News Release

Title

Blockade of EGFR improves responsiveness to PD1 blockade in EGFR-mutated non-small cell lung cancer

Key Points

・ Epidermal growth factor receptor (EGFR) mutations are found in 50% of lung adenocarcinomas in East Asia, including Japan.

・ Low clinical efficacy of anti-PD-1 mAbs against EGFR-mutated NSCLC has been reported, while the promising results of immune checkpoint blockade (ICB) in NSCLC treatment was observed,

・ In this study, we explored the immunological status of the EGFR-mutated lung adenocarcinomas and identified a unique immunological status, leading to a novel cancer immunotherapy.

Summary

The clinical efficacy of anti-PD-1 mAb against cancers with oncogenic driver gene mutations, which often harbor a low tumor mutation burden, is variable, suggesting different contributions of each driver mutation to immune responses. In this study, we investigated the immunological phenotypes in the tumor microenvironment of epidermal growth factor receptor (EGFR)-mutated lung adenocarcinomas, for which anti-PD-1 mAb is largely ineffective. While EGFR-mutated lung adenocarcinomas had a non-inflamed tumor microenvironment (TME), $CD4^+$ effector regulatory T (Treg) cells, which are generally present in the inflamed TME, exhibited high infiltration. The EGFR signal activated cJun/JNK and reduced IRF1; the former increased CCL22, which recruits $CD4^+$ effector Treg cells, and the latter decreased CXCL10 and CCL5, which induce $CD8^+$ T cell infiltration. EGFR inhibitor, erlotinib decreased $CD4^+$ effector Treg cell infiltration in the TME, and the combination with anti-PD-1 mAb showed better antitumor effects than either treatment alone. Our results suggest that EGFR inhibitors when used in combination with anti-PD-1 mAb could increase the antitumor efficacy of immunotherapy in lung adenocarcinomas.

The study has been published online in Science Immunology on Jan 31st, 2020.

Research Background

Alterations in several oncogenic driver genes, including genes encoding epidermal growth factor receptor (EGFR) have been reported in non-small cell lung cancer (NSCLC). The activating *EGFR* mutation is found in about 50% of lung adenocarcinomas (LUADs) in East Asia, including Japan. Recently, immune checkpoint blockade (ICB), including monoclonal antibodies (mAbs) against programmed cell death 1 (PD-1) has demonstrated impressive antitumor effects in NSCLC, opening a new era in NSCLC treatment. However, the promising results of ICB in NSCLC, a low clinical efficacy of anti-PD-1 mAbs against *EGFR*-mutated NSCLC has been reported. In this study, we explored the immunological status of the *EGFR*-mutated lung adenocarcinomas (LUADs) and identified an intriguing immunological status.

Research Results

First, whole exome sequencing was performed with LUAD samples. Nonsynonymous single nucleotide variants and frameshift mutations, which can reflect the number of gene alteration-associated neoantigens and are associated with clinical efficacy of anti-PD-1 mAbs, were significantly higher in *EGFR* wild-type LUADs than in *EGFR*-mutated LUADs (Fig. 1).

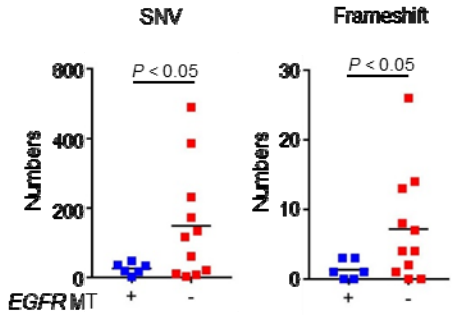

Fig 1. Tumor mutation burden are decreased in *EGFR*-mutated LUADs.

Tumor mutation burden according to *EGFR* gene status. Both single nucleotide variants and frameshift mutations were examined.

IHC assays with surgically resected LUADs were performed for detailed immune profiling. In *EGFR*-mutated LUADs, the frequency of $CD8^+$ T cells was lower than that of the *EGFR* wild-type LUADs.

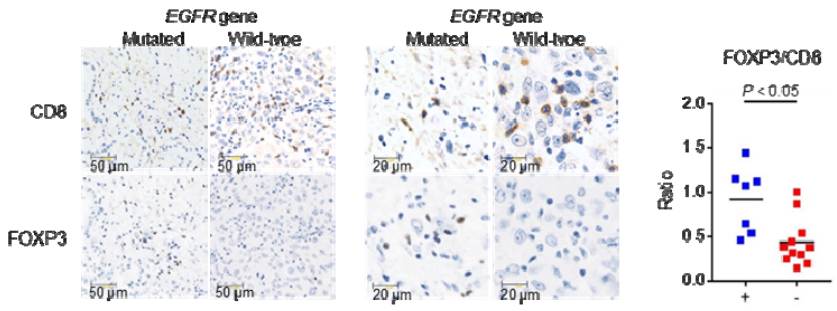

Fig 2. Tregs highly infiltrate into EGFR-mutated LUADs using IHC assay.

Representative IHC for CD8 and FOXP3 according to *EGFR* gene status (left). Summary of the ratio of FOXP3/CD8 (right).

To validate these data, we also investigated tumor infiltrating lymphocytes (TILs) with flow cytometry and CyTOF assays. We defined effector regulatory T cells (eTregs) with strong immune suppressive function as $CD45RA^-FOXP3^{high}CD4^+$ T cells. TIL analyses confirmed that

the frequency of CD8⁺ T cells was lower in *EGFR*-mutated LUADs than in *EGFR* wild-type LUADs. The frequency of tumor-infiltrating eTregs and the eTreg/CD8⁺ T cell ratio were significantly higher in *EGFR*-mutated LUADs than in *EGFR* wild-type LUADs, corresponding to the data of IHC (Fig. 3). These findings suggest that Tregs infiltrate into the TME despite the low levels of CD8⁺ effector T cells in *EGFR*-mutated LUADs.

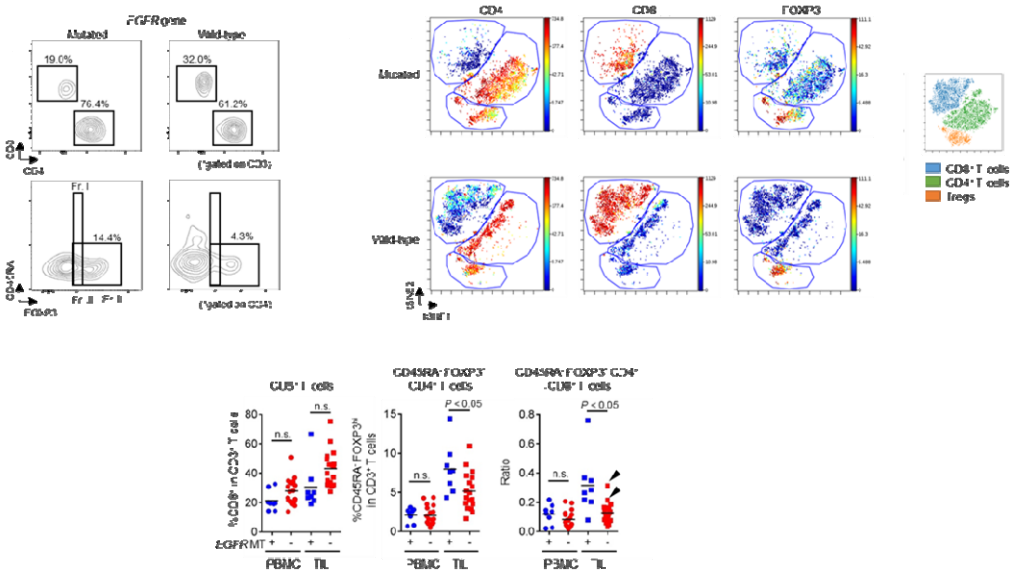

Fig 3. eTregs highly infiltrate into *EGFR*-mutated LUADs using Flowcytometry and CyTOF assay.
(Left) Representative flow cytometry staining (CD4/CD8 for T cells and CD45RA/FOXP3 for CD4⁺ T cells) and (Right) CyTOF staining of TILs from *EGFR*-mutated and wild-type LUADs. (Bottom) Summary of the frequency of the indicated T cell fractions in surgically resected LUADs. EGFR MT, *EGFR* mutations.

