Identifying New Biomarkers and Targeted Molecules for Immunotherapy Using Targeted RNA Next Generation Sequencing

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Methods
We used RNA sequencing of 1385 genes to profile tissues from solid tumors and lymphomas, and correlated RNA levels with PD-L1 expression as detected by IHC in tumor and inflammatory cells.

Key Points
• Targeted RNA NGS can reliably be used in predicting PD-L1 IHC expression.
• 20 genes in addition to CD274 (PD-L1) showed high correlation with PD-L1 protein expression and potentially can be used in predicting PD-L1 expression.
• CD274, PLAU, and RAC1 are independent predictors of PD-L1 protein expression.
• A model incorporating CD274, RAC1, PLAU, and tumor type can significantly improve prediction of PD-L1 expression.
• Targeting PLAU and RAC1 in combination with PD-L1 inhibitors may potentially augment the therapeutic effects of anti-PD-L1.

Results
After normalization, adjusting for group effect and multiple hypothesis testing, 21 genes correlated with PD-L1 expression; 14 genes correlated positively and 7 correlated negatively. Using the first principle component, we demonstrated that these 21 genes are highly correlated with PD-L1 expression; 14 genes correlated positively and 7 correlated negatively.

Transcripts Correlated with PD-L1 IHC Protein Expression

LASSO Model Refinement – Predicting PD-L1 Expression
A. Model 1 (CD274 alone). The predicted expression level is on the x-axