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Infiltration of tumor-associated macrophages is involved in tumor programmed death-ligand 1 expression in early lung adenocarcinoma. Cancer

Shima T, Shimoda M, Shigenobu T, Ohtsuka T, Nishimura T, Emoto K, Hayashi Y, Iwasaki T, Abe T, Asamura H, Kanai Y.
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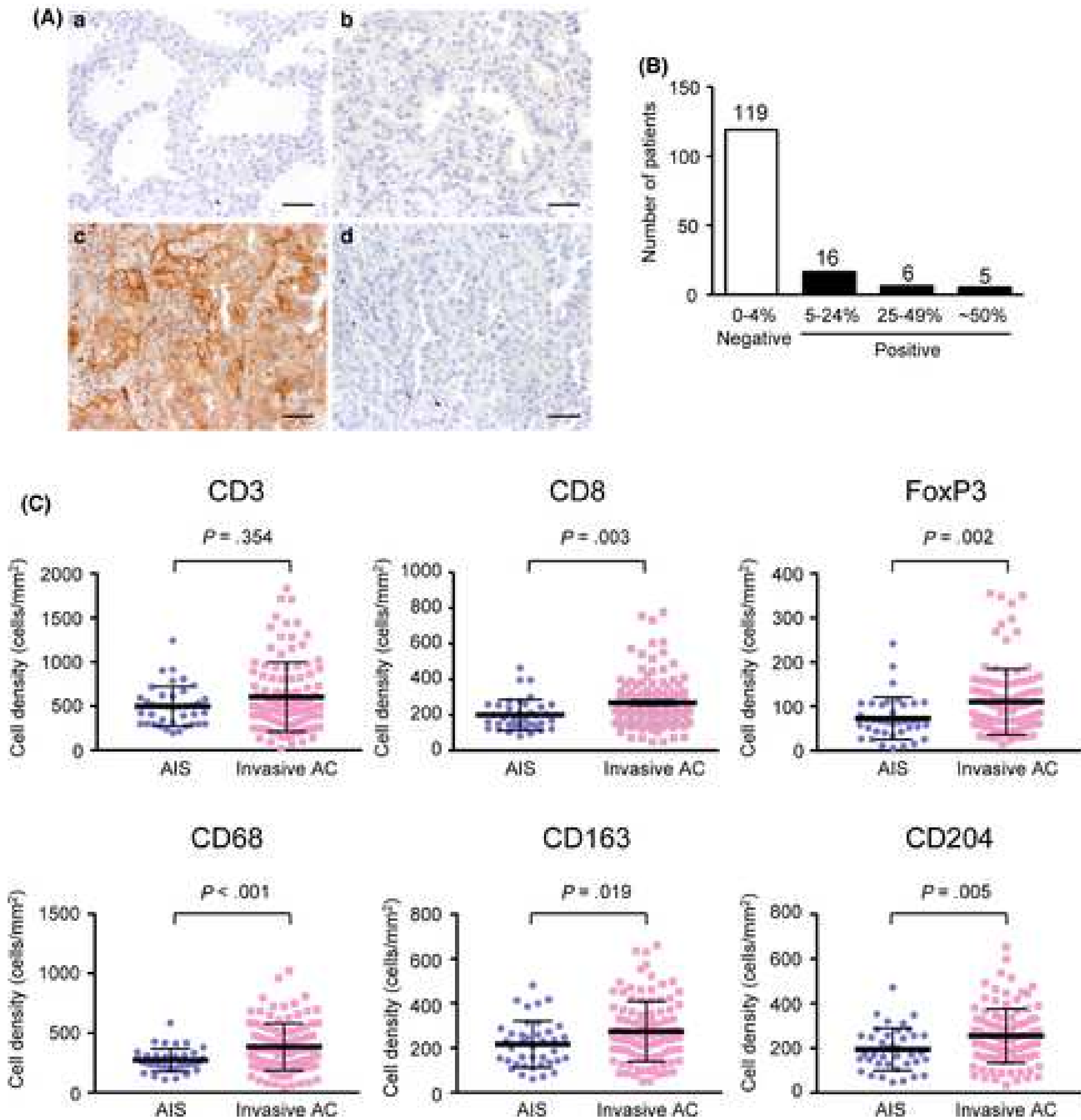
要旨：
PD-L1 は T リンパ球の PD-1 に結合し、免疫抑制を促進する免疫調整物質である。腫瘍細胞の PD-L1 の発現は抗 PD-L1 抗体の臨床反応と関連すると示されているが、その制御メカニズムについては未だ解明されていない。本研究では、免疫細胞による腫瘍細胞の PD-L1 発現の外因性制御について調べるために 146 例の初期の肺腺癌（AC）においての腫瘍細胞の PD-L1 発現と免疫細胞の浸潤パターンの関連性を評価した。症例は 0-ⅠA 期の AC 患者で、18.5%に腫瘍細胞の PD-L1 発現を認めた。腫瘍細胞の PD-L1 発現は免疫担当細胞の浸潤と優位に関連しており、腫瘍関連マクロファージ（TAM）や CD8＋細胞傷害性 T 細胞、FoxP3+制御性 T 細胞の増加を伴っていた。このうち、TAM と CD8＋細胞傷害性 T 細胞は PD-L1 陽性の腫瘍細胞領域に集積していた。CD8＋細胞傷害性 T 細胞は INF-γを産生することで腫瘍細胞の PD-L1 発現を誘導することが知られている。TAM に関しては CD8＋細胞傷害性T細胞の浸潤とは別に腫瘍細胞の PD-L 1 の発現と関連していた。In vitro の実験で、M2 に分化したマクロファージと肺癌細胞株の共培養により PD-L1 の発現は上昇した。さらに M2 の産生する TGF-βを阻害すると腫瘍細胞の PD-L1 の発現はベースラインまで低下した。これらの結果より、腫瘍に浸潤した TAM は TGF-βにより腫瘍細胞の PD-L1 の発現を調整する外的調整因子となることを示した。よって、腫瘍細胞の PD-L1 と TAM の両方を標的とした併用療法が今後の肺癌の効果的な治療法となる可能性を示した。

