HHLA2, a New Immune Checkpoint Member of the B7 Family, Is Widely Expressed in Human Lung Cancer and Associated with EGFR Mutational Status

Haiying Cheng1, Murali Janakiram1, Alain Borczuk2, Juan Lin3, Wanglong Qiu4, Huijie Liu1, Jordan M. Chnai5, Balazs Halmos1, Roman Perez-Soler1, and Xingxing Zang1,5

Abstract

Purpose: Immuno therapy with antibodies against B7/CD28 family members, including PD-1, PD-L1, and CTLA-4 has shifted the treatment paradigm for non–small cell lung carcinoma (NSCLC) with improved clinical outcome. HHLA2 is a newly discovered member of the family. By regulating T-cell function, HHLA2 could contribute to tumor immune suppression and thus be a novel target for cancer immunotherapy. There is limited information and critical need to characterize its expression profile and clinical significance in NSCLC.

Experimental Design: We performed IHC with an HHLA2-specific antibody (clone 566.1) using tissue microarrays constructed from 679 NSCLC tumor tissues, including 392 cases in the discovery set and 287 cases in the validation cohort. We also studied clinicopathologic characteristics of these patients.

Results: Overall, HHLA2 was not detected in most of normal lung tissue but expressed in 66% of NSCLC across different subtypes. In particular, EGFR-mutated NSCLC was significantly associated with higher tumor HHLA2 expression in both discovery (EGFR vs. WT: 76% vs. 53%, P = 0.01) and validation cohorts (89% vs. 69%, P = 0.01). In one of the two cohorts, HHLA2 expression was higher in lung adenocarcinoma as compared with squamous and large cell histology, non-Hispanic White versus Hispanics, and tumors with high tumor-infiltrating lymphocyte (TIL) density. In the multivariate analysis, EGFR mutation status and high TIL intensity were independently associated with HHLA2 expression in lung adenocarcinoma.

Conclusions: HHLA2 is widely expressed in NSCLC and is associated with EGFR mutation and high TILs in lung adenocarcinoma. It is potentially a novel target for lung cancer immunotherapy.

Clinical Cancer Research; 23(3); 825–32. ©2016 AACR.

Introduction

Lung cancer is the most common cause of cancer-related mortality in the United States, accounting for more deaths than breast, prostate, and colorectal cancers combined in 2015 (1). The treatment for non–small cell lung cancer (NSCLC) has substantially changed over the past decade with the identification of driver mutations and the recent approval of immune checkpoint inhibitors. State-of-the-art treatment paradigms now incorporate histology-driven approaches (2) and targeted treatment of molecularly defined subsets in lung cancer (3). The ability to evade the immune system is considered one of the hallmarks of cancer (4, 5). One of the mechanisms of immune evasion in lung cancer is the expression of immune checkpoints on tumor cells. When an antigen is presented through the MHC complex, a simultaneous signal is required which determines the type of T-cell response; a costimulatory signal causing activation of the T cell while a coinhibitory signal results in T-cell inhibition. When tumor cells express coinhibitory ligands, this results in T-cell exhaustion and subsequently a blunted immune response which represents one of the mechanisms of tumor immune evasion (6–8).

The CD28 family of receptors and the B7 family of ligands provide the T-cell costimulatory and coinhibitory signals (6, 9). The well-established members of the CD28 receptor family include CD28, CTLA-4, ICOS, and PD-1, whereas the B7 ligands for these corresponding receptors are B7-1, B7-2, ICOS-L, PD-L1, and PD-L2. Other B7 ligands have been discovered in the past decade namely B7-H3 (CD276; ref. 10) and B7x (B7-H4/B7S1; refs. 11–13) and have been shown to suppress tumor immune response when expressed in various cancers (6, 8, 14–19). HHLA2 is the most recently discovered ligand of the B7 family (20–23), and was first demonstrated as a T-cell coinhibitory molecule by us (20, 24). Blocking immune checkpoints has become an important

Note: H. Cheng, M. Janakiram, and A. Borczuk contributed equally to this article.

