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Original Article

Osimertinib (AZD9291) decreases programmed death ligand-1 in EGFR-mutated non-small cell lung cancer cells

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Abstract

Osimertinib (AZD9291) is a third-generation epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) that has been approved for the treatment of EGFR-mutated non-small cell lung cancer (NSCLC). In NSCLC patients, an EGFR mutation is likely to be correlated with high levels of expression of programmed death ligand-1 (PD-L1). Here, we showed that osimertinib decreased PD-L1 expression in human EGFR mutant NSCLC cells *in vitro*. Osimertinib (125 nmol/L) markedly suppressed PD-L1 mRNA expression in both NCI-H1975 and HCC827 cells. Pretreatment with the N-linked glycosylation inhibitor tunicamycin, osimertinib clearly decreased the production of new PD-L1 protein probably due to a reduction in mRNA. After blocking transcription and translation processes with actinomycin D and cycloheximide, respectively, osimertinib continued to reduce the expression of PD-L1, demonstrating that osimertinib might degrade PD-L1 at the post-translational level, which was confirmed by a cycloheximide chase assay, revealing that osimertinib (125 nmol/L) decreased the half-life of PD-L1 from approximately 17.8 h and 13.8 h to 8.6 h and 4.6 h, respectively, in NCI-H1975 and HCC827 cells. Pretreatment with the proteasome inhibitors (MG-132 or bortezomib) blocked the osimertinib-induced degradation of PD-L1, but an inhibitor of autophagy (chloroquine) did not. In addition, inhibition of GSK3β by LiCl prevented osimertinib-induced PD-L1 degradation. The results demonstrate that osimertinib reduces PD-L1 mRNA expression and induces its protein degradation, suggesting that osimertinib may reactivate the immune activity of T cells in the tumor microenvironment in EGFR-mutated NSCLC patients.

Keywords: non-small cell lung cancer; EGFR; osimertinib; PD-L1; ubiquitin-proteasome system; MG-132; bortezomib; GSK3β; LiCl

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Introduction

Currently, targeted therapy with epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKI) has become a standard first-line therapy for the treatment of patients with EGFR-mutated non-small-cell lung cancer (NSCLC)[1, 2]. Despite high tumor response rates with first-line EGFR-TKI therapy, the disease ultimately progresses in most patients after approximately 1 year of treatment, usually leading to an acquired resistance with an EGFR T790M mutation[3, 4]. Osimertinib is an oral, irreversible, third generation EGFR-TKI. It forms a covalent bond with residue C797 in the EGFR kinase domain and targets both EGFR-sensitizing mutations and the T790M mutant[5]. It has been approved by the US Food and Drug Administration (FDA) for the treatment of patients with EGFR T790M mutation-positive NSCLC.

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Cancer cells evade immune destruction by using immune checkpoints to interact with the host immune system in the tumor microenvironment, which eventually allows the tumor to escape the organism's immune surveillance[6, 7]. Programmed cell death ligand 1 (PD-L1) is a transmembrane glycosylated protein that is often overexpressed in a variety of cancer cells[7, 8]. As an immune checkpoint, it interacts with programmed cell death protein 1 (PD-1), a T cell inhibitory immune-checkpoint receptor, to help tumors to escape from immune activity in the tumor microenvironment[6, 7]. Recently, immunotherapy using antibodies against PD-1 or PD-L1 has been widely studied for the treatment of a variety of cancers because of their impressive and durable clinical responses[9-15]. Currently, several PD-1 or PD-L1 antibodies (nivolumab, pembrolizumab and atezolizumab) are being used to treat patients with NSCLC[16].

The molecular mechanisms involved in the regulation of PD-L1 are diverse. In the tumor microenvironment, surface expression of PD-L1 on tumors can be induced by interferon

gamma that is released from activated T cells[17]. In addition, dysregulation of intrinsic oncogenic pathways, such as anaplastic lymphoma receptor tyrosine kinase (ALK) rearrangement[18, 19] and MYC overexpression[20], contribute to the constitutive expression of PD-L1. High levels of expression of PD-L1 in advanced NSCLC patients are likely related to EGFR mutations[21-23]. In EGFR-mutated NSCLC cells, gefitinib, a first-generation EGFR TKI, down-regulates PD-L1 expression at the mRNA level[24-26]. However, the effects of osimertinib on PD-L1 remain unclear in EGFR-driven NSCLC cells.

In the current study, we demonstrated for the first time that inhibition of EGFR phosphorylation by osimertinib not only decreased the expression of PD-L1 mRNA but also induced proteasomal degradation of PD-L1, thereby shedding new light on the molecular mechanisms of PD-L1 regulation by EGFR-TKIs.

Material and methods

Reagents

Osimertinib, rociletinib, gefitinib, erlotinib, MG-132, bortezomib, chloroquine, Z-VAD-FMK and necrosulfonamide (NSA) were obtained from Selleck Chemicals (Houston, TX, USA). Tunicamycin, actinomycin D, dimethyl sulfoxide (DMSO), Hoechst 33342, LiCl and 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) were obtained from Sigma (St Louis, MO, USA). Cycloheximide was purchased from Cell Signaling Technology (Beverly, MA, USA).

