohistochemistry

Immunohistochemical staining was performed by using anti-mouse Gr1, or anti-Foxp3 (eBioscience), as described previously (22). Images were viewed with a microscope (DMIRB; Leica Microsystems) and processed with Adobe Photoshop software (Adobe Systems).

Western blot

Activation of ERK, JNK, p38, IκB, and expression of COX-2 in 3LL lung cancer cells stimulated with or without Jo2-Ab were detected using the assay kit according to manufacturer's instructions. Blots were probed for 1 h with 1/1000 diluted, then incubated with 1/2000 diluted HRP-conjugated anti-rabbit or goat Ig G. Proteins were visualized using SuperSignal West Femto Maximum Sensitivity Substrate, as instructed by the manufacturer (Pierce).

Statistical analysis

The comparison of mean values between groups was determined by the Student's t test. Statistical analysis of survival data was performed by the Kaplan-Meier method and analyzed by using the log-rank test, p < 0.05 was considered to be statistically significant.

Results

Fas signal in lung cancer cells promotes lung cancer growth in vivo

As shown in Fig. 1A, 3LL Lewis lung cancer cells constitutively express Fas at mRNA and protein levels. Upon stimulation of 3LL cells with agonistic anti-Fas Ab Jo2, no significant apoptotic cells were observed, even high concentration of Jo2 (5 µg/ml) was used (Fig. 1B). These data indicate that 3LL lung cancer cells are resistant to Fas-induced apoptosis. To investigate the effects of Fas signal on the lung cancer growth, we first detect the in vitro 3LL growth in the presence of Jo2 and anit-Fasl Ab by MTT assay, but both Jo2 and anti-Fasl Ab does not affect the 3LL growth (data not shown). To observe Fas-induced 3LL growth in vivo, we transfected Fas-WT or Fas-DN into 3LL cells, and obtained stable 3LL transfectant clones expressing Fas-WT (3LL/Fas-WT) or Fas-DN (3LL/Fas-DN). Forced overexpression of Fas-WT or Fas-DN did not affect the in vitro growth of 3LL cells (Fig. 1D). However, when 3LL/Fas-WT, 3LL/Fas-DN, or parental 3LL cells were s.c. inoculated into C57Bl6/J mice, we found the accelerated growth of tumor formed by inoculation with 3LL/Fas-WT cells, as compared with that of parental 3LL cells or 3LL/Fas-DN cells. Accordingly, the survival of the mice bearing 3LL/Fas-WT cells was reduced more significantly than that of mice bearing parental 3LL cells or 3LL/Fas-DN cells (Fig. 1E). So, the results indicate that Fas signal in lung cancer cells can promote lung cancer growth in vivo. To confirm the role of Fas signal in the promotion of in vivo growth of lung cancer, we s.c. inoculated 3LL/Fas-WT cells, 3LL/Fas-DN cells, or parental 3LL cells into Fas-L-deficient gld mice, as described previously (23). Then the TIL were stained with FITC-conjugated CD4 with PE conjugated Foxp3 for Treg cells analysis, respectively by flow cytometry.

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FIGURE 3. Fas-ligated lung cancer cells chemoattract MDSC but not Treg cells in vitro. A, 3LL cells (1 × 10^6/ml) were stimulated with 1 µg/ml Jo2 for 12 h, and C, 3LL/Fas-WT, 3LL/Fas-DN and parental 3LL cells (1 × 10^6/ml) were cultured for 24h, then the tumor cell supernatants were five-fold diluted and used to observe the chemoattracting effect on MDSC and Treg cells in vitro. The CD11b^+ Gr1^+ MDSC and CD4^+CD25^+ Treg cells were purified and used as described in Materials and Methods. The chemoattracted cells in the low chamber were counted by FACSCalibur in low rate within 90 s and analyzed with CellQuest software. Data are showed as mean ± SD of three independent experiments with similar results, **, p < 0.01.

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and found there was no significant difference in the tumor growth and survival of tumor-bearing mice among these groups (Fig. 1F). Collectively, Fas signal in lung cancer cells promotes lung cancer growth in vivo, which is dependent on the Fas/FasL interaction.

Increased MDSC and subsequent Foxp3+ Treg cell accumulation in Fas-overexpressing tumor

