**MET-Oncogenic and JAK2-Inactivating Alterations Are Independent Factors That Affect Regulation of PD-L1 Expression in Lung Cancer**

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**Abstract**

**Purpose:** The blockade of immune checkpoints such as PD-L1 and PD-1 is being exploited therapeutically in several types of malignancies. Here, we aimed to understand the contribution of the genetics of lung cancer to the ability of tumor cells to escape immunosurveillance checkpoints.

**Experimental Design:** More than 150 primary non–small cell lung cancers, including pulmonary sarcomatoid carcinomas, were tested for levels of the HLA-I complex, PD-L1, tumor-infiltrating CD8⁺ lymphocytes, and alterations in main lung cancer genes. Correlations were validated in cancer cell lines using appropriate treatments to activate or inhibit selected pathways. We also performed RNA sequencing to assess changes in gene expression after these treatments.

**Results:** MET-oncogenic activation tended to associate with positive PD-L1 immunostaining, whereas STK11 mutations were correlated with negative immunostaining. In MET-altered cancer cells, MET triggered a transcriptional increase of PD-L1 that was independent of the IFNγ-mediated JAK/STAT pathway. The activation of MET also upregulated other immunosuppressive genes (PDCD1LG2 and SOCS1) and transcripts involved in angiogenesis (VEGFA and NRP1) and in cell proliferation. We also report recurrent inactivating mutations in JAK2 that co-occur with alterations in MET and STK11, which prevented the induction of immune-response-related genes following treatment with IFNγ.

**Conclusions:** We show that MET activation promotes the expression of several negative checkpoint regulators of the immune response, including PD-L1. In addition, we report inactivation of JAK2 in lung cancer cells that prevented the response to IFNγ. These alterations are likely to facilitate tumor growth by enabling immune tolerance and may affect the response to immune checkpoint inhibitors. Clin Cancer Res. 24(18): 4579–87. ©2018 AACR.

**Introduction**

A hallmark of cancer biology is the ability of tumors to avoid host immune surveillance. This characteristic has turned out to be therapeutically exploitable in novel immunotherapies (1).

Several alterations in cancer cells are known to impair immune surveillance. These include defects in tumor immunorecognition, such as downregulation of the HLA-I complex due to gene alterations of its components (either B2M or HLA-A) or at molecules involved in its maturation process (e.g., CALR, TAP1/2, and TAPBP, refs. 2–4). An abnormal increase in the expression of negative controllers of the immune response, such as constitutively high levels of programmed death ligand-1 (PD-L1) expression, also contributes to immune tolerance (1).

The programmed cell death-1 (PD-1) receptor is expressed in activated T and B cells and regulates the cellular and humoral immune responses by interacting with its cognate ligand, PD-L1, which is expressed in the macrophage lineage and in some carcinomas, including lung cancer (5). The interaction of PD-1 with PD-L1 attenuates the immune response by inhibiting T-cell proliferation and functions (1, 5). The blockade of these checkpoints is the means by which therapeutic agents are intended to enhance tumor immunologic responses. In non–small cell lung cancer (NSCLC), anti–PD-1/PD-L1 therapies have yielded clinical responses and increases in overall survival in subsets of patients (6–8). This clinical benefit makes it crucial to identify factors that determine the sensitivity or refractoriness to immune checkpoint inhibitors (ICIs). Several functionally interrelated tumor biomarkers have been proposed as predictors of the response to ICIs, including high tumor mutational load, DNA mismatch repair deficiency, strong intratumoral T-cell infiltration,
Translational Relevance

The ability of tumors to avoid host immune surveillance through the blockade of PD-L1 and PD-1 is being exploited therapeutically. However, few biomarkers are currently well-established predictors of the response to these treatments. Here we found that activation of MET in lung cancer triggers a transcriptional increase of PD-L1. This was framed within a more generic transcriptional activation of immunosuppression-related molecules. We also report recurrent inactivating mutations at JAK2 that impair the upregulation of immunoresponsiveness-related transcripts when treated with IFNγ. Therefore, MET and JAK2 alterations in lung cancer contribute to promote immunotolerance and may constitute biomarkers for predicting responsiveness to immunotherapies in this type of cancer.