Corresponding Authors: Haiying Cheng, Albert Einstein College of Medicine/Montefiore Medical Center, 1300 Morris Park Avenue, Chanin Building 407, Bronx, NY 10461. E-mail: hcheng@montefiore.org; and Xingxing Zang, xing-xing.zang@einstein.yu.edu


©2016 American Association for Cancer Research.
Treatment option in the management of NSCLC and inhibition of the PD-1/PD-L1 pathway has been shown to have a therapeutic benefit (7, 25). The FDA has approved PD-1 inhibitors, nivolumab and pembrolizumab, as second-line treatments for advanced NSCLC, because they have been shown to improve overall survival as compared with conventional second-line chemotherapy (26, 27). Hence, this is a therapeutic approach of great interest and it is important to explore other immune checkpoints which could contribute to immune evasion, apart from PD-1/PD-L1. The expression and clinical significance of the newest immune checkpoint, HHLA2, in human cancer is unclear. In this report, we hereby explored its expression and its association with clinical outcome in human NSCLC.

Assessment of tumor-infiltrating lymphocytes
As described previously (30), the scoring of tumor-infiltrating lymphocyte (TIL) was performed in the same slides used for HHLA2 staining with hematoxylin and eosin (H&E). Slightly modified from the published method (30), the TIL system was read using a three-tiered scale based on the visual estimation of the proportion of lymphocytic infiltration in each histospot. A score of “Absent” indicated absence of TILs, “Low” was defined as 1% to 30%, and “High” was defined as >30% (Fig. 1A).

Statistical analysis
For categorical variables after checking for assumptions, χ² or Fisher exact test were appropriately used to compare the distribution of each categorical variables between HHLA2 expression positive and negative groups. For pairwise comparisons, two-sample proportion test was performed to test whether the frequency of positive HHLA2 expression is greater in one mutational, histologic, or ethnic subgroup than another. Wilcoxon rank sum test was performed to test whether the median HHLA2 H-score was greater in one mutational, histologic, or ethnic subgroup than another. Logistic regression models were used for multivariate analysis. For survival analysis, comparison of the Kaplan–Meier survival curves between HHLA2 expression positive and negative groups or different degree of TIL groups were performed using log-rank test. All tests were two-sided using the SAS 9.2 software (SAS Institute Inc.). All P values ≤0.05 were considered significant.

Results
Clinical and pathologic features of patients and tumors
The majority of samples from the TMA sets are well annotated. In the discovery cohort (n = 392 patients), the mean age of the patient population was 67.6 years. Among them, 85% (334/392) had stage I–III NSCLC disease and underwent surgical resection from 2000 to 2012 whereas 0.5% (2/392) patients had stage IV disease. Race was documented for 280 patients, including 82% (230/280) of non-Hispanic White, 9% (25/280) of Hispanic, 6.4% (18/280) of African American, and 1.2% (7/280) of Asian patients. Histology was available for all the cases: the cohort consisted of 74% (290/392) lung adenocarcinoma, 8%...
(31/392) squamous, and 4.1% (16/392) large-cell NSCLC. Mutational status was known for 49% (191/392) of samples, including 41 patients with EGFR mutation and 62 patients with KRAS mutation.

To validate our findings from the initial cohort, we also performed similar studies using a separate patient TMA set (n = 287 patients). In this validation cohort, the mean age was 70 years and 76% (218/287) of patients underwent surgical resection for stage I–III NSCLC. As the information on race was not documented for most of cases in this cohort, race was not included in the analysis. The validation set differed from the discovery group in histology as it included very few cases of large-cell NSCLC, 1% (3/287). In addition, mutational status was known for 64% (185/287) of cases.

Immunohistochemical analysis of overall HHLA2 expression in lung cancer and normal lung tissues

To examine the expression profile of HHLA2 in primary lung tumors, we performed IHC and stained the TMA sets of NSCLC tumor tissues with the HHLA2-specific mAb clone 566.1. Some of the TMA sections did not have sufficient tumor tissue for accurate assessment and hence were not scored. Similar to a previous report (29), positive cases were defined as samples with positive membranous and cytoplasmic HHLA2 staining (intensity scores of 1, 2, or 3; Fig. 1B). In the discovery set, HHLA2 was found to be widely expressed in 62.8% (n = 228/363) NSCLC and HHLA2 was scored as 0 in 37.2% (n = 135/363), 1 in 31.7% (n = 115/363), 2 in 24.8% (n = 90/363), 3 in 6.3% (n = 23/363) of cases, respectively. Similarly, HHLA2 expression was positive in 71% (n = 185/262) of the cases in the validation cohort. Overall, HHLA2 was expressed in 66% of NSCLC cases when combining both cohorts.