Cell lines and cell culture

Human EGFR-mutated NSCLC cell lines NCI-H1975 and HCC827 were obtained from the Shanghai Cell Bank (Shanghai, China). All cell lines were cultured in RPMI-1640 supplemented with 10% fetal bovine serum and antibiotics (100 μg/mL streptomycin and 100 units/mL penicillin). All cells were maintained at 37 °C in a humidified incubator containing 5% CO_2.

Western blot

Cells were harvested and lysed in RIPA lysis buffer (Beyotime Biotechnology, Shanghai, China) containing the protease inhibitor Phenylmethylsulfonyl fluoride (PMSF) and phosphatase inhibitor cocktail PhosSTOP (Thermo Fisher Scientific Inc, MA, USA). After incubation on ice for 20 min, the cell lysate was centrifuged at 15 000 revolutions per minute for 20 min at 4 °C. The protein content of the supernatant was determined using the BCA™ protein assay kit (Pierce, Rockford, IL, USA). A total of 20 μg of protein was separated by SDS-PAGE, transferred onto polyvinylidene fluoride membranes, and then blocked with 5% nonfat milk in PBST for 1 h at room temperature. The membranes were probed with primary antibodies against PD-L1 (#13684), p-EGFR (Y1068) (#3777), LC3B (#2775), c-MYC (#5605), p-GSK3β (S9) (#5558) and GAPDH (#2118) overnight at 4 °C. All antibodies were purchased from Cell Signaling Technology (Beverly, MA, USA). The membranes were washed with PBST for 20 min and then incubated with anti-rabbit IgG HRP-conjugated secondary antibody at room temperature for 1 h. Signals were detected using an ECL advanced Western blot detection kit (GE Healthcare, Uppsala, Sweden). The relative protein levels of PD-L1 were quantified using Image Lab™ software (Bio-Rad, CA, USA) compared with GAPDH.

Immunofluorescence

The immunofluorescence assay was performed according to the manufacturer's instructions. Briefly, cells cultured in chamber slides (Thermo Fisher Scientific Inc, MA, USA) were washed with PBS three times, fixed with 4% paraformaldehyde for 15 min at room temperature, and then permeated in ice-cold methanol for 10 min at –20 °C. After blocking with 0.5% BSA for 1 h, the cells were incubated with primary antibody to PD-L1 (#86744, Cell Signaling Technology, Beverly, MA, USA) overnight at 4 °C. After rinsing with PBS, the cells were incubated with anti-rabbit IgG (H+L), F(ab')2 fragment (Alexa Fluor® 488 Conjugate) (#4412, Cell Signaling Technology, Beverly, MA, USA) at room temperature for 1 h in the dark. After staining with Hoechst 33342 for 10 min at room temperature, the cells were photographed using a confocal laser scanning microscope (Leica TCS SP8, Solms, Germany).

Flow cytometric analysis

Cells were collected by centrifugation at 1000 revolutions per minute for 5 min, fixed with 4% formaldehyde for 10 min at 37 °C, and then incubated with 0.5% BSA at room temperature for 10 min. The cells were probed with PD-L1 (#86744, Cell Signaling Technology) and a matched isotype control at room temperature for 1 h. After washing three times with PBS, the cells were incubated with anti-rabbit IgG (H+L), F(ab')2 fragment (Alexa Fluor® 488 Conjugate) (#4412, Cell Signaling Technology) for 30 min in the dark. The cells were analyzed using flow cytometry (Becton Dickinson FACS Canto, Franklin Lakes, NJ, USA), and data analysis was performed using FlowJo VX.

MTT assay

Cells were seeded on 96-well plates at 5000 cells per well overnight. The cells then were treated with different concentrations of osimertinib for 6 h. The supernatant was discarded, and 100 μL of MTT solution was added to each well (1 mg/mL) and then incubated for 4 h. Cell viability was determined by the addition of 100 μL of DMSO and then shaken for 10 min in the dark to solubilize formazan. The absorbance at 570 nm was recorded using a SpectraMax M5 microplate reader (Molecular Devices, Sunnyvale, CA, USA).

RNA extraction and quantitative real-time PCR

Total RNA was extracted from cells using TRIzol reagent (Life Technologies, Shanghai, China), and cDNA was synthesized using a Transcriptor First Strand cDNA Synthesis Kit (Roche, Germany) according to the manufacturer's instructions. Quantitative real-time PCR was performed using FastStart Universal SYBR Green Master (Roche, Germany) and analyzed on a Stratagene Mx3005P multiplex quantitative PCR system (Agilent Technologies, USA). For PCR, primers were obtained from Invitrogen Life Technologies (Shanghai, China). The

following primers were used for the amplification of PD-L1: 5′-CAATGTGACCAGCACACTGAGAA-3′ (forward) and 5′-GGCATAATAAGATGGCTCCCAGAA-3′ (reverse); and for GAPDH: 5′-GCGACACCCACTCCTCCACCTTT-3′ (forward) and 5′-TGCTGTAGCCAAATTCGTTGTCATA-3′ (reverse). The relative expression of PD-L1 was normalized to GAPDH, and $2^{-\Delta\Delta CT}$ values were normalized to control levels. All experiments were conducted in triplicate.

Cycloheximide chase assay

Cycloheximide, a protein synthesis inhibitor, was used to evaluate the stability of PD-L1. Cells were co-treated with or without osimertinib for 0–24 h. Proteins were extracted at the indicated time, and Western blot was performed to detect the expression of PD-L1 protein. The half-life of PD-L1 protein was calculated using GraphPad Prism software 6 (GraphPad Software, Inc, CA, USA).