Materials and Methods

Tumor samples and cancer cell lines

Tumor specimens were collected from patients newly diagnosed with NSCLC at the time of surgical resection from the Tumour Bank of the Spanish National Cancer Research Centre and from the Hopital Universitaire de Grenoble. Informed consent was obtained from patients, and the study was approved by the respective institutional review boards. Patient studies were conducted according to the Declaration of Helsinki, the Belmont Report, and the U.S. Common Rule. Information about tumors and patients is presented in Supplementary Table S1. Genetic analytical data were collated from our previous studies (19) and were used as well-established predictors of the response to these treatments. We considered PD-L1 positivity when ≥5% of the tumor cells showed membranous staining. To determine the degree of CD8 T-lymphocyte intratumoral infiltration (TIL), we followed some of the previously defined guidelines (13). For immunofluorescence, cells were fixed in a 4% formaldehyde solution (Electron Microscopy Sciences) and blocked with 20% goat serum. Samples were kept with primary antibodies overnight at 4°C, washed, and incubated with 1:500 diluted Alexa Fluor-488 and -568 fluorochrome-conjugated secondary antibody (Life Technologies) for 1 hour. Cell nuclei were stained with DAPI (0.1 μg/μL).

Antibodies, Western blots, and immunostainings

References concerning the antibodies and scoring systems used, as well as the details and concentrations of the various treatments are presented in Supplementary Table S2. Western blots and immunohistochemistry were performed using previously described protocols (4). We used previously described criteria to determine B2M, HLA-I, and PD-L1 levels by immunohistochemistry (4). We considered PD-L1 positivity when ≥5% of the tumor cells was stained. All statistical analyses were performed in R using the R package (40). The Student test (or Fisher exact test). Continuous variables were summarized as means and standard deviations, and group differences examined with two-tailed paired Student t tests, as appropriate. We considered any tests to be significant for values of P < 0.05.
Results
Wild-type STK11 and activated MET tumors are associated with positive PD-L1 protein

We investigated correlations between the genetic profile and immunologically related markers in a cohort of 155 primary NSCLCs, including 24 pulmonary sarcomatoid carcinomas (PSC). This information included the genetic status for bona fide oncogenes and tumor-suppressor genes and the immunostaining of various proteins involved in immunorespose, i.e., the PD-L1, TILs, and HLA-I and B2M proteins (HLA-I complex; refs. 4, 20–22). We found a mutually exclusive presence of alterations in the KRAS, EGFR, MET, and ALK oncogenes, but one tumor bore simultaneous ERBB2 amplification and MET exon 14 skipping (METex14) mutation (Fig. 1A; ref. 22). As we previously reported (4), a statistically significant correlation was observed between a positive PD-L1 and high levels of TILs and B2M/HLA-I staining in the entire cohort (Table 1; Fig. 1B). Tumors harboring MET activation were more likely to be positive for PD-L1 staining (P = 0.05). In lung adenocarcinomas (LAD), STK11 (hereafter, LKB1 for its protein product) mutations were correlated with a negative PD-L1 (P = 0.012) and showed low levels of TILs. The observations were validated using publicly available data from a TCGA cohort of primary LADs (Fig. 1C). The levels of the PD-L1 transcript (hereafter CD274) were reduced in tumors exhibiting STK11 inactivation (P = 0.0001) and increased in tumors with MET activation (P = 0.001; Fig. 1C).

The strong association between STK11 inactivation and low PD-L1 levels prompted us to investigate a possible functional relationship. We restituted LKB1 expression in five LKB1-deficient lung cancer cells but found no changes in the abundance of PD-L1 (Supplementary Fig. S1A). The signaling through IFNγ, a cytokine that induces immunorespose and upregulates PD-L1 through the JAK/STAT pathway (6, 23), did not show any dependence on LKB1. A proficient immunorespose requires the expression and correct positioning of the HLA-I complex on the cell surface (1). Here, we were also able to discount the possibility that the lack of LKB1 affects the expression or localization of the HLA-I complex component, B2M (Supplementary Fig. S1B and S1C). Hence, LKB1 is not directly involved in the transcriptional regulation of PD-L1 or in the appropriate expression or localization of the HLA-I complex.