We also reviewed 68 cases of normal lung tissues and did not detect HHLA2 expression in alveolar type 1 and type II cells, endothelial cells and smooth muscle in blood vessels. Occasional alveolar macrophages and club cells (also known as bronchiolar exocrine cells) were 1+ HHLA2 positive. Thus, in contrast to its wide expression in tumor specimens, HHLA2 protein was not expressed in most of normal lung tissues (Fig. 1C).

Figure 1.
A, Representative specimens showing TIL score of "0": absence of TILs, "Low": 1%–30%; and "High": >30%. A three-tiered scale based on the visual estimation of the proportion of lymphocytic infiltration in each histospot was used. B, Representative specimens demonstrating varying HHLA2 expression in lung cancer by IHC. The level of HHLA2 expression was graded as intensity of IHC staining: 0, absent staining; 1, weak staining; 2, moderate staining; 3, strong staining. C, Representative HHLA2 staining in normal lung tissues.
We then investigated the relationship between tumor HHLA2 expression and clinicopathologic variables in lung cancer (Table 1). There was no significant correlation between age and gender with tumoral HHLA2 expression.

**HHLA2 expression by race**

For the diverse racial groups in the discovery set, 36% (9/25) of Hispanic, 42.9% (3/7) of Asian, 55.6% (10/18) of African American, and 62.2% (143/230) of non-Hispanic white patients had HHLA2-positive lung cancers, respectively (Table 1). When pairwise comparisons were performed, the tumor expression of HHLA2 in non-Hispanic White patients was significantly higher than that in Hispanic patients ($P = 0.01$). Given lack of information in the validation cohort, similar analysis was not performed.

**HHLA2 expression and histology**

There are three major histologic subtypes for NSCLC: adenocarcinoma, squamous cell carcinoma, and large cell carcinoma. We analyzed HHLA2 expression in different subtypes. In the discovery cohort, 66.3% (167/252) of stage I, 51.1% (127/248) of stage II, and 69% (86/121) of stage III NSCLC cases were positive for HHLA2, respectively (Table 1). The expression of HHLA2 in adenocarcinoma specimens was significantly higher than that in squamous cell (P = 0.0002) and large cell NSCLC (P = 0.0001). In the validation cohort, the majority of adenocarcinoma (69%) was positive for HHLA2, which was similar to the initial cohort. However, there were very cases of large-cell NSCLC (n = 3) and HHLA2 expression was present in 73% of squamous cell cases. Thus, there was no difference among histology groups in this table (Table 1).

**HHLA2 expression is associated with EGFR mutation status**

EGFR and KRAS mutations are among the most common driver oncogenic alterations in NSCLC. Lung cancer patients with these mutations have distinct pathogenesis, clinical features, and disease course (3). We analyzed HHLA2 expression in different mutational categories. In the discovery cohort, positive HHLA2 staining was found in 75.6% (31/41) of EGFR-mutated, 62.9% (39/62) of KRAS-mutated, and 52.7% (48/91) of WT/WT (EGFR and KRAS wild-type) patients (Table 1). Among them, the HHLA2 expression in EGFR-mutated lung cancer was significantly higher than that in WT/WT patients ($P = 0.015$; Fig. 2). Similarly, in the validation cohort, EGFR-mutant tumors had significantly higher expression of HHLA2 when compared with WT/WT (89% vs. 69%, $P = 0.01$; Fig. 2).

**HHLA2 expression and TIL density**

The relationship between TILs and HHLA2 expression was examined. In the discovery set, HHLA2 was positive in 60% (144/238) of TILlow cases and 68% (73/108) of TILhigh groups but the difference was not significant. However, a significantly higher TIL score correlated with higher HHLA2 positivity in the validation cohort (TILlow vs. TILhigh, 67% vs. 86%, $P = 0.03$).
positive HHLA2 expression in the subsets with different nodal status, N1, N2, and N3 \( (P = 0.35) \). Similarly, no relationship between HHLA2 expression and different stages or lymph node status was identified in the validation cohort.

**Comparison of H-scores for different groups**

Further analysis with Wilcoxon rank sum test was performed to test whether the median HHLA2 H-score was greater in one group than another. In the discovery cohort (Table 2), the median HHLA2 H-scores for the EGFR-mutated lung cancers were significantly higher than that of WT/WT \( (P = 0.015) \), whereas no significant difference was found between KRAS-mutated and WT/WT groups \( (P = 0.123) \). Histology wise, adenocarcinoma specimens had significantly higher median H-scores than tumors with squamous \( (P < 0.001) \) or large-cell histology \( (P < 0.001) \). In addition, non-Hispanic White patients had significantly higher median HHLA2 tumor scores than Hispanic patients \( (P = 0.034) \). Moreover, the TIL\textsubscript{high} group tended to have higher HHLA2 H-scores than the TIL\textsubscript{low}, although not significantly in the whole discovery set. If only adenocarcinoma histology was considered, then HHLA2 scores were significantly higher in the TIL\textsubscript{high} group \( (P = 0.02) \).

Similarly to the initial set, the median HHLA2 H-scores was significantly higher for the EGFR-mutated tumors than that of WT/WT \( (P = 0.007) \) in the validation cohort (Table 2). Furthermore, the TIL\textsubscript{high} group had significantly higher HHLA2 H-scores than the TIL\textsubscript{low} group in this set \( (P = 0.02; \text{Table 2}) \).

The tumor HHLA2 expression was also evaluated as mean H-scores and it was 81 for the discovery cohort and 60 for the validation set. The mean scores for each category are listed in Table 3. Taken together, our data suggest that HHLA2 is widely expressed in NSCLC and its tumoral expression is associated with EGFR mutation status in both cohorts.

**Survival analysis in correlation with tumor HHLA2 expression and TIL scores in lung cancer**

Survival data were available for 250 of the 393 patients in the discovery set. Neither tumor HHLA2 expression nor TIL scores was significantly associated with overall survival in NSCLC. In the EGFR-mutated subset, there was a trend toward higher HHLA2 expression with decreased overall survival \( (P = 0.19) \) but this was

---

**Table 2.** Wilcoxon rank sum test was performed to test whether the median HHLA2 H-score was greater in one group than the other.

<table>
<thead>
<tr>
<th>Discovery cohort</th>
<th>Mutation</th>
<th>EGFR Median H-score (interquartile range) 75 (20–200)</th>
<th>WT/WT 25 (0–100)</th>
<th>WT/WT 0.015</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mutation</td>
<td>KRAS Median H-score (interquartile range) 67.5 (0–150)</td>
<td>WT/WT 25 (0–100)</td>
<td>WT/WT 0.123</td>
</tr>
<tr>
<td></td>
<td>Histology</td>
<td>Adeno Median H-score (interquartile range) 75 (0–200)</td>
<td>Squamous 0 (0–30)</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Histology</td>
<td>Adeno Median H-score (interquartile range) 75 (0–200)</td>
<td>Large cell 0 (0–0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Histology</td>
<td>Squamous Median H-score (interquartile range) 0 (0–0)</td>
<td>Large cell 0 (0–0)</td>
<td>0.0802</td>
</tr>
<tr>
<td></td>
<td>Race</td>
<td>Non-Hispanic White Median H-score (interquartile range) 60 (0–125)</td>
<td>Hispanic 0 (0–100)</td>
<td>0.0340</td>
</tr>
<tr>
<td></td>
<td>TIL</td>
<td>High Median H-score (interquartile range) 82 (0–200)</td>
<td>Low 50 (0–125)</td>
<td>0.1060</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Validation cohort</th>
<th>Mutation</th>
<th>EGFR Median H-score (interquartile range) 75 (27.5–137.5)</th>
<th>WT/WT 45 (0–100)</th>
<th>WT/WT 0.007</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mutation</td>
<td>KRAS Median H-score (interquartile range) 25 (0–100)</td>
<td>WT/WT 45 (0–100)</td>
<td>WT/WT 0.389</td>
</tr>
<tr>
<td></td>
<td>Histology</td>
<td>Adeno Median H-score (interquartile range) 40 (0–100)</td>
<td>Squamous 25 (0–50)</td>
<td>0.339</td>
</tr>
<tr>
<td></td>
<td>TIL</td>
<td>High Median H-score (interquartile range) 70 (22.5–100)</td>
<td>Low 30 (0–100)</td>
<td>0.02</td>
</tr>
</tbody>
</table>
including its different subgroups. Lung cancer is considered as an association with clinicopathologic characteristics and clinical frequent expression of HHLA2 in human lung cancer and its on dendritic cells or T cells. In this study, we described the highly after stimulation with lipopolysaccharide (LPS) and IFN on the surface of human monocytes and was induced on B cells.