Statistical analysis

Data in bar graphs are expressed as the mean±SD from three independent experiments. Statistical analysis was performed using the Student's unpaired *t*-test or one-way analysis of variance relative to the control groups. All statistical analyses were performed using the GraphPad Prism software 6 (GraphPad Software, Inc, CA, USA). A P value <0.05 was considered statistically significant.

Results

Osimertinib decreased PD-L1 expression in NCI-H1975 cells

EGFR T790M mutant NCI-H1975 cells were treated with different concentrations of osimertinib. After treatment for 24 h, osimertinib clearly inhibited EGFR phosphorylation and significantly reduced the protein levels of PD-L1 (Figure 1A). NCI-H1975 cells were then incubated with 125 nmol/L osimertinib for different durations (3, 6, 12 and 24 h). As shown in Figure 1B, osimertinib down-regulated PD-L1 expression at 6 h. Furthermore, immunofluorescence was used to localize PD-L1 in NCI-H1975 cells. Compared with the osimertinib-untreated group, cell membranes exhibited weak PD-L1 signals at 6 and 24 h (Figure 1C). Consistently, the reduction of PD-L1 on the membranes was confirmed further by flow cytometry after treatment with osimertinib for 6 and 24 h (Figure 1D). To exclude the massive suppression of PD-L1 mRNA and protein expression caused by cell death, we performed MTT assays to examine the cell viability after treatment with osimertinib. We found that osimertinib could not trigger cell death in NCI-H1975 cells at 6 h (Figure 1E), which was further verified in HCC827 cells (data not shown). In addition, the apoptosis inhibitor Z-VAD-FMK and the necroptosis inhibitor NSA failed to reverse the osimertinib-triggered decrease of PD-L1 in NCI-H1975 cells (Figure 1F). Collectively, these findings demonstrate that osimertinib reduces PD-L1 expression in NCI-H1975 cells independent of cell death.

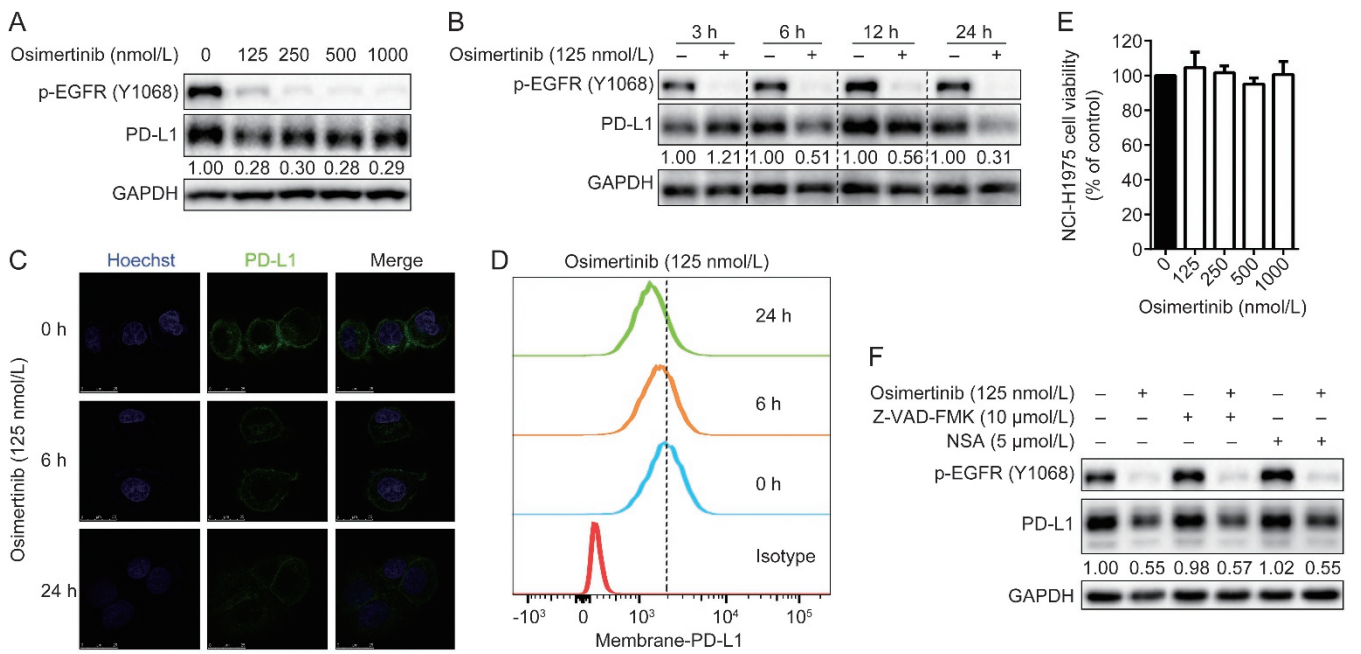

Figure 1. Osimertinib down-regulates PD-L1 expression in NCI-H1975 cells. (A) Western blot analysis of PD-L1 expression after NCI-H1975 cells were treated with different doses of osimertinib for 24 h. (B) The protein expression level of PD-L1 was determined by Western blot assays in NCI-H1975 cells after treatment with 125 nmol/L osimertinib for 3, 6, 12 and 24 h. (C) The localization of PD-L1 was determined by immunofluorescence after treatment with 125 nmol/L osimertinib for 6 h and 24 h in NCI-H1975 cells. Scale bar, 25 μm. (D) Membrane expression of PD-L1 on NCI-H1975 cells was evaluated by flow cytometry in the presence of osimertinib (125 nmol/L) for 6 h and 24 h. (E) NCI-H1975 cells were treated with different doses of osimertinib for 6 h, and cell viability was determined using the MTT assay. (F) Western blot assays were preformed to determine PD-L1 expression in NCI-H1975 cells that were pretreated with Z-VAD-FMK or NSA for 1 h, followed by treatment with osimertinib for 6 h.