MET activation promotes an increase of PD-L1 expression levels

We sought, at the molecular level, a possible role for MET in regulating PD-L1 levels. We selected six cancer cell lines with different MET status, including MET amplification, METex14 mutations, and MET wild-type (Fig. 2A). METex14 mutations are known to increase MET protein stability, which have oncogenic potential and confer sensitivity to crizotinib, a MET-tyrosine kinase inhibitor (24, 25).

First, we tested the levels of phosphoMET (pMET), MET, and PD-L1 in these cells. Here, it is worth mentioning that the Western blot of PD-L1 distinguished two separate bands, the most

Figure 1.
Correlation between gene alterations and protein levels of immunorespose-related proteins. A, Oncoplot of the presence of alterations in the indicated genes and the levels of the indicated proteins, determined by immunohistochemistry. LADs, lung adenocarcinomas; LSCCs, lung squamous cell carcinomas; PSC, pulmonary sarcomatoid carcinomas. B, Representative negative and strong immunostaining of CD8 (scale bar, 100 μm), PD-L1 (scale bar, 50 μm), HLA-I (scale bar, 50 μm), and B2M (scale bar, 50 μm) in lung primary tumors. C, mRNA levels of CD274 (PD-L1) in a cohort of LADs gathered from the TCGA provisional, according to the status of STK11 (inactivating mutations and homozygous deletions) and of MET (METex14 mutations and gene amplification). Probabilities are those associated with a two-tailed Student t test.
Table 1. Distribution of the immunostaining of PD-L1 among lung tumors, by histopathology and genetic and molecular characteristics.

<table>
<thead>
<tr>
<th>All histopathologies</th>
<th>PD-L1 negative</th>
<th>PD-L1 positive</th>
<th>P</th>
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<tr>
<td>All LSCCs</td>
<td>155</td>
<td>79 (51%)</td>
<td>76 (49%)</td>
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<tr>
<td>GA or METext14</td>
<td>7 (5%)</td>
<td>1 (7%)</td>
<td>6 (4%)</td>
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<tr>
<td>Wild-type</td>
<td>13 (95%)</td>
<td>71 (52%)</td>
<td>60 (43%)</td>
</tr>
<tr>
<td>HLA-I/B2M</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strong</td>
<td>48 (36%)</td>
<td>18 (33%)</td>
<td>30 (22%)</td>
</tr>
<tr>
<td>Weak</td>
<td>29 (22%)</td>
<td>10 (8%)</td>
<td>19 (14%)</td>
</tr>
<tr>
<td>Negative</td>
<td>57 (42%)</td>
<td>41 (31%)</td>
<td>16 (12%)</td>
</tr>
<tr>
<td>CD8/TILs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>43 (38%)</td>
<td>14 (12%)</td>
<td>29 (25%)</td>
</tr>
<tr>
<td>Low</td>
<td>71 (62%)</td>
<td>43 (38%)</td>
<td>28 (25%)</td>
</tr>
<tr>
<td>All LADs</td>
<td>59</td>
<td>30 (51%)</td>
<td>29 (49%)</td>
</tr>
<tr>
<td>KRAS</td>
<td></td>
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<tr>
<td>Mutant</td>
<td>14 (20%)</td>
<td>8 (14%)</td>
<td>6 (10%)</td>
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<tr>
<td>Wild-type</td>
<td>45 (80%)</td>
<td>23 (47%)</td>
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<td>6 (11%)</td>
<td>2 (4%)</td>
<td>4 (7%)</td>
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<tr>
<td>Wild-type</td>
<td>49 (89%)</td>
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<td>9 (16%)</td>
<td>1 (2%)</td>
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<tr>
<td>Wild-type</td>
<td>46 (82%)</td>
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<td>26 (46%)</td>
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<tr>
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<td>4 (7%)</td>
<td>3 (5%)</td>
<td>1 (2%)</td>
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<tr>
<td>Wild-type</td>
<td>55 (93%)</td>
<td>27 (46%)</td>
<td>28 (47%)</td>
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<tr>
<td>All LSCCs</td>
<td>62</td>
<td>35 (57%)</td>
<td>27 (43%)</td>
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<tr>
<td>FGFR1</td>
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<td>GA</td>
<td>9 (15%)</td>
<td>7 (12%)</td>
<td>2 (5%)</td>
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<tr>
<td>Wild-type</td>
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<td>27 (45%)</td>
<td>24 (40%)</td>
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<tr>
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<td>1 (2%)</td>
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<tr>
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<td>25 (47%)</td>
<td>24 (44%)</td>
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<tr>
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<td>17 (71%)</td>
</tr>
<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>Mutant</td>
<td>4 (23%)</td>
<td>0 (0%)</td>
<td>4 (26%)</td>
</tr>
<tr>
<td>Wild-type</td>
<td>13 (77%)</td>
<td>6 (37%)</td>
<td>6 (37%)</td>
</tr>
</tbody>
</table>

