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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, SRC, JAK3, ERBB4, ERBB2, and SMAD4. 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 Biosciences).

Results

The level of PD-L1 expression was significantly (P=0.0005) lower in colorectal cancer as compared with NSCLC. The NSCLC cohort had significantly (P<0.0001) 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 if it was dichotomized at 20% (P=0.0003) 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 carcinoma 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 two 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 or 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 inducibly expressed on both hematopoietic and non-hematopoietic cells following cell-specific stimulation.
- Aberrant expression of PD-L1 on tumor cells inhibits anti-tumor immune responses. Tumor expression of PD-L1 has been linked to poorer 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 carcinoma, melanoma and urothelial carcinoma.
- 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

200 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 (Quiagen) from unstained slides (5-10 sections at 5-10 um thickness) according to the manufacturer recommendation. Tumor was circled by a pathologist and tumor tissue was scraped.

Next Generation Sequencing

NGS was performed using an Illumina MiSeq system (San Diego, CA); NGS, amplification, and indexing were performed as recommended by the manufacturer. Amplicons were confirmed for each sample by running an agarose gel. Samples were pooled and the experiment sheet was generated using Illumina Experiment Manager. An amplicon-based primers set was validated for the following genes: EGFR, AKT1, ERBB2, SMAD4, ALK, ERBB4, HRAS, APC, SMO, ATM, FGFR1, NRAS, BRAF, FGFR2, JAK3, PDGFRA, STK11, FGFR3, KDR, PIK3CA, TP53, KIT, PTEN, GNA11, KRAS, CTNNB1, GNAQ, and MET. Library preparation and sequencing using MiSeq or NextSeq was performed 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, NextGene software (SoftGenetics, State College, PA) was also used. Hot spots and abnormalities were confirmed using Integrative Genomics Viewer (IGV). Average sequencing coverage across the entire coding regions was 4,000 in 94% of the sequenced amplicons. Uniformity was >90% for any run to be acceptable. Allele frequency for mutation was set at 5%. Positive, negative and sensitivity control (5%) were used with each batch.

AKT1	APC	BRAF	EGFR	ERBB2	ERBB4
FGFR1	FGFR2	FGFR3	HRAS	JAK2	JAK3
KIT	KRAS	MET	NOTCH1	NRAS	PDGFRA
PIK3CA	PTEN	SMAD4	SMO	SRC	TP53

PD-L1 Immunohistochemistry

Formalin-fixed paraffin-embedded tissue sections were stained with an anti-PD-L1 primary antibody (clone SP142) and a polymer-technology based system was used for detection. Stains were scored by a pathologist using manual microscopy. The percentage of tumor cells with membrane staining is reported and the intensity of staining is scored as follows: 0, absent; 1+, weak; 2+, moderate; and 3+, strong.

Conclusions

- There is significant difference between NSCLC and colorectal cancers in PD-L1 expression levels.
- 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=0.0005)

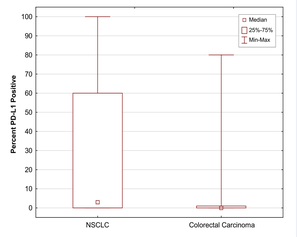
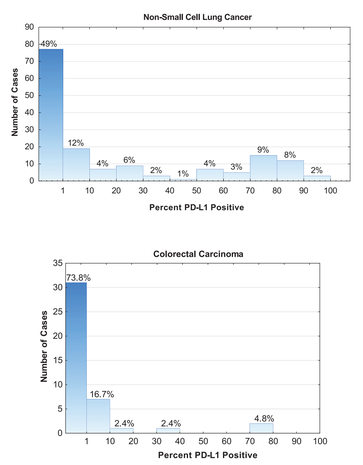


Figure 2. Characteristics of lung and colorectal samples analyzed



The majority of samples of each type were negative for PD-L1 staining. Percentages at the top of each bar represent the proportion of samples that had that result.

Figure 3. NSCLC has more mutated genes than colorectal cancer (P<0.0001)

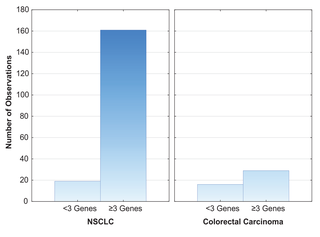


Figure 4. No significant difference in TP53 mutation rate between NSCLC and colorectal cancer (P=0.9)

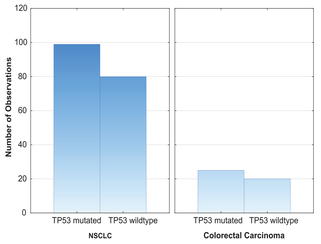
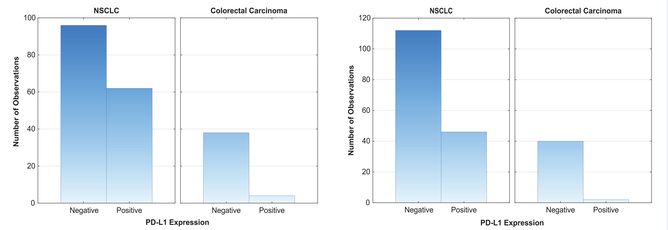


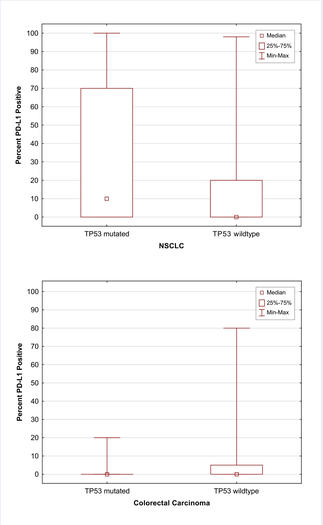
Figure 5. PD-L1 expression is higher in NSCLC than in colorectal cancer using either 20% or 40% cut-off for PD-L1 positivity



PD-L1 expression is higher in NSCLC (P=0.0003), cut-off 20%

PD-L1 expression is higher in NSCLC (P=0.001), cut-off 40%

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



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