Osimertinib down-regulated the mRNA levels of PD-L1

Previous studies indicated that gefitinib could decrease the expression of PD-L1 mRNA depending on the inhibition of EGFR activity[25, 26]. To investigate whether osimertinib could down-regulate PD-L1 mRNA levels, quantitative real-time PCR was used to detect the expression of PD-L1 mRNA after treatment with osimertinib. Similarly, osimertinib also caused greater than 70% reduction of PD-L1 mRNA levels in NCI-H1975 cells (Figure 2A). Recently, it was found that PD-L1 is a highly glycosylated protein with a long half-life in breast cancer cells[8]. To determine whether the down-regulation of PD-L1 mRNA levels affects the expression of its non-glycosylated form, tunicamycin, an N-linked glycosylation inhibitor, was used to disrupt glycosylation of PD-L1. As shown in Figure 2B, a significant portion of non-glycosylated PD-L1 appeared after treatment with tunicamycin for 6 h. Osimertinib clearly decreased the expression of non-glycosylated PD-L1 in NCI-H1975 cells, indicating that osimertinib reduces the production of new PD-L1 protein, which is likely because of the reduction of its mRNA. Furthermore, the expression of both mRNA levels and non-glycosylated PD-L1 was down-regulated after treatment with osimertinib in HCC827 cells, another EGFR mutant NSCLC cell line (Figure 2C and 2D).

Osimertinib caused PD-L1 protein degradation by proteasomes

Interestingly, osimertinib not only down-regulated the non-glycosylated PD-L1 after pretreatment with tunicamycin but also down-regulated its glycosylated form (Figure 2C and 2D); therefore, we questioned whether osimertinib also causes PD-L1 protein degradation. To test this hypothesis, the transcription inhibitor actinomycin D was used to block the formation of RNA. In the presence of actinomycin D, the expression of c-MYC with a short half-life was completely inhibited, and the glycosylated PD-L1 was diminished after treatment with osimertinib for 6 h in NCI-H1975 and HCC827 cells (Figure 3A and 3B), suggesting that it exerts post-transcriptional regulation. In addition, after pretreatment with cycloheximide, a protein synthesis inhibitor, osimertinib still reduced the PD-L1 expression, indicating that osimertinib indeed induced PD-L1 protein degradation (Figure 3C and 3D). Moreover, we performed cycloheximide chase assays to evaluate the half-life of PD-L1 protein when co-treated with or without osimertinib in the NCI-H1975 and HCC827 cell lines. The half-life of PD-L1 was approximately 17.8 h and 13.8 h in NCI-H1975 and HCC827 cells, respectively, while osimertinib clearly reduced its half-life to 8.6 h and 4.6 h, respectively (Figure 3E and 3F). Thus, these results verify that osimertinib induces PD-L1 pro-