Next, we wanted to know what's the mechanism for the promotion of lung cancer growth in vivo by Fas signal in lung cancer cells. Because MDSC and Treg accumulation in tumor are involved in the tumor immune escape and tumor progression, we analyzed the accumulation of MDSC and Treg in the tumor tissues 7 and 14 days after cancer cell inoculation. Among three groups, more markedly infiltration of MDSC was observed in the tumor formed by 3LL/Fas-WT than that in the tumor formed by 3LL/Fas-DN mice (Fig. 2B). We also counted the absolute numbers of MDSC (Gr1+ CD11b+) and Tregs (CD4+ Foxp3+) in TIL. There were ~5.3 × 10^5, 1.8 × 10^5, and 1.0 × 10^5 MDSC and 2.3 × 10^5, 8 × 10^4, and 5 × 10^4 Tregs in TILs derived from 3LL/Fas-WT, 3LL, or 3LL/Fas-DN in C57BL/6 mice, respectively. Similar to that in 3LL/Fas-DN from C57BL/6 mice, the absolute numbers of MDSC and Tregs were ~1.2 × 10^5 MDSC and 5.5 × 10^4 Tregs in TILs derived from 3LL/Fas-WT of gld mice. However, in vivo administration of anti-Gr1 Ab could significantly reduce the absolute numbers of MDSC and Tregs, with the ~2 × 10^4 MDSC and 1 × 10^4 Tregs, respectively. Therefore, more MDSC and Tregs were accumulated in 3LL/Fas-WT tumor tissue, but not in tumors in gld mice (Fig. 2D). Together with above data, we conclude that Fas signal in lung cancer cells can recruit more MDSC and Foxp3+ Treg cells into tumor tissues.

MDSC have been shown to be expanded rapidly in the tumor-bearing host. Considering that MDSC can induce generation of Treg cells (24) and the Treg accumulation in the Fas-overexpressing tumor was later than MDSC accumulation as observed above,
we wondered whether the rapid accumulation of MDSC could induce subsequent accumulation of Treg cells in the Fas-overexpressing tumor. By in vivo depleting Gr-1+ cells in the mice bearing Fas-overexpressing tumor, we found the number of tumor-infiltrating Treg cells significantly decreased (Fig. 2E), consistent with the observations of the reduced tumor growth and prolonged survival of tumor-bearing mice (Fig. 2, F and G).

Fas-ligated lung cancer cells chemoattract MDSC but not Treg cells in vitro

As more accumulation of MDSC and Treg cells was observed in Fas-overexpressing tumor, we examined whether Fas-ligated 3LL lung cancer cells can chemoattract more MDSC or Treg cells in vitro. For parental 3LL cells, we found the supernatant of Jo2 stimulated-parental 3LL cells was more effective in chemoattracting CD11b+Gr1+ MDSC than the control supernatant derived from isotype-treated parental 3LL cells, but did not chemoattract more CD4+Foxp3+ Treg cells than controls (Fig. 3A). However, for 3LL/Fas-DN cells, Fas ligation did not enhance the chemotraction effect of the supernatants on MDSC (Fig. 3B). Furthermore, compared with the supernatants from parental 3LL cells and 3LL/Fas-DN cells, supernatant derived from 3LL/Fas-WT cells exhibited more potent ability to chemoattract MDSC (Fig. 3C). So, Fas signal can enhance the ability of 3LL lung cancer cells to chemoattract MDSC but not Treg cells.

PGE2 derived from the Fas-ligated lung cancer cells contributes to the enhanced chemoattraction of MDSC

Then, we went further to look for which factor(s) derived from Fas-ligated 3LL lung cancer cells responsible for the enhanced chemoattraction for MDSC. As reported, Fas signaling is associated with inflammation by increasing chemokine secretion and chemoattracting inflammatory cells (25, 26). VEGF and MCP-1 have been shown to be crucial for MDSC migration (27, 28). So, we detected the expression of VEGF and MCP-1 in the supernatant of 3LL lung cancer cells stimulated by Jo2, however, no increase of MCP-1 and VEGF secretion was found (data not shown). Then, we detected the proinflammatory cytokines IL-1β, IL-10, TGF-β, and PGE2 in the supernatant of Jo2-stimulated 3LL lung cancer cells. No secretion of IL-1β, IL-10, in 3LL cells and no significant increase of TGF-β were observed in Fas-ligated 3LL cells (data not shown). As shown in Fig. 4A, we found Fas ligation could significantly induce 3LL lung cancer cells to produce PGE2 in a dose- and time-dependent manner. Accordingly, Fas ligation also significantly increased COX-2 expression in 3LL cells (Fig. 4B).

As PGE2 can promote migration of cells including DC (29, 30) and endothelial cells (31), then we tested whether the increased production of PGE2 by Fas-ligated cancer cells was responsible for the increased chemoattraction of MDSC. We found that the anti-PGE2 Ab profoundly reduced the chemoattraction of MDSC by the supernatant of Fas-ligated 3LL lung cancer cells (Fig. 4C). Furthermore, COX-2 selective inhibitor SC-58125 (32) was used to treat the Fas-ligated 3LL cancer cells, then we found the increased chemoattraction of MDSC by Fas-ligated 3LL cancer cells was also abrogated (Fig. 4D). To further confirm the conclusion in vivo, we detected COX-2 expression in tumor tissues formed by 3LL/Fas-WT and 3LL/Fas-DN, and found that COX-2 expression in 3LL/Fas-WT tumor tissue was higher than that in 3LL/Fas-DN tumor tissue (data not shown). In vivo i.p. injection of COX-2 inhibitor SC58125 significantly reduced accumulation of MDSC in the tumor tissue of 3LL/Fas-WT-bearing mice (Fig. 4E) and inhibited tumor growth and prolonged survival of tumor-bearing mice (data not shown). Furthermore, we analyzed the apoptosis of 3LL cells stimulated with Jo2 in different concentrations and found

Jo2 combined with SC58125 (70 nM, 700 nM) did not induce 3LL cell apoptosis, thus excluding the possibility that cell death induction may account for the abrogation (Fig. 4F). These data suggest that Fas signal can induce lung cancer cells to secrete more PGE2, which in turn contributes to the enhanced chemoattraction of MDSC by Fas-ligated lung cancer cells.