To gain insight into the mechanism(s) for this immunological status of *EGFR*-mutated LUADs (high eTreg infiltration despite low CD8⁺ effector T-cell infiltration), we investigated the effect of EGFR signaling on CD8⁺ effector T cells and eTreg infiltration with two *EGFR*-mutated cell lines (PC-9 and HCC827) and an *EGFR* wild-type cell line (H322) treated with erlotinib and EGF, respectively. Consistently, we found CCL5 and CXCL10, which reportedly recruit CD8⁺ T cells, were downregulated by EGFR signaling. Additionally, CCL22, which recruits Tregs, was elevated with activation of EGFR signaling (*EGFR*-mutated cell lines without erlotinib and *EGFR* wild-type cell line with EGF) (Fig. 4). This elevation was abrogated by inhibition of EGFR signaling with an erlotinib in *EGFR*-mutated cell lines.

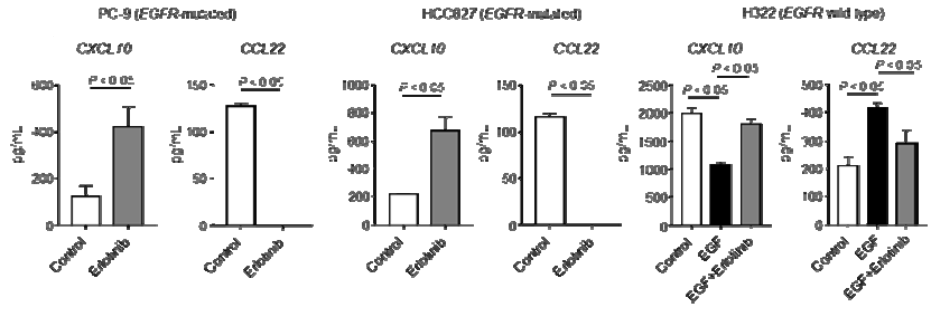

Fig 4. CXCL10 recruiting CD8+ effector T cells is down-regulated and CCL22 recruiting Tregs is up-regulated by EGFR signal in *EGFR*-mutated LUADs.

CXCL10 and CCL22 expression levels in the *EGFR*-mutated cell lines (PC-9 and HCC827) treated with/without erlotinib and the *EGFR* wild-type cell line (H322) treated with/without EGF and erlotinib was evaluated by quantitative real-time reverse transcription PCR.

To further examine chemokine changes by EGFR signaling, we examined the transcriptional regulation of these chemokines. Since the JNK/cJun pathway has been reported to increase CCL22 expression, JUN expression was examined. JUN expression was augmented along with CCL22 expression. JUN knockdown decreased CCL22 expression but not CXCL10 expression. A luciferase assay using CCL22 promoter regions also demonstrated that JUN knockdown decreased CCL22 luciferase activity, suggesting that EGFR signaling increases CCL22 expression via JNK/cJun activation. To investigate the mechanism(s) of CCL5 and CXCL10 reduction, we found that IRF1 expression was concurrently changed with CXCL10 expression and was downregulated by the activation of EGFR signaling. Additionally, IRF1 knockdown resulted in the downregulation of CXCL10, but not CCL22, at the mRNA level and in luciferase assays, indicating that the EGFR signaling decreased CXCL10 expression via IRF1 inhibition (Fig. 5). We propose the EGFR signaling plays an important role in driving high Treg infiltration despite low $CD8^+$ effector T-cell infiltration in EGFR-mutated LUADs via CCL22 upregulation through JNK/cJun and CXCL10 downregulation mediated by IRF1.