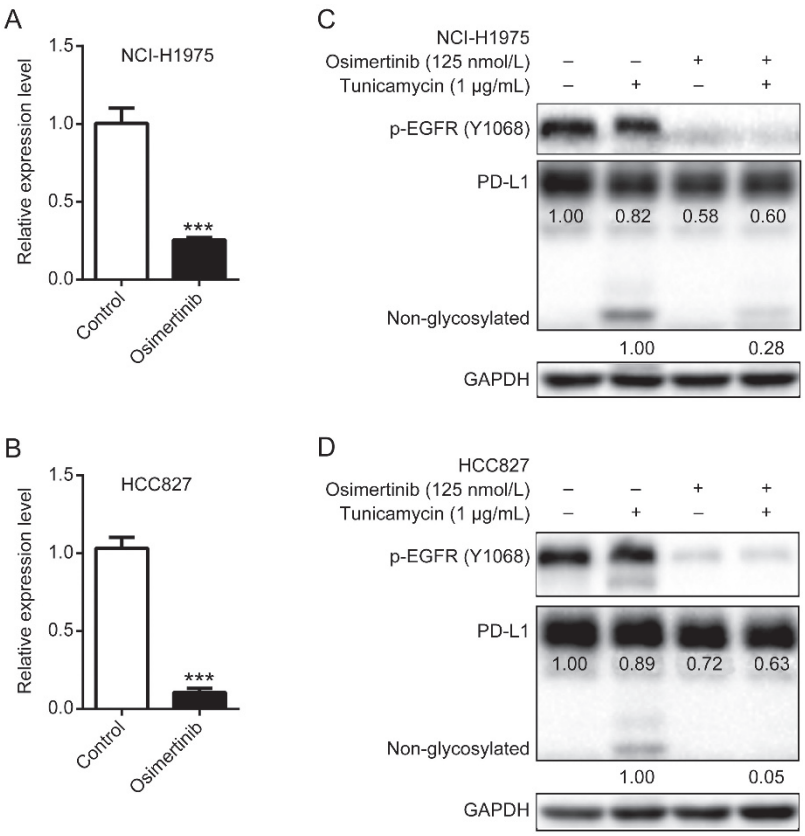

Figure 2. Osimertinib reduces the mRNA level of PD-L1. (A and B) The levels of PD-L1 mRNA in NCI-H1975 and HCC827 cells were determined by RT-qPCR after treatment with osimertinib for 6 h. $^{***}P<0.001$. (C and D) Western blot assays were preformed to evaluate the expression of non-glycosylated PD-L1 in NCI-H1975 and HCC827 cells that were pretreated with tunicamycin for 1 h, followed by treatment with osimertinib for 6 h.

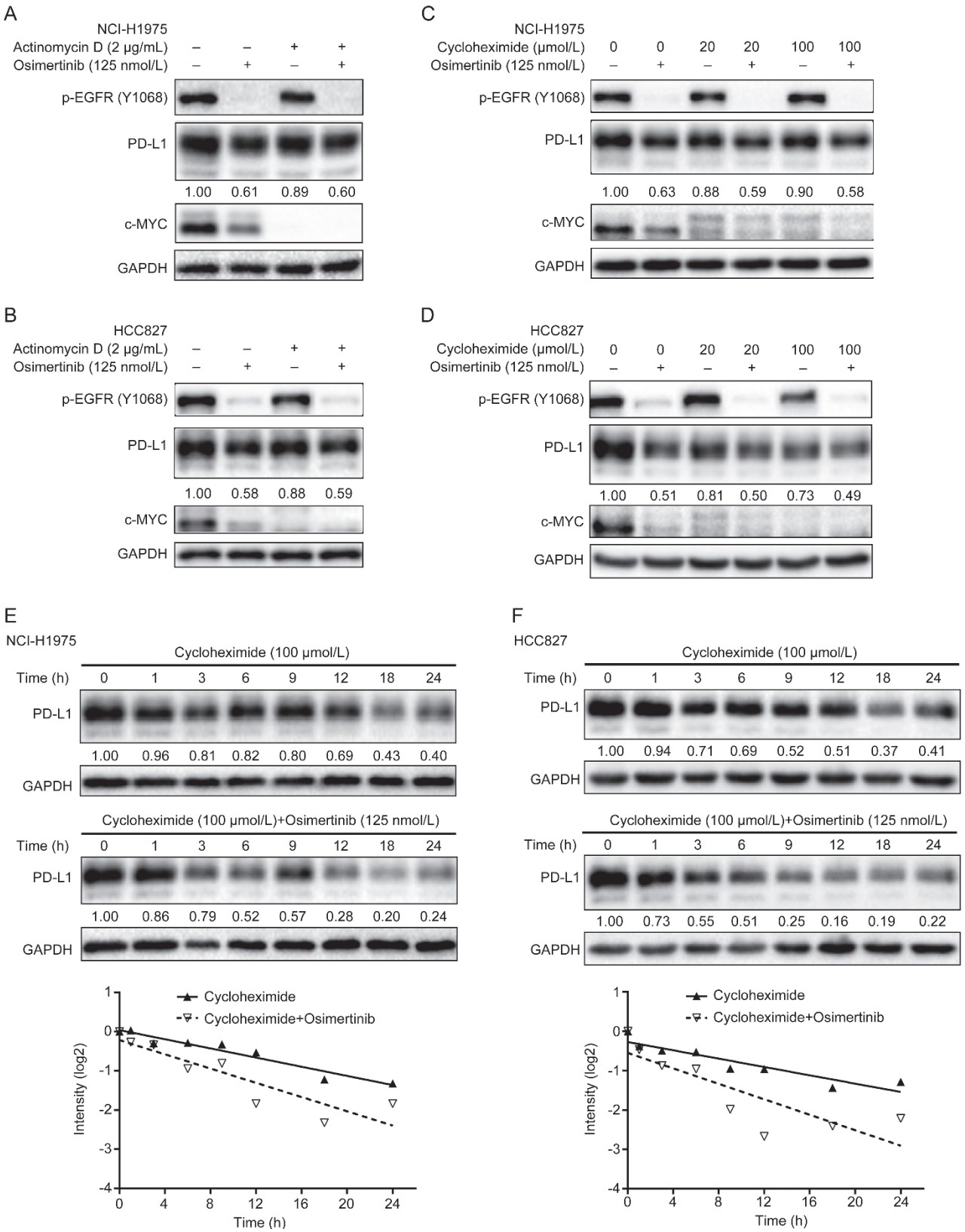

Figure 3. Osimertinib reduces PD-L1 protein at the post-translation level. (A and B) The protein expression levels of PD-L1 were detected by Western blot assays in NCI-H1975 and HCC827 cells pretreated with actinomycin D for 1 h followed by treatment with osimertinib for 6 h. (C and D) Western blot analysis of PD-L1 expression in NCI-H1975 and HCC827 cells pretreated with cycloheximide for 1 h, followed by treatment with osimertinib for 6 h. (E and F) Western blots were performed to detect PD-L1 protein expression in NCI-H1975 and HCC827 cells after cycloheximide and co-treatment with or without osimertinib for 0–24 h.