In the discovery set, both the HHLA2 expression varied by ethnic background and there was a trend of higher expression in tumors from Caucasians than Hispanics. There could be at least two possible explanations: (i) evolutionary origin and (ii) carcinogenic exposure. Smoking is a carcinogen and PD-L1 is more highly expressed in smokers than in nonsmokers and whether this is a confounding factor in the higher expression of HHLA2 needs to be evaluated in a larger study. HHLA2 was also more highly expressed in adenocarcinoma than in the other subtypes of lung cancer in the discovery set although the trend was not significant in the validation cohort. This tendency of dissimilarity could be due to differences in the expression of genes involved in immune or inflammatory response between adenocarcinoma and squamous/large-cell cancers such as CFI, β-2 microglobulin, MHC class I and class II, serglycin, and MCP-3 or due to differences in neoantigen presentation between the subtypes resulting from a differential expression of MHC class I and II genes including HLA-DRB1, HLA-DQ, HLA-DR, HLA-DPR1, HLA-E, and β2-microglobulin (31).

The sensitizing EGFR mutations in adenocarcinoma of the lung are associated with an excellent response to EGFR tyrosine kinase inhibitors (TKI). EGFR activation results in the upregulation of the same checkpoint molecule, PD-L1, through the p-ERK1/2/p-c-Jun pathway (32, 33) and hence a similar mechanism could be postulated for HHLA2 overexpression in EGFR-mutated tumors. Indeed, we found that EGFR mutational status was significantly associated with higher HHLA2 expression in both discovery and validation cohorts. In addition, in the EGFR-mutated subgroup, patients with high HHLA2 tumors trended toward poorer survival, although this finding was not statistically significant. This could be important as HHLA2 expression might help define a subpopulation of patients who may do poorly despite TKIs for EGFR-mutated tumors.

TILs have been shown to be prognostic in some cancers but the predictive value of TILs remains controversial in lung cancer. In some studies, a higher density of TILs has been shown to have a lower disease-free and recurrence-free survival in early-stage lung cancer (34) but in other studies there was no correlation noted (30). Hence, we explored the correlation between TILs and HHLA2 expression. There was no association between the density of TILs and overall survival. However, in lung adenocarcinoma, there was a significant association between high TIL infiltration and HHLA2 expression after adjusting for mutational status. This suggests that there may be different factors involved in TILs recruitment and HHLA2 expression may at least partly be a response to TIL infiltration in lung adenocarcinoma.