Take home message
・初期肺腺癌において腫瘍PD-L1の発現は免疫細胞の浸潤と相関する（CD8+T細胞、TAM）
・CD 8 +T 細胞は INF-γ、TAM は M2 由来の TGF-β を介して腫瘍細胞の PD-L1 発現を促進する
・今後 PD-1/PD-L1 抗体に加えて、腫瘍細胞の PD-L1 発現の外因性調節因子である TAM も治療標的となりうる

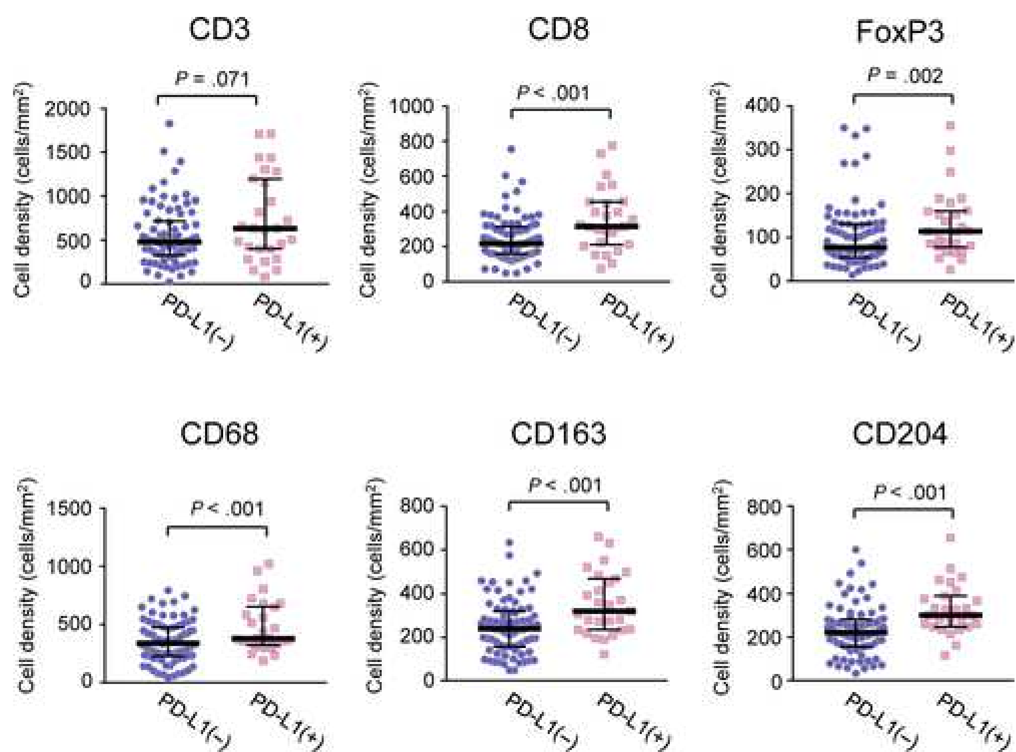
抄読者　鹿内 俊介

Figure1



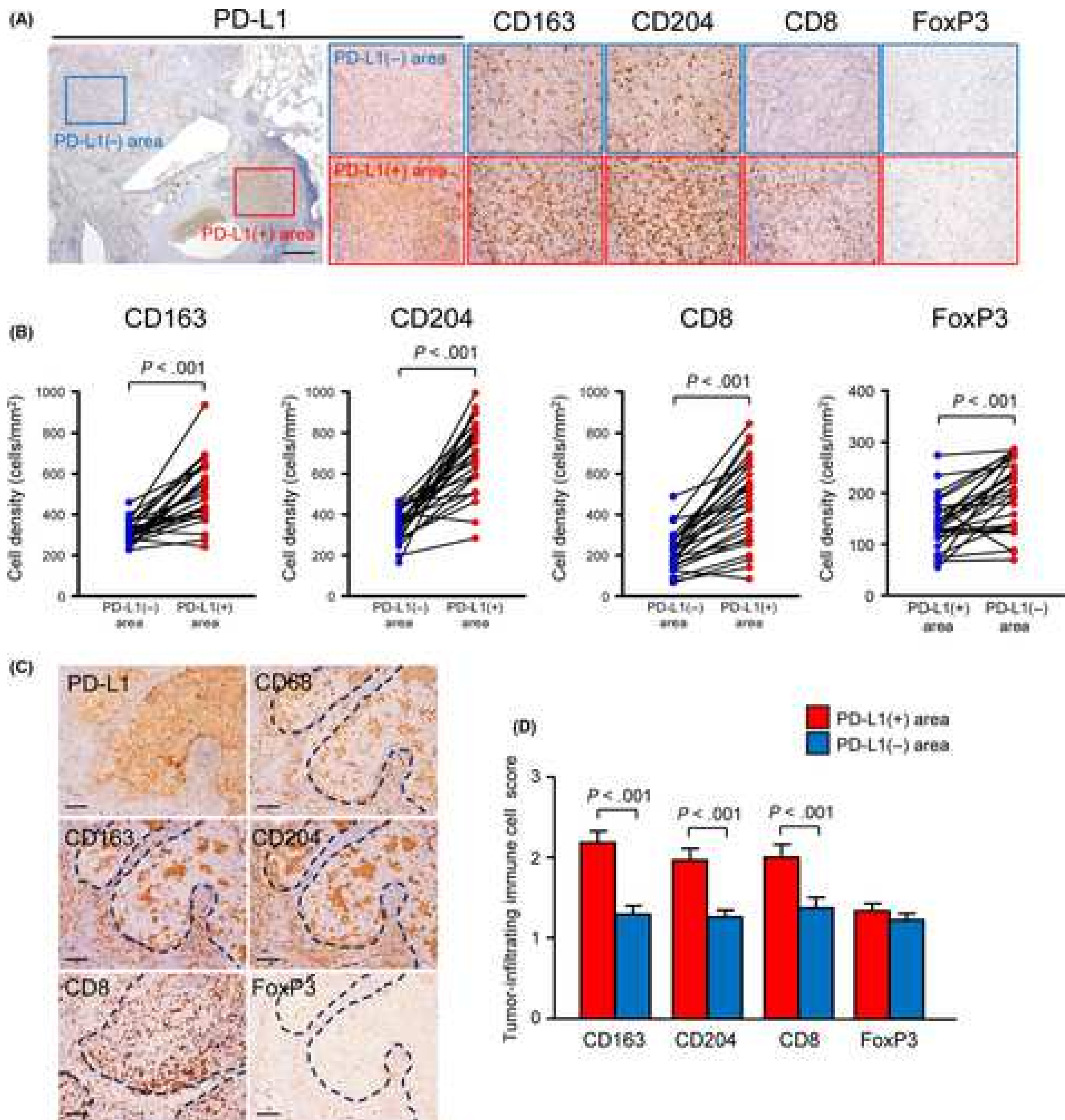
Immunohistochemical analysis of tumor programmed death-ligand 1 (PD-L1) membranous expression. A, Representative images of immunohistochemical staining for PD-L1 of adenocarcinoma in situ (AIS) (a), PD-L1-negative invasive adenocarcinoma (AC) (b) and PD-L1-positive invasive AC (c). (d) shows PD-L1-positive invasive AC immunostained with non–immune IgG. Scale bars, 50 μm. B, Number of patients in groups classified according to the tumor PD-L1 positivity. C, Relationship between stromal invasion (AIS vs invasive AC) and CD3−, CD8−, FoxP3−, CD68−, CD163− or CD204− immunostained immune cell densities (AIS, n = 39; Invasive AC, n = 107). A Mann-Whitney *U* test was performed