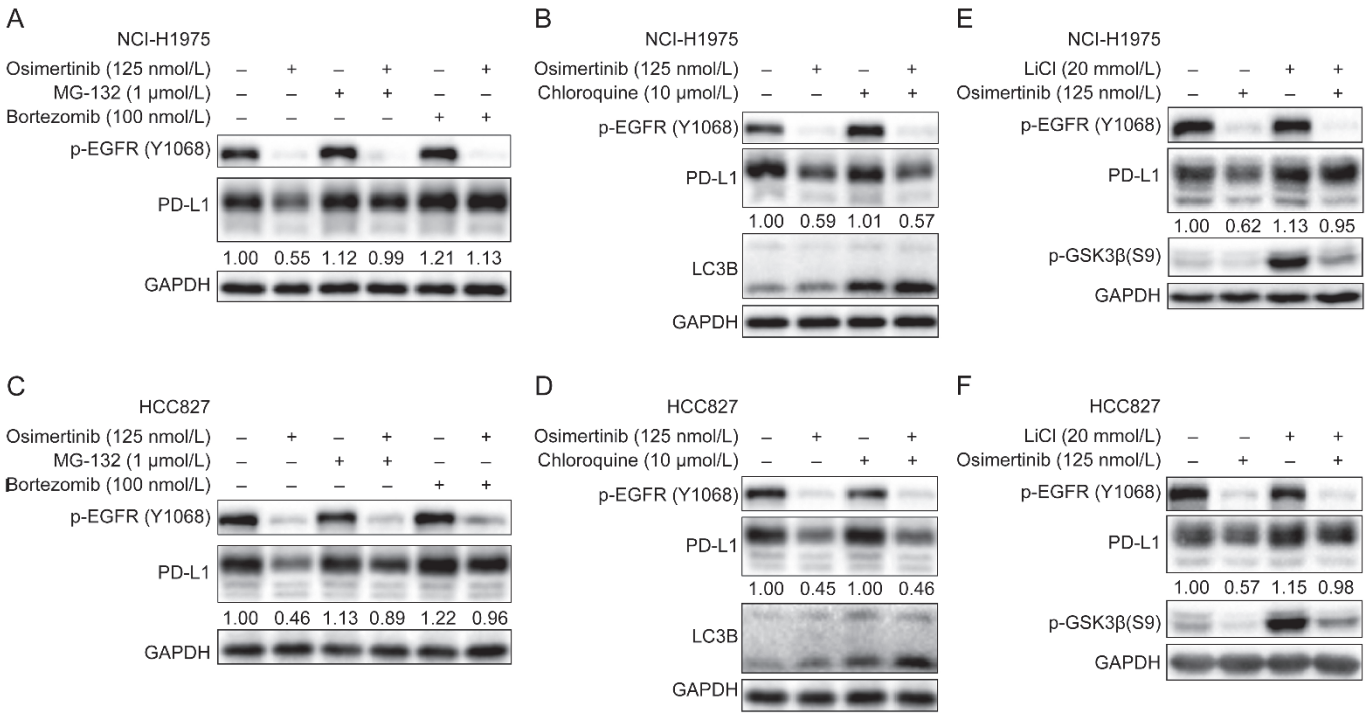

Figure 4. GSK3β mediates osimertinib-induced PD-L1 degradation via proteasomes. (A and C) Protein expression levels of PD-L1 were analyzed by Western blot analysis in NCI-H1975 and HCC827 cells treated with MG-132 and bortezomib for 1 h prior to 6 h of treatment with osimertinib. (B and D) Protein expression levels of PD-L1 were examined by Western blots in NCI-H1975 and HCC827 cells pretreated with chloroquine for 1 h followed by treatment with osimertinib for 6 h. (E and F) Western blot analysis of PD-L1 and p-GSK3β in NCI-H1975 and HCC827 cells that were treated with LiCl for 1 h prior to 6 h of treatment with osimertinib.

tein degradation.

As is known, two pathways, the ubiquitin–proteasome system and the lysosome, are involved in intercellular protein degradation[27]. To investigate which pathway modulates osimertinib-induced PD-L1 degradation, we treated cells with lysosome and proteasome inhibitors. As shown in Figure 4A, pretreatment with proteasome inhibitors (MG-132 and bortezomib) clearly reversed osimertinib-induced PD-L1 degradation compared with osimertinib alone. Although the lysosome inhibitor chloroquine disrupted lysosomal function, as supported by the up-regulation of LC3-II, it cannot attenuate PD-L1 degradation by osimertinib in NCI-H1975 cells (Figure 4B). Thus, these results indicate that osimertinib induces proteasomal degradation of PD-L1, which was further confirmed in HCC827 cells (Figure 4C and 4D).

GSK3β mediated osimertinib-induced PD-L1 degradation

It has been reported that glycogen synthase kinase 3β (GSK3β) could induce PD-L1 degradation via the proteasome pathway[8]. GSK3β is a serine/threonine protein kinase that was originally associated with glycogen metabolism. Importantly, GSK3β often phosphorylates a variety of substrates, such as c-MYC, that were recognized by ubiquitin E3 ligase for proteasomal degradation[8, 28]. In addition, EGFR mediates AKT activity to inhibit GSK3β activity by Ser9 phosphorylation[29]. In NCI-H1975 and HCC827 cells, inactivation of EGFR by osimertinib significantly activated GSK3β by its dephosphorylation. Moreover, osimertinib-induced PD-L1 down-regulation could be clearly attenuated by pretreatment with the GSK3β inhibitor LiCl (Figure 4E and 4F). Collectively, these results indicate that osimertinib induces proteasomal degradation of PD-L1 that is mediated by GSK3β.