NOTE: Nonevaluable cases are not included in the table.

Abbreviations: GA, gene amplification; LADs, lung adenocarcinomas; LSCCs, lung squamous cell carcinomas; PSCs, pulmonary sarcomatoid carcinomas.

abundant at 45 kDa and the other at 33 kDa. Both correspond to the PD-L1 protein, and the upper band corresponds to an extensively N-glycosylated protein (26). The H596, Hs746T, and EBC1 cells had strong basal PD-L1, whereas the remaining three had low (H1993) to undetectable levels of PD-L1 protein (Fig. 2B). The two cell lines with undetectable PD-L1 were the H1437, which is MET wild-type, and the H1573, which has moderate pMET/MET protein levels.

As previously described, pMET was stimulated in the H596 cells by treatment with HGF (24). We observed that administration of HGF also stimulated PD-L1 levels in an equivalent manner to the upregulation triggered by IFNγ (Fig. 2C). In both cases, there was an increase of the 33-kDa band at 6 hours, which progressively disappeared in favor of the 45-kDa band (24 and 36 hours), presumably due to the accumulation of N-glycosylation. HGF barely increased the levels of pMET or PD-L1 in the MET-amplified and MET-independent Hs746T and EBC1 cells (Fig. 2D). In both cells, the administration of IFNγ upregulated PD-L1 levels, especially the 33-kDa band. In the case of the H1993 cells, which harbor MET amplification, although the basal levels of PD-L1 protein were low, there was an increase upon administration of HGF (Fig. 2D). The treatment with crizotinib prevented the MET-dependent upregulation of PD-L1 in all these cells. However, in the MET wild-type H1437 cells, PD-L1 expression was triggered only upon IFNγ stimulation. Interestingly, the H1993 and H1573 cells did not increase PD-L1 levels in response to IFNγ. We noticed that these cells carry a genetic inactivation of the Janus kinase 2, JAK2 (COSMIC and CCLE databases). Such mutations constitute a mechanism for acquired resistance to ICI and confer lack of reactive PD-L1 expression in melanoma patients (10, 11).

We also performed immunofluorescence that validated the observations and provided evidence of increased levels of PD-L1 on the cell surface (Fig. 2E). Finally, we measured the levels of CD274 and found that the effects of MET activity upon the levels of PD-L1 occur through transcriptional regulation (Fig. 2F).

In order to discard that the effect of crizotinib upon the levels of PD-L1 was unspecific, we depleted the expression of MET, using shRNAs. Compared with scramble RNA, depletion of MET with the different shMET did decrease the levels of PD-L1 in the EBC1 cells or prevented the upregulation of PD-L1 following treatment with HGF, in the H596 cells, further supporting the existence of a MET-dependent mechanism that upregulates PD-L1 levels in these cells (Fig. 2C).