Figure2



Relationship between tumor programmed death-ligand 1 (PD-L1) expression and CD3−, CD8−, FoxP3−, CD68−, CD163− or CD204− immunostained immune cell densities in invasive adenocarcinomas (AC). A Mann-Whitney *U* test was performed (PD-L1-negative invasive AC, n = 80; PD-L1-positive invasive AC, n = 27)

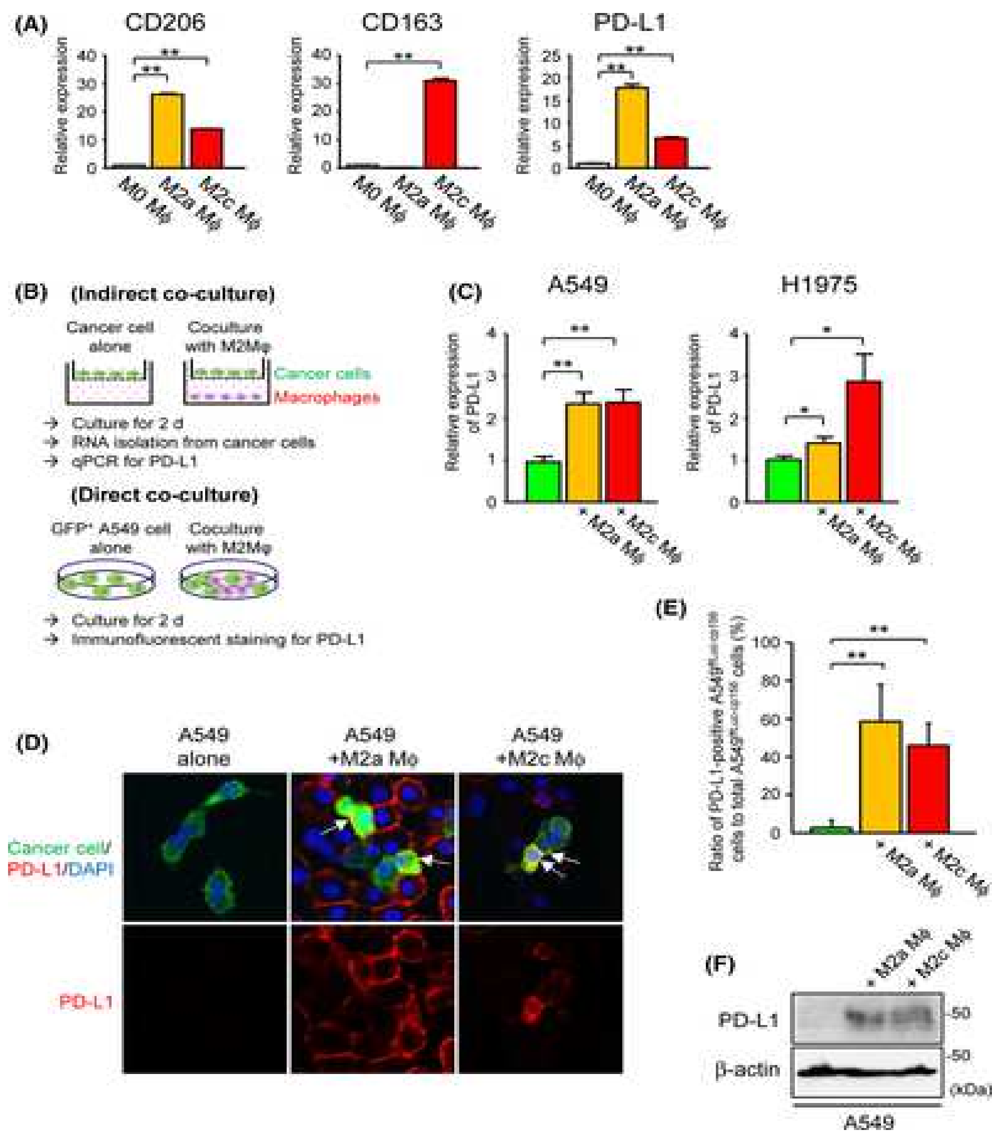
Figure3



A, Representative images of immunohistochemical staining for PD-L1, CD163, CD204, CD8 or FoxP3 in PD-L1-low/no (PD-L1^{-}) or PD-L1-high (PD-L1^{+}) expression areas in PD-L1-positive invasive adenocarcinoma. The PD-L1-stained section is shown in the left panel and the rectangle PD-L1^{-} and PD-L1^{+} areas are magnified to the right. Scale bars, 500 μm. B, Association between tumor PD-L1 expression status and the