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, SMAD4, AKT1, MET, PIK3CA, APC, SMO, NOTCH1, NRAS, BRAF, EGFR, AKT1, ERBB4, HRAS, TP53, NRAS, MET, PIK3CA, and EGFR. The amplicons were confirmed for each sample by running an agarose gel. Samples were pooled and the experiment sheet was generated using Illumina Experiment Manager. The experimental run was performed with the following primer sets: EGFR, AKT1, ERBB2, SMAD4, AKH, ERBB3, HRAS, APC, SM0, ATN, FGFR1, NRAS, BRAF, EGFR, AKT1, ERBB4, HRAS, PIK3CA, MET, PIK3CA, and EGFR. The sample for each primer set was validated for the following genes: EGFR, AKT1, ERBB2, SMAD4, AKH, ERBB3, HRAS, APC, SM0, ATN, FGFR1, NRAS, BRAF, EGFR, AKT1, ERBB4, HRAS, PIK3CA, MET, PIK3CA, and EGFR. An amplicon-based primers set was validated for the following genes: EGFR, AKT1, ERBB2, SMAD4, AKH, ERBB3, HRAS, APC, SM0, ATN, FGFR1, NRAS, BRAF, EGFR, AKT1, ERBB4, HRAS, PIK3CA, MET, PIK3CA, and EGFR. A Reporter was used for analysis and Variant Studio was used for calling. For confirmation of variant calling, Nextseq software (Illumina, San Diego, CA) was used. Hotspots and aminoacids were confirmed using Integrative Genomics Viewer (IGV). Average sequencing coverage across the entire coding regions was 4,000-5,000 of the sequenced amplicons. Uniformity was >80% for any run to be acceptable. Allele frequency for mutation was set at 5%. Positive, negative, and wildtype regions was 4,000 in 94% of the sequenced amplicons. Uniformity was >90% for the expression of PD-L1 was, 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.

Results

There were no significant differences in PD-L1 expression 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 (P=0.03). There was no correlation between PD-L1 expression and the presence or absence of TP53 mutation in the NSCLC cohort, but not in the colorectal carcinoma cohort (P=0.34). 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). In addition, PD-L1 expression was significantly (P=0.0005) lower in colorectal cancer than in the NSCLC cohort. The NSCLC cohort had significantly (P<0.0001) more tumors with PD-L1 expression than the colorectal carcinoma cohort. The NSCLC cohort had significantly (P=0.01) more tumors with PD-L1 expression than the colorectal carcinoma cohort.

Conclusions

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Methodology

Specimen Cohort

DNA-immortalized cell line derived from non-small cell lung carcinoma

DNA Extraction

DNA Extraction from FFPTE tissue was performed using GeneRead DNA FFPE Kit (QIAGEN) 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.

Tissue was ground into powder and lysed with lysis buffer. DNA was extracted and concentrated using a commercial kit (QIAGEN) 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

Next Generation Sequencing was performed using a 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, AKH, ERBB3, HRAS, APC, SM0, ATN, FGFR1, NRAS, BRAF, EGFR, AKT1, ERBB4, HRAS, PIK3CA, MET, PIK3CA, and EGFR. A Reporter was used for analysis and Variant Studio was used for calling. For confirmation of variant calling, Nextseq software (Illumina, San Diego, CA) was used. Hotspots and aminoacids were confirmed using Integrative Genomics Viewer (IGV). Average sequencing coverage across the entire coding regions was 4,000-5,000 of the sequenced amplicons. Uniformity was >80% for any run to be acceptable. Allele frequency for mutation was set at 5%. Positive, negative, and wildtype regions was 4,000 in 94% of the sequenced amplicons. Uniformity was >90% for the expression of PD-L1 was, 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.

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