HHLA2 binds to its putative receptors on a variety of immune cells, including T cells and antigen-presenting cells and subsequently inhibits proliferation and cytokine production of both human CD4 and CD8 T cells. TMIGD2 (29), also called immunoglobulin-containing and proline-rich receptor-1 (ICPR-1; ref. 35) or CD28 homology (CD28H; ref. 21), is one of receptors for HHLA2. We have recently proposed a model that tumor-expressed HHLA2 interacts not only with an unidentified receptor on activated T cells which leads to T-cell inhibition, but also with immunogenic tumor and the expression of coinhibitory molecules represents one mechanism of tumor immune evasion. Unlike PD-L1, which is usually focally expressed in lung cancer, HHLA2 was homogeneously expressed in most (>90%) of the tumor cells in a given lung tumor sample and there was minimal variation in the staining intensity in a given sample. HHLA2 was expressed in 66% of lung cancers examined. In the discovery cohort, HHLA2 expression varied by ethnic background and there was a trend of higher expression in tumors from Caucasians than Hispanics. There could be at least two possible explanations: (i) evolutionary origin and (ii) carcinogenic exposure. Smoking is a carcinogen and PD-L1 is more highly expressed in smokers than in nonsmokers and whether this is a confounding factor in the higher expression of HHLA2 needs to be evaluated in a larger study. HHLA2 was also more highly expressed in adenocarcinoma than in the other subtypes of lung cancer in the discovery set although the trend was not significant in the validation cohort. This tendency of dissimilarity could be due to differences in the expression of genes involved in immune or inflammatory response between adenocarcinoma and squamous/large-cell cancers such as CFI, β-2 microglobulin, MHC class I and class II, serglycin, and MCP-3 or due to differences in neoantigen presentation between the subtypes resulting from a differential expression of MHC class I and II genes including HLA-DRB1, HLA-DQ, HLA-DR, HLA-DPR1, HLA-E, and β2-microglobulin (31).

The sensitizing EGFR mutations in adenocarcinoma of the lung are associated with an excellent response to EGFR tyrosine kinase inhibitors (TKI). EGFR activation results in the upregulation of the same checkpoint molecule, PD-L1, through the p-ERK1/2/p-c-Jun pathway (32, 33) and hence a similar mechanism could be postulated for HHLA2 overexpression in EGFR-mutated tumors. Indeed, we found that EGFR mutational status was significantly associated with higher HHLA2 expression in both discovery and validation cohorts. In addition, in the EGFR-mutated subgroup, patients with high HHLA2 tumors trended toward poorer survival, although this finding was not statistically significant. This could be important as HHLA2 expression might help define a subpopulation of patients who may do poorly despite TKIs for EGFR-mutated tumors.

TILs have been shown to be prognostic in some cancers but the predictive value of TILs remains controversial in lung cancer. In some studies, a higher density of TILs has been shown to have a lower disease-free and recurrence-free survival in early-stage lung cancer (34) but in other studies there was no correlation noted (30). Hence, we explored the correlation between TILs and HHLA2 expression. There was no association between the density of TILs and overall survival. However, in lung adenocarcinoma, there was a significant association between high TIL infiltration and HHLA2 expression after adjusting for mutational status. This suggests that there may be different factors involved in TILs recruitment and HHLA2 expression may at least partly be a response to TIL infiltration in lung adenocarcinoma.

HHLA2 binds to its putative receptors on a variety of immune cells, including T cells and antigen-presenting cells and subsequently inhibits proliferation and cytokine production of both human CD4 and CD8 T cells. TMIGD2 (29), also called immunoglobulin-containing and proline-rich receptor-1 (ICPR-1; ref. 35) or CD28 homology (CD28H; ref. 21), is one of receptors for HHLA2. We have recently proposed a model that tumor-expressed HHLA2 interacts not only with an unidentified receptor on activated T cells which leads to T-cell inhibition, but also with

not statistically significant. In addition, the logistic regression analysis revealed that the patients with EGFR/KRAS wild-type had significantly higher chance of death comparing to patients with EGFR mutation after adjusting for TIL score [OR 3.402; 95% confidence interval (CI), 1.028–11.26; P = 0.0153].

EGFR and TIL status are independently associated with HHLA2 expression in lung adenocarcinoma

The multivariate analysis was performed in cases with lung adenocarcinoma because mutation tests, such as EGFR status, are not routinely performed in squamous cell lung cancer per standard of care. In the discovery set, both EGFR mutation status (WT/WT vs. EGFR; OR 0.32; 95% CI, 0.13–0.76; P = 0.0079) and TIL score (high vs. low; OR 2.441; 95% CI, 1.143–5.211; P = 0.0021) were independently associated with HHLA2 expression in lung adenocarcinoma (n = 289). Similarly, both EGFR mutation status (WT/WT vs. EGFR; OR 0.22; 95% CI, 0.13–0.22; P = 0.015) and TIL score (high vs. low; OR 3.282; 95% CI, 1.01–10.28; P = 0.0486) were also independently associated with HHLA2 expression in the group of lung adenocarcinoma (n = 186) in the validation cohort.