densities of CD163-, CD204-, CD8- or FoxP3-immunostained immune cells within the tumor (n = 27). A paired Student *t* test was performed. C, Representative images of PD-L1^{+} carcinoma cell nests immunostained for PD-L1, CD68, CD163, CD204, CD8 or FoxP3. Note that CD163^{+} or CD204^{+} TAM and

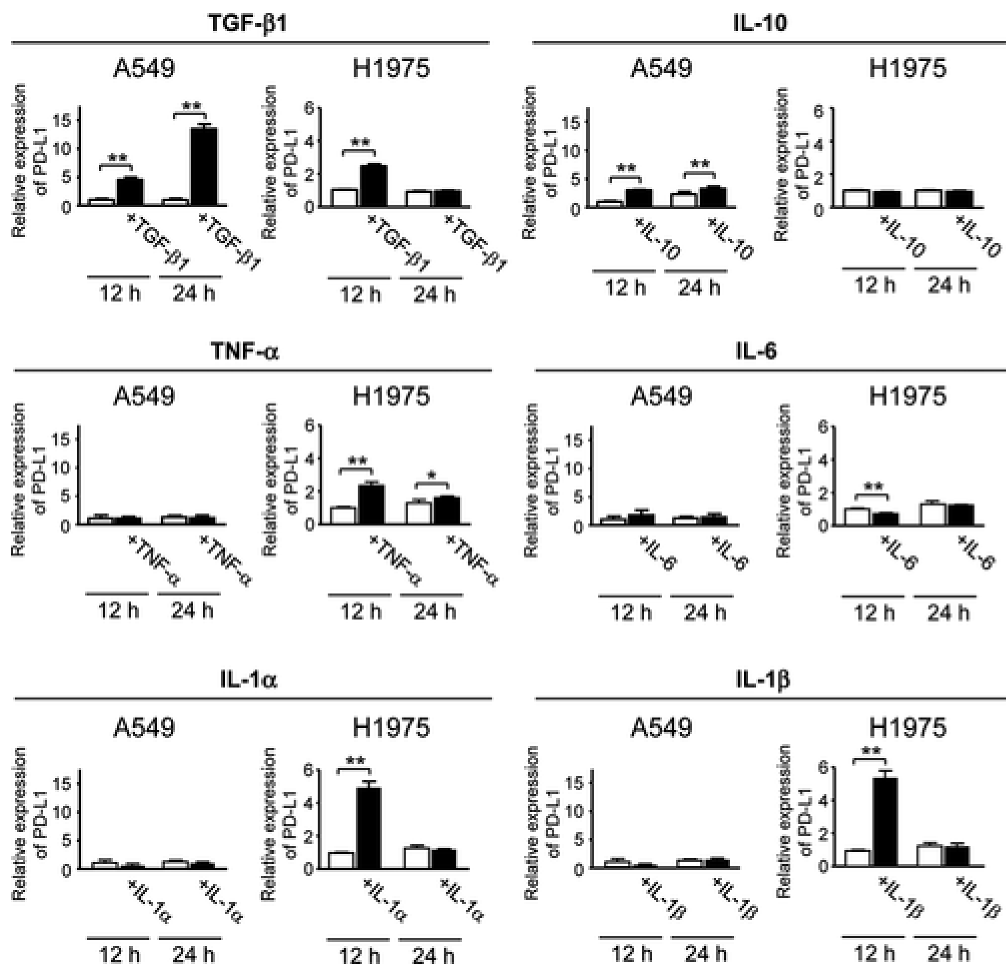
CD8+ T cells were accumulated in PD-L1+ carcinoma cell nests, whereas FoxP3+ T cells were mainly observed in the tumor stroma, even in PD-L1+ areas. Dotted lines indicate PD-L1+ cancer cell nests. Scale bar, 100 μm. D, Comparison of tumor-infiltrating immune cell scores between PD-L1− and PD-L1+ areas within the tumor (n = 27). The tumor-infiltrating immune cell score was defined as described in "Section 2". A paired Student t test was performed