Inhibition of EGFR signaling by EGFR TKI-induced PD-L1 degradation by proteasomes

Similar to the osimertinib-induced degradation of PD-L1, inhibition of the EGFR pathway by gefitinib destabilized PD-L1 and enhanced anti-tumor T cell immunity in breast cancer syngeneic mouse models, prompting us to propose a hypothesis that the inhibition of EGFR activity by other EGFR TKIs also promoted proteasome degradation of PD-L1. To this end, several EGFR TKIs (rociletinib, gefitinib and erlotinib) were used to treat EGFR mutant NSCLC cells. In EGFR TKI-sensitive HCC827 cells, all of the EGFR TKIs down-regulated PD-L1 expression (Figure 5A-5C). Because EGFR T790M mutant NCI-H1975 cells are insensitive to the first-generation EGFR TKIs (gefitinib and erlotinib), they cannot inhibit EGFR activity and decrease PD-L1 expression, while rociletinib can (Figure 5D-5F). Additionally, bortezomib clearly diminished EGFR TKI-mediated PD-L1 degradation (Figure 5A-5D), indicating that the inhibition of EGFR activity by EGFR TKIs induces proteasomal degradation of PD-L1 in EGFR mutant

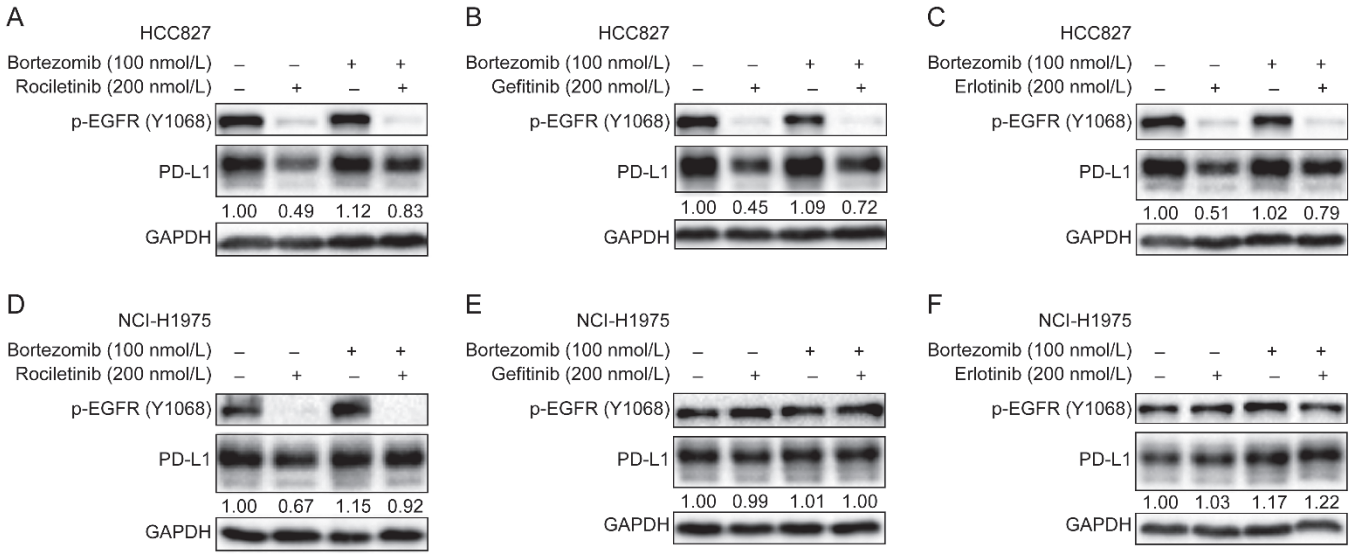

Figure 5. EGFR TKIs causes PD-L1 degradation by proteasomes. (A to F) Western blot analysis of PD-L1 in NCI-H1975 and HCC827 cells pretreated with bortezomib for 1 h, followed by treatment with rociletinib, gefitinib and erlotinib for 6 h.

NSCLC cells.

Discussion

Osimertinib is a third-generation EGFR-TKI and has been approved for the treatment of EGFR T790M mutant NSCLC patients. Compared to second-line standard chemotherapy, osimertinib exhibited superior progression-free survival and safety profiles for EGFR T790M positive NSCLC patients whose disease had progressed following first-line EGFR TKI therapy[30]. Moreover, the duration of response, objective response rates and disease control rates also achieved significant improvement compared to chemotherapy. Furthermore, there is an ongoing phase III study of the safety and efficacy of osimertinib compared to gefitinib and erlotinib as first-line treatments in patients with EGFR mutant NSCLC (NCT02296125). Clinical studies indicated that high levels of expression of PD-L1 were likely in EGFR mutant NSCLC patients[21-23]. PD-L1 could be located on the surface of immune cells as well as tumor cells and interacts with the T cell receptor PD-1 to help tumors evade immune destruction[6, 7]. Here, we demonstrated for the first time that inhibition of EGFR activity by osimertinib not only decreased PD-L1 mRNA expression but also caused PD-L1 degradation via the proteasome pathway in EGFR-driven NSCLC cells (Figure 6).