MET-dependent regulation of PD-L1 expression is independent of EGFR signaling but is mediated by AKT

We had previously observed that genetic activation of MET triggers activation of EGFR in EBC1 cells (27). Because it has been proposed that oncogenic EGFR promotes an increase in PD-L1 levels (14, 16), we decided to test whether the effects of MET on the expression of PD-L1 in the EBC1 and H596 cells are mediated by EGFR activity. The EBC1 cells had high basal levels of active EGFR, measured by the presence of phosphorylated EGFR at Tyr1068 (pEGFR) and the administration of EGFR did not increase pEGFR or PD-L1 levels (Fig. 3A). Consistent with our previous observations (27), crizotinib leads to a strong decrease in pEGFR, suggesting that EGFR phosphorylation is mediated by MET activity in these cells, either directly or through downstream targets. In the HGF-dependent H596 cells, the activation of EGFR was dependent on EGFR, but the levels of PD-L1 remained unaltered. Further, the treatment with erlotinib, an EGFR inhibitor, did not decrease PD-L1 in any of the cells. These observations indicate that MET-dependent upregulation of PD-L1 in cancer cells is not mediated by EGFR.

A recent study shows that the PD-L1 expression is controlled by the AKT/mTOR pathway (28). Because AKT is a signal transduction molecule acting downstream of the MET receptor (24, 27), we decided to examine whether the effects of MET on PD-L1 expression were mediated by AKT. We treated the H596 and EBC1 cells with uprosertib, an ATP-competitive-AKT inhibitor. The inhibition of the AKT kinase activity by uprosertib was demonstrated by the strong decreased in the phosphorylation levels of its direct target, GSK3B (Fig. 3B). An increase of pAKT levels could also be observed, likely due to feedback mechanisms, as described previously (29). Pharmacologic inhibition of AKT led to a decrease in PD-L1 protein levels, supporting that AKT mediates the MET-dependent upregulation of PD-L1 expression, in these cells.

MET-dependent regulation of PD-L1 expression is independent of the JAK2 pathway

The binding of IFNγ to its cognate receptor activates JAK1 and JAK2 and the signal transducers and activators of...
transcription (STAT), resulting in changes in the expression of a large set of genes, including PD-L1 (30). We explored a possible common node between the MET and the IFNγ pathways in the upregulation of PD-L1. As expected, the administration of IFNγ triggered an increase of pSTAT1 in the MET-activated and IFNγ-responsive H596 and EBC1 cells, but not in the JAK2-mutant, H1993 (Fig. 3B; ref. 30). Treatment with ruxolitinib, a JAK1/JAK2 inhibitor that targets the ATP-binding pocket (31), inhibited pSTAT1 and prevented IFNγ-dependent, but not MET-dependent, upregulation of PD-L1. Ruxolitinib, nonetheless, did not affect the levels of pSTAT1 or PD-L1 in the H1993 cells. Intriguingly, these cells had basal pSTAT1 that was inhibited by crizotinib treatment, suggesting that MET is activating STAT1 in these cells (Fig. 3C). RT-QPCR confirmed that the changes in PD-L1 were due to transcriptional regulation (Fig. 3D). These observations indicate that, in MET-activated cancer cells, MET-dependent upregulation of PD-L1 appears to be independent of the JAK2 pathway.
MET-dependent increase in PD-L1 expression levels in cancer cells with MET activation is independent of the EGFR or JAK2 pathways. A, Western blot of the indicated proteins and cell lines upon treatment with HGF, EGF, IFNγ, crizotinib, erlotinib, or various combinations for 6 hours. ACTIN and TUBULIN, protein-loading controls. B, Western blot of the indicated proteins and cell lines upon treatment with HGF (50 nmol/L), crizotinib (crizo; 100 nmol/L), uprosertib (upro; 200 nmol/L), or combinations for 6 hours. ACTIN and TUBULIN, protein-loading controls. C, Western blot of the indicated proteins and cell lines upon treatment with HGF (50 nmol/L), crizotinib (crizo; 100 nmol/L), erlotinib (erlo; 100 nmol/L), IFNγ (50 nmol/L), ruxolitinib (ruxol; 500 nmol/L), or combinations for 6 hours. ACTIN and TUBULIN, protein-loading controls. Below, quantification of the PD-L1 protein levels using densitometry analysis (DU, densitometry units). D, mRNA levels assessed by real-time quantitative PCR of CD274 (relative to ACTIN) in the indicated cell lines in basal condition and after administration of the treatments, as in C. Results are presented as the median of independent biological triplicates. E, Profile of gene alterations, including some coding for proteins involved in immunorecognition and IFNγ response, in a panel of lung cancer cell lines (see also Supplementary Fig. S4). Black and white squares indicate alterations and wild-type genes, respectively. The sensitivity or refractoriness to IFNγ is also indicated for some of the cells. *, Partial response to IFNγ (increase of pSTAT1 but not of PD-L1 levels). Asterisks denote statistical significance ( *, P < 0.05; **, P < 0.01; ***, P < 0.005; ****, P < 0.0001) from two-tailed Student’s t tests. Data are presented as mean ± SD. Comparisons: basal conditions versus treated counterparts, HGF-treated cells versus treated counterparts, or IFNγ-treated cells versus treated counterparts.