Figure4



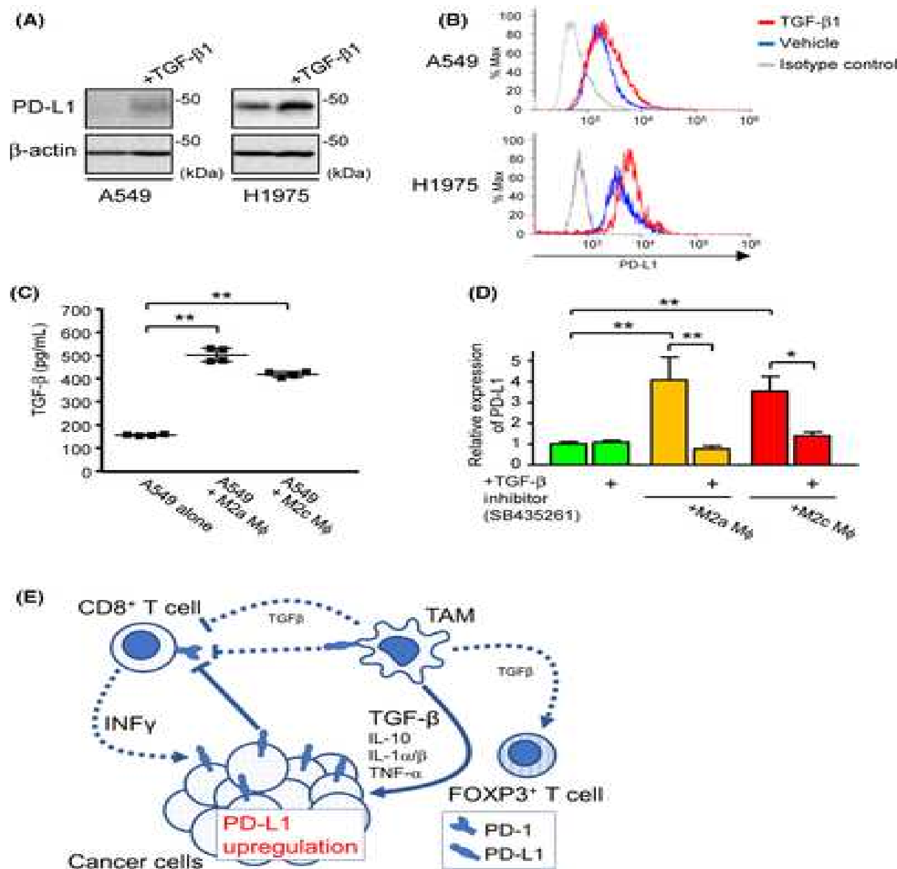
Upregulation of tumor programmed death-ligand 1(PD-L1) expression by co–culture with peripheral blood mononuclear cell (PBMC)-derived M2-differentiated macrophages. A, Relative expression of CD206, CD163 or PD-L1 in human PBMC-derived M0-differentiated, M2a-differentiated and M2c-differentiated macrophages by real-time quantitative PCR (qPCR) (n = 4). B, Description of co–culture schemes. For all experiments, human PBMC were differentiated into M2a or M2c macrophages prior to co–culture with human lung cancer cell lines. Following indirect or direct co–culture with these macrophages for 2 d, PD-L1 expression in human lung cancer cell lines was evaluated by qPCR or immunofluorescent staining. C, Relative expression of PD-L1 in A549 or H1975 cells indirectly co–cultured with human PBMC-derived M2a-differentiated or M2c-differentiated macrophages by qPCR (n = 4). D, Representative images of immunofluorescent staining for PD-L1 in Venus-expressing $A549^{ffLuc-cp156}$ cells directly co–cultured with PBMC-derived M2a-differentiated or M2c-differentiated macrophages. Arrows indicate $A549^{ffLuc-cp156}$ cells showing positive membrane staining for PD-L1. E, Ratio of PD-L1-positive $A549^{ffLuc-cp156}$ cells to total $A549^{ffLuc-cp156}$ cells in the presence or absence of PBMC-derived M2a- or M2c-differentiated macrophages (n = 5). F, Representative immunoblot of PD-L1 in A549 cells indirectly co–cultured with human PBMC-derived M2a-differentiated or M2c-differentiated macrophages. β-actin was used as a loading control. $^{*}P < 0.05$, $^{**}P < 0.01$