The biological effects of PGE2 in the target cells are mediated by four different G protein-coupled receptor subtypes, EP1, EP2, EP3, and EP4 (33). Of PGE2 receptors, EP2 and EP4 were known to be essential for PGE2-mediated cell migration (30, 31), then we detected EP2/EP4 expression in MDSC and 3LL cells. RT-PCR results showed that EP2/EP4 were expressed in both MDSC and 3LL cells, especially highly expressed in MDSC (Fig. 4G).

Fas-activated p38 signaling pathway is responsible for the increased PGE2 production by Fas-ligated lung cancer cells

Finally, we wanted to know which pathway(s) is responsible for the increased COX-2 expression and PGE2 production in Fas-ligated lung cancer cells. Activation of MAPK and NF-κB pathways has been shown to contribute to the Fas-mediated proinflammatory factor production (34, 35) and to be associated with tumor growth and invasion (20). As shown in Fig. 5, Jo2 stimulation can activate the ERK, p38, and NF-κB pathways in 3LL lung cancer cells (Fig. 5A). To elucidate which pathway(s) was essential for the increased production of PGE2 by Fas ligation, specific inhibitors for signaling pathways were used to pretreat 3LL lung cancer cells before Jo2 stimulation. We found p38 MAPK-specific inhibitor SB203580 could markedly suppress the Jo2-induced PGE2 production (Fig. 5B), indicating Fas-activated p38 MAPK signaling
pathway is responsible for the increased PGE$_2$ production by Fas-ligated lung cancer cells.

**Discussion**

Fas (CD95/APO-1) belongs to the TNF receptor superfamily and is expressed in a variety of normal and neoplastic cells (14). Triggering of Fas signaling by FasL or agonistic Ab Jo2 results in rapid induction of apoptosis in susceptible cells. Although, other than apoptosis induction, Fas also can transduce activating signal and induce cell proliferation, differentiation, and inflammation (17–20, 36, 37). Hepatocytes are very sensitive to apoptosis induction by agonistic Ab against Fas, however, in vivo administration of agonistic Ab against Fas after partial hepatectomy could promote the proliferation of mouse hepatocytes and contribute to the liver regeneration and healing (38). In addition, similar phenomena have been observed in neurite outgrowth and neuron regeneration following injury (39). It is thus evident that Fas signaling can promote the cell growth in some tissues and conditions. For tumor cells, previous studies demonstrate Fas activation promotes tumor growth in vitro, and their promoting effect results from the direct influence of Fas on cancer cells (17–20). In this study, we demonstrate for the first time that Fas signal promotes lung cancer growth in vivo by enhancing the MDSC and Treg accumulation in tumor, contributing to the immune escape of lung cancer.

Cancer can grow and progress by disrupting the surveillance of host immune system and escaping from the attack of immune cells. During this progress, induction of MDSC and Treg generation, triggering of MDSC and Treg accumulation in tumor microenvironment are tightly associated with tumor progression. In our study, we found Fas signal in lung cancer can promote the accumulation of MDSC and Foxp3$^+$ Treg cells in tumors, and depletion of MDSC in vivo reduces tumor growth and prolongs survival of 3LL/Fas-WT-bearing mice, indicating that the accumulation of MDSC and Foxp3$^+$ Treg cells may contribute to Fas signal-induced lung cancer growth in vivo. Immunohistochemical staining showed that MDSC appeared in the tumor 7 days after 3LL inoculation, while no Foxp3$^+$ Treg cells was found until 14 days later. Jo2 stimulation enhanced the chemotraction for MDSC but not Tregs in vitro, suggesting that MDSC were recruited to the tumor, while Foxp3$^+$ Treg cells may contribute to Fas signal-induced lung cancer growth in vivo. PGE$_2$ production in 3LL, and tumor-derived PGE$_2$ is required for increased expression of Arginase I and CD11b$^+$ DCs expressing Fas ligands (40). Thereby, blocking Fas signal induces MDSC and Treg infiltration in tumors (40). In our study, we found Fas signals promote PGE$_2$ production in 3LL, and tumor-derived PGE$_2$ is required for MDSC chemotraction, providing new mechanistic explanation for inflammation and tumor immune escape. Our results also further confirm that blockade of COX-2/PGE$_2$ is a powerful strategy to the design of new approaches to cancer treatment.

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**Disclosures**

The authors have no financial conflict of interest.

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