Saigi et al.

The two IFNγ-refractory lung cancer cell lines in our study exhibited inactivation of JAK2. We explored public databases (cBioportal, CCLE, and COSMIC) and found JAK2 mutations in about 3% of primary NSCLCs, half of them truncating alterations (Supplementary Fig. S3). We found additional lung cancer cell lines with homozygous JAK2 alterations, some of which were validated by Sanger sequencing (Supplementary Table S4; Supplementary Fig. S3). JAK2 mutations were concomitant with alterations at other cancer genes, including STK11 and MET, but not with genetic deficiencies at antigen presentation-related proteins (i.e., B2M, HLA-A, PDIA3, CALR, and TAP1; ref. 4; Fig. 3E). Two mutations at JAK1 were also found, although these were heterozygous and, thus, of uncertain relevance. The response to IFNγ, measured as the ability to increase pSTAT1 and to increase PD-L1 protein levels, was impaired in the JAK2-mutant cells but not in lung cancer cells with alterations at genes involved in immunorecognition (Supplementary Fig. S4; Fig. 3E). Of particular interest were the H23 cells, which are JAK2 wild-type but partially responsive to IFNγ (an increase in pSTAT1 but not in PD-L1).

MET activation enhances the expression of immunosuppressive cytokines

Treatment with IFNγ triggers the transcriptional activation of genes involved in antigen presentation, the production of chemokines that attract T cells and the promotion of tumor growth inhibition and apoptosis (30). In this context, the upregulation of PD-L1 by IFNγ is part of a feedback inhibition that ensures a controlled immunologic response. Here, we compared the changes in gene expression following MET activation and IFNγ treatment in lung cancer cells to identify possible shared nodes in the upregulation of PD-L1. We performed RNA sequencing of the H596, EBC1, H1993, and H1573 cells, subjected to IFNγ, HGF, and/or crizotinib treatments. Unsuservised hierarchical clustering segregated cells on the basis of cell identity (Fig. 4A). Further, administration of IFNγ generated an additional independent cluster in the H596 and EBC1 cells, but not in H1993 and H1573 cells, providing further evidence of the IFNγ-unresponsive nature of the latter two cell lines. Next, we established the MET activation (MET-Sign) and the IFNγ treatment (IFN-Sign) signatures (Fig. 4B;...
Supplementary Table S6) to identify shared patterns in gene expression. The MET-Sign was enriched in genes involved in proliferation (e.g., MYC and FOSL1) and the processes of angiogenesis and response to wounding (e.g., VEGFA and NRP1), whereas the IFN-Sign mainly consisted of immunoresponse- and inflammation-related genes (Supplementary Fig. S5). The MET-Sign includes the upregulation of additional negative regulators of immunoresponse processes, such as PDCD1LG2 (PD-L2) and suppressor of the cytokine signaling family (SOCS1), which were also upregulated in the IFN-Sign (Fig. 4C). However, none of the proimmunoresponse transcripts found in the IFN-Sign were present in the MET-Sign. Collectively, these observations suggest that MET activation promotes the transcriptional activation of multiple inhibitory checkpoints that attenuate the immunoresponse.