Figure5



Effect of macrophage-producing cytokines on programmed death-ligand 1 (PD-L1) expression in human lung adenocarcinoma cell lines. Relative expression of PD-L1 in A549 or H1975 cells by treatment with transforming growth factor-β1 or interleukin (IL)-10, tumor necrosis factor-α, IL-6, IL-1α or IL-1β for 12 or 24 h by real-time quantitative PCR (n = 4). $^{*}P < 0.05$, $^{**}P < 0.01$

Figure6



Transforming growth factor (TGF)-β1 is involved in tumor-associated macrophage (TAM)-mediated programmed death-ligand 1 (PD-L1) expression in human lung cancer cell lines. A, Representative immunoblot of PD-L1 in A549 or H1975 cells by treatment with TGF-β1 or vehicle alone for 72 h. β-actin was used as a loading control. B, Representative flow cytometry data showing cell surface PD-L1 expression in A549 or H1975 cells by treatment with TGF-β1 or vehicle alone for 48 h. C, The concentration of TGF-β in the conditioned media from A549 cells alone or indirectly co–cultured with human peripheral blood mononuclear cell (PBMC)-derived M2a or M2c macrophages for 3 d, determined by ELISA (n = 4). D, Relative expression of PD-L1 in A549 cells co–cultured with human PBMC-derived M2a-differentiated or M2c-differentiated macrophages in the presence of a TGF-β inhibitor, SB431542 or vehicle alone by real-time quantitative (n = 3). E, Schematic presentation of the possible role of TAM in the tumor microenvironment. In this study, we showed that infiltration of TAM is involved in tumor PD-L1 induction through production of cytokines such as TGF-β in early lung adenocarcinoma. Upregulated PD-L1 in macrophages during M2 differentiation may also directly contribute to immune suppression. In addition, TGF-β is known to promote FoxP3^{+} regulatory T cell development and inhibit CD8^{+} T cell functions, suggesting that TAM infiltration within the tumor is a key factor for creation of the immunosuppressive tumor microenvironment. $^{*}P < 0.05$, $^{**}P < 0.01$