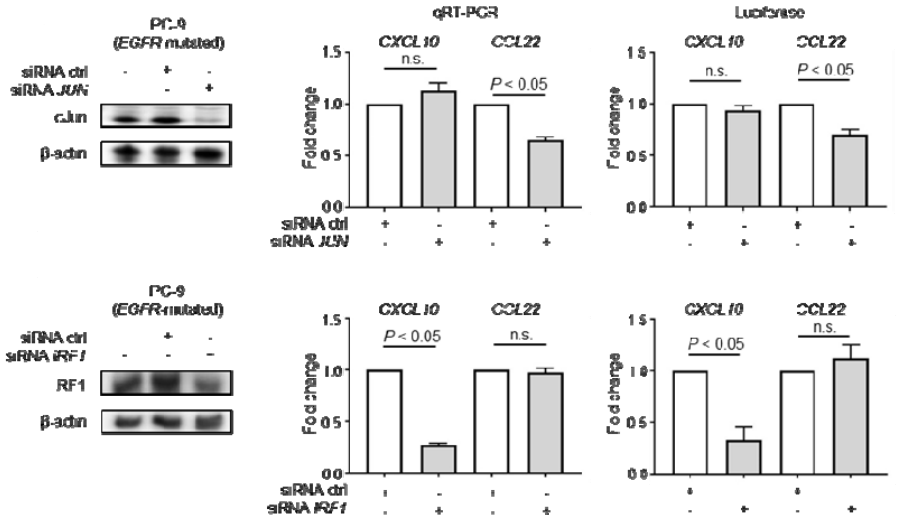

Fig 5. EGFR signaling controls the transcription factors cJun/JNK and IRF1 for the immune phenotype of *EGFR*-mutated LUADs.

(Top) (Left) JUN expression by PC-9 was knocked down by siRNA, and protein expression was confirmed with western blotting. (Right) CXCL10 and CCL22 gene expression was examined by quantitative real-time

reverse transcription PCR, and luciferase activity of the CXCL10 and CCL22 promoter regions was examined by luciferase assays. (Bottom) (Left) IRF1 expression by PC-9 was knocked down by siRNA, and protein expression was confirmed with western blotting. (Right) CXCL10 and CCL22 gene expression was examined by quantitative real-time reverse transcription PCR, and luciferase activity of the CXCL10 and CCL22 promoter regions was examined by luciferase assays.

We next addressed whether EGFR signal inhibition altered the immunological status (high Treg infiltration despite low $CD8^+$ effector T cell infiltration) of *EGFR*-mutated LUADs and prevented tumor growth/progression. We then employed human *EGFR* mutant (exon 19 deletion)-transfected mouse cell lines to examine the *in vivo* antitumor activity. The combination of erlotinib and anti-PD-1 mAb significantly inhibited tumor growth compared with the control or either single treatment after transfection MC-38ex19del (Fig. 6). Our results suggest that combination treatment with EGFR tyrosine kinase inhibitor such as erlotinib and anti-PD-1 can be a promising strategy for the treatment of *EGFR*-mutated LUADs.

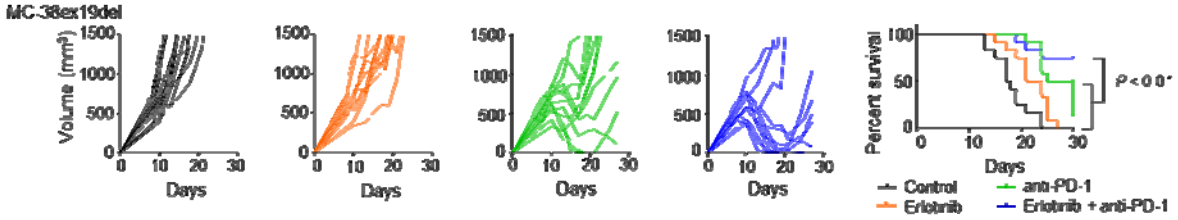

Fig 6. The combination treatment with erlotinib and anti-PD-1 mAb effectively induces tumor growth inhibition in *EGFR*-mutated LUADs

Mice were inoculated with MC-38Ex19del and treated with/without erlotinib, anti-PD-1 mAb or the combination (erlotinib + anti-PD-1 mAb). Tumor growth and the survival curve are shown.

Research Summary and Future Perspective

In this study, we found a unique immunological status in the *EGFR*-mutated LUADs: high Treg infiltration which are induced by *EGFR* mutations and related to resistance to cancer immunotherapies. Driver gene alterations represented by *EGFR* mutations therefore play an important role in cell growth and/or survival as well as the development of immune escape machineries, warranting further tests in cancer immunotherapies combined with molecular-targeted therapies against cancers with driver gene alterations.

Publication

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Science Immunology（Jan 31st, 2020）
Blockade of EGFR improves responsiveness to PD1 blockade in EGFR-mutated non-small cell lung cancer
DOI：10.1126/sciimmunol.aav3937

Japanese ver.
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