Discussion

Our results indicate that at least two independent mechanisms regulate PD-L1 expression levels in lung cancer cells: one extrinsic, through IFNγ, and the other intrinsic, through MET activation. At present, information about the involvement of MET activity in the control of the immunoresponse is scarce. High levels of MET activity were correlated with high PD-L1 levels in renal cell carcinoma (32), and acquired MET amplification, in erlotinib-resistant NSCLC cells, was associated with an increase in PD-L1
Here, we demonstrate that, in MET-activated cancer cells, MET promotes PD-L1 expression in a cell-autonomous manner and independently of EGFR and JAK2. The activation of MET induced the expression of negative controllers of the immunoresponsive (i.e., CD274, SOCS1, and PDCD1LG2), and of angiogenesis and vasculogenesis factors (VEGFA and NRP1), essential for establishing immunosuppression (1). Some of these negative controllers of immunoresponse were also upregulated by IFNγ, although in this case it is reactive to ensure a controlled immunoresponse (6). As opposed to the IFN-γ-Sign, no positive regulators of the immunoresponse were present in the MET-Sign (1, 30).

Altogether, our observations suggest that establishing an immunosuppressive environment is one of the oncogenic features of MET. However, despite exhibiting high PD-L1 levels, preliminary clinical observations reported that IC1 treatment is ineffective in METex14 NSCLCs, whereas targeting MET yields better outcomes (34). Thus, endeavors to determine how MET activation (both MET gene amplification and METex14 mutations) affects the response to immunotherapy and whether the combination of immunotherapy with anti-MET treatments provides clinical benefits are, thus, justified.

Inactivation of JAK2 has been associated with primary and acquired resistance to IC1 in melanoma patients (11). Here, we report recurrent inactivating mutations of JAK2 in lung cancer that co-occurred with MET and STRK1 alterations. JAK2, together with JAK1, mediates the signaling of IFNγ (30) and, accordingly, we found that JAK2 inactivation impairs the upregulation of immunoresponsive-related transcripts when treated with IFNγ. Genes coding for proteins related to the HLA-I complex, such as HLA-A, have been shown to be mutated in lung cancer and in melanomas and have been associated with acquired resistance to immunotherapy (4, 10, 11). We found that alterations of the HLA-I complex or of IFNγ signaling, which prevent appropriate host immune surveillance and thereby facilitate tumor growth, are mutually exclusive. However, their low frequency in lung cancer, less than 10%, means that we cannot draw definitive conclusions (Fig. 4D). Most of these abnormalities predict low levels of immune response-related proteins (HLA-I complex, TILs, and PD-L1), a characteristic present in about 30% of NSCLCs (4). Taken together, these observations suggest the presence of as yet unidentified gene alterations that prevent the tumor immunoresponse.

Finally, here we ruled out the involvement of LKB1 in the control of PD-L1 expression or in the maturation of the HLA-I complex, in a cell-autonomous manner. Despite no direct functional relationship, the strong correlation between the presence of mutations at LKB1 and low levels of PD-L1 and of CD8/TILs, in lung cancer specimens, supports an involvement of LKB1 in the regulation of the tumor immune response. In a previous work, an increase in the expression of T-cell exhaustion markers and tumor-promoting cytokines were observed in tumors from Lkb1-deficient/Kras-mutated mice as compared with their Kras-mutated counterparts (35). These observations suggest that LKB1 inactivation influences the immune milieu of the tumor microenvironment.

Collectively, our observations imply that MET activation restrains the immunoresponsive through the transcriptional control of immunosuppressive molecules. We also report the presence of JAK2-inactivating mutations in lung cancer that prevent appropriate host immune surveillance via IFNγ signaling. Our results identify other alterations, which can be added to the list of those already described, that contribute to the ability of tumor cells to escape immunosurveillance.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: M. Saigi, J.J. Alburquerque-Bejar, E. Nadal, E. Brambilla, M. Sanchez-Cespedes
Development of methodology: M. Saigi, J.J. Alburquerque-Bejar, E. Pros, O.A. Romero, N. Baixeras
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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M. Saigi, O.A. Romero, A. Esteve-Codina, E. Nadal, M. Sanchez-Cespedes
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The RNA-sequencing raw data produced by this study are available at Gene Expression Omnibus (GEO) under the accession code GSE109720.

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References
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