PD-L1 Expression Correlates with TP53 Gene Mutation Status in Lung Cancer but not in Colorectal Cancer

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Background
Expression of PD-L1 is, in general, associated with response to immunotherapy. However, it is believed that additional intrinsic factors play a role in determining the potential of response to immunotherapy. Toward this goal we investigated the relationship between mutation profile and PD-L1 expression in lung and colorectal cancers.

Methods
Molecular profiling using a panel of 24 genes was performed by next generation sequencing (NGS) on 158 non-small cell lung cancer (NSCLC) and 42 colorectal cancers. The genes studied included ERBB2, FGFR1, FGFR2, FGFR3, JAK2, ERBB4, RARB, and SMARCB. In addition, these tumors were studied for the expression of PD-L1 using immunohistochemistry (IHC). PD-L1 expression was performed using standard IHC approach using SP142 clone (Spring Bioscience).

Results
The level of PD-L1 expression was significantly (P=0.003) lower in colorectal cancer as compared with NSCLC. The NSCLC cohort had significantly (P=0.005) more cases with 3 or more genes mutated as compared with colorectal cancer. However, there was no significant difference in TP53 mutation frequency between the two tumor types. There was no correlation between PD-L1 expression and the presence or absence of 3 or more gene mutations in either NSCLC or colorectal cancer. PD-L1 expression was higher in NSCLC cohort than in the colorectal carcinoma cohort regardless of whether it was evaluated as a continuous variable or it was dichotomized at 20% (P=0.003) or 40% (P=0.001) cutoffs. In addition, PD-L1 expression was significantly (P=0.01) higher in tumors with TP53 mutation in the NSCLC cohort, but not in the colorectal cancer cohort (P=0.34).

Conclusions
There is significant difference between NSCLC and colorectal cancers in PD-L1 expression levels. More importantly, TP53 mutation in NSCLC correlates with the expression of PD-L1 protein, but not in colorectal cancer despite similar rate of mutation of the TP53 between the tumor types. This suggests a possible difference in the mechanism of regulating PD-L1 expression between the two tumor types.

Introduction
PD-L1 is a transmembrane protein that downregulates immune responses by binding to PD-1 on T-cells and B7.1, which is expressed on T-cells and antigen presenting cells. PD-L1 plays a role in maintenance of peripheral tolerance. PD-L1 is induced in both hematopoietic and non-hematopoietic cells following cell-adaptive stimuli. 

• Absent expression of PD-L1 on tumor cells inhibits anti-tumor immune responses.
• Tumor expression of PD-L1 has been linked to poor prognosis and shorter survival in some tumor types.

Expression of PD-L1 is, in general, associated with response to immunotherapy. Several anti-PD-L1 therapies have recently been FDA-approved for treatment of non-small cell lung cancers, melanomas and hematologic malignancies.

• However, it is believed that additional intrinsic factors play a role in determining the potential of response to immunotherapy.
• We investigated the relationship between mutation profile and PD-L1 expression in lung and colorectal cancers.

Methodology
Specimen Cohort
DNA was extracted from formalin-fixed, paraffin-embedded tumor specimens.

• 158 non-small cell lung carcinomas
• 42 colorectal carcinomas

DNA Extraction
DNA Extraction from FFPE tissue was performed using GeneRead DNA FFPE kit (Qiagen). DNA was quantified using Qubit fluorometer, and quality was checked by gel electrophoresis. 

DNA Sequencing
DNA Sequencing was performed using Illumina MiSeq system (San Diego, CA). NGS libraries were prepared according to the manufacturer’s instructions. Human genome build 19 (hg19) was used for alignment. MiSeq Reporter was used for analysis and Variant Studio was used for calling. For confirmation of variant calling, Nextseq studio (Illumina, San Diego, CA) was also used. Hot spots and abnormalities were confirmed using Integrative Genomics Viewer (IGV).

NGS Analysis
Average sequencing coverage across the entire coding regions was ≥ 4,000 in 98% of the sequenced amplicons. Uniformity was ≥95% for any run to be acceptable. Allele frequency for mutation was set at 5%.

Conclusions
PD-L1 Expression Correlates with TP53 Gene Mutation Status in Lung Cancer but not in Colorectal Cancer.

• TP53 mutation in NSCLC correlates with the expression of PD-L1 protein, but not in colorectal cancer despite similar rate of mutation of the TP53 between the two tumor types.

• This suggests a possible difference in the mechanism of regulating PD-L1 expression between the two tumor types.

Results
Figure 1. PD-L1 expression is significantly lower in colorectal cancer than NSCLC (P=0.0003)

Figure 2. Characteristic of lung and colorectal samples analyzed

Figure 3. NSCLC has more mutated genes than colorectal cancer (P=0.001)

Figure 4. No significant difference in TP53 mutation rate between NSCLC and colorectal cancer (P=0.01)

Figure 5. PD-L1 expression is higher in NSCLC than in colorectal cancer using either 20% or 40% cut-off for PD-L1 positivity

Figure 6. PD-L1 expression is higher in TP53-mutated NSCLC (P=0.01) but not colorectal cancer (P=0.34) with PD-L1 as continuous variable

*P values < 0.05 are considered significant.