**Discussion**

Analogous to other B7 molecules such as PD-L1, PD-L2, B7x, and B7-H3, HHLA2 belongs to the B7 family of coinhibitory molecules. These coinhibitory members of the B7 family of ligands interact with the CD28 family of receptors such as PD-1 and CTLA-4 with resultant T-cell inhibition. In our previous study (20), we examined HHLA2 expression on hematopoietic cells and found that HHLA2 protein was constitutively expressed on the surface of human monocytes and was induced on B cells after stimulation with lipopolysaccharide (LPS) and IFNγ, but not on dendritic cells or T cells. In this study, we described the highly frequent expression of HHLA2 in human lung cancer and its association with clinicopathologic characteristics and clinical outcome.

We revealed that HHLA2 was expressed widely in lung cancer, including its different subgroups. Lung cancer is considered as an

<table>
<thead>
<tr>
<th>Table 3. Mean tumor HHLA2 H-scores for each category in lung cancers</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parameter</strong></td>
</tr>
<tr>
<td>--------------</td>
</tr>
<tr>
<td><strong>H-score</strong></td>
</tr>
<tr>
<td>Gender</td>
</tr>
<tr>
<td>Male (n = 141)</td>
</tr>
<tr>
<td>Histology</td>
</tr>
<tr>
<td>Squamous (n = 31)</td>
</tr>
<tr>
<td>Stage</td>
</tr>
<tr>
<td>I (n = 252)</td>
</tr>
<tr>
<td>II (n = 47)</td>
</tr>
<tr>
<td>III (n = 35)</td>
</tr>
<tr>
<td>TIL</td>
</tr>
<tr>
<td>Low (n = 238)</td>
</tr>
<tr>
<td>High (n = 108)</td>
</tr>
<tr>
<td>Mutation status</td>
</tr>
<tr>
<td>KRAS (n = 62)</td>
</tr>
<tr>
<td>WT/WT (n = 91)</td>
</tr>
</tbody>
</table>
HHLA2 in Lung Cancer

Authors' Contributions

Conception and design: H. Cheng, M. Janakiram, R. Perez-Soler, X. Zang
Development of methodology: H. Cheng, M. Janakiram, W. Qiu
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): H. Cheng, M. Janakiram, A. Borczuk, W. Qiu
Experimental design: H. Cheng, M. Janakiram, A. Borczuk, W. Qiu
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): H. Cheng, M. Janakiram, A. Borczuk, J. Lin, B. Halmos, R. Perez-Soler
Writing, review, and/or revision of the manuscript: H. Cheng, M. Janakiram, A. Borczuk, B. Halmos, R. Perez-Soler, X. Zang
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): H. Cheng, A. Borczuk, H. Liu
Study supervision: H. Cheng, R. Perez-Soler

Grant Support

The work was supported by the NIH Paul Calabresi Career Development Award for Clinical Oncology SK12CA132787-03 (to R. Perez-Soler and H. Cheng), the LUNGevity Foundation Targeted Therapies Award (to H. Cheng and B. Halmos), NIH RO1CA175495 (to X. Zang), NIH RO1DK10052S (to X. Zang), and Department of Defense PC131008 (to X. Zang).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References


HHLA2, a New Immune Checkpoint Member of the B7 Family, Is Widely Expressed in Human Lung Cancer and Associated with EGFR Mutational Status

Haiying Cheng, Murali Janakiram, Alain Borczuk, et al.


Access the most recent version of this article at:

This article cites 35 articles, 9 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/23/3/825.full#ref-list-1

This article has been cited by 10 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/23/3/825.full#related-urls

Sign up to receive free email-alerts related to this article or journal.

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

To request permission to re-use all or part of this article, use this link
http://clincancerres.aacrjournals.org/content/23/3/825.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.