Previous studies have shown that several transcriptional factors could regulate PD-L1 expression. Casey *et al* reported that MYC directly regulated PD-L1 mRNA expression in human tumor and mouse tumor cells[20]. Although osimertinib significantly reduced MYC expression, knock-down of MYC could not decrease PD-L1 mRNA and protein expression in NCI-H1975 and HCC827 cells (data not shown), which was in line with another study [31]. Moreover, p65 was involved in the gefitinib-induced reduction of PD-L1 mRNA expression in EGFR-driven NSCLC cells, which was inconsistent with the effect of osimertinib as supported by the p65 signaling inhibitor (data not shown). In addition, it was reported that the signal transducer and activator of transcription 3 (STAT3) signaling pathway could regulate PD-L1 expression in both EGFR-mutated[26] and ALK positive NSCLC[19]. However, we demonstrated that osimertinib could inhibit STAT3 signaling in HCC827 cells but not, remarkably, in NCI-H1975 cells (data not shown), suggesting that STAT3 is not a key transcriptional factor in the osimertinib-triggered down-regulation of PD-L1 mRNA expression. Although we indicated that osimertinib indeed decreased the mRNA level of PD-L1, the transcriptional factor that is involved in this process needs to be studied further.

With the inhibition of transcription or translation processes, osimertinib still reduced PD-L1 expression, demonstrating that osimertinib might induce PD-L1 protein degradation. In the tumor microenvironment, tumor necrosis factor alpha induced PD-L1 stabilization via transactivation of COP9 signalosome subunit 5 (CSN5) by p65, while inhibition of CSN5 by curcumin caused ubiquitination and degradation of PD-L1[32]. Lastwika *et al* showed that inhibition of AKT-mTOR signaling increased the lysosomal degradation of PD-L1[33]. However, we found that the lysosome inhibitor chloroquine cannot reverse PD-L1 degradation, though treatment with high concentration of osimertinib induced autophagy in EGFR-driven NSCLC cells[34]. Inconsistently, it was reported that GSK3β-mediated PD-L1 degradation through the ubiquitin–proteasome system[8] and that GSK3β inactivation was required for PARP inhibitor-induced PD-L1 up-regulation[35]. Similarly, our experiments demonstrated that osimertinib-induced PD-L1 degradation could be reversed by proteasome inhibitors. Moreover, inactivation of GSK3β by LiCl attenuated the osimertinib-induced down-regulation of PD-L1. Collectively, osimertinib activated GSK3β and induced PD-L1 degradation

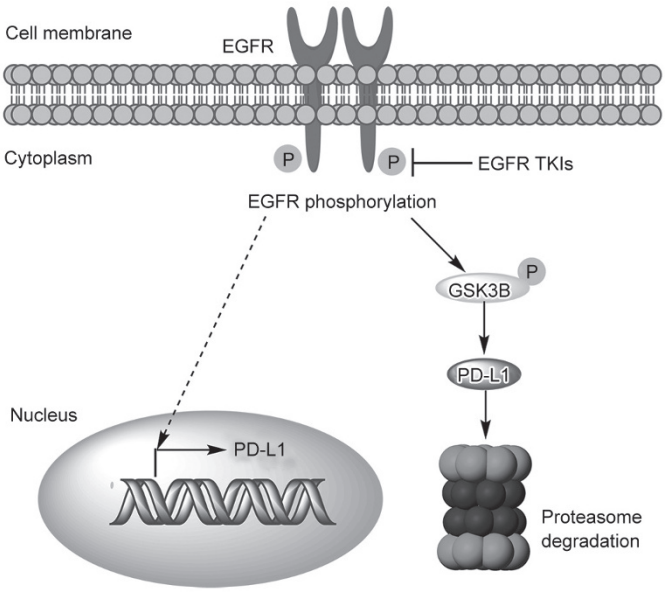

Figure 6. Schematic representation of the proposed molecular regulatory mechanisms of osimertinib-mediated PD-L1 expression in EGFR-driven NSCLC cells. Inhibition of p-EGFR by osimertinib not only causes reduction of PD-L1 mRNA but also activates GSK3β, resulting in PD-L1 degradation by the proteasome pathway.

by proteasomes.

In summary, we demonstrated that the inactivation of EGFR by osimertinib reduced PD-L1 by two mechanisms in EGFR mutant NSCLC cells. Specifically, osimertinib caused significant down-regulation of PD-L1 mRNA expression and induced the proteasomal degradation of PD-L1. However, the function of osimertinib-modulated PD-L1 reduction in the tumor microenvironment requires further study.

Acknowledgements

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Author contribution

Xiao-Ming JIANG and Jin-Jian LU designed the research; Xiao-Ming JIANG, Yu-Lian XU and Mu-Yang HUANG performed the experiments; Xiao-Ming JIANG, Jin-Jian LU, Le-Le ZHANG, Min-Xia SU and Xiuping CHEN contributed to data analysis; Xiao-Ming JIANG and Jin-Jian LU wrote the manuscript. All authors provided final approval of the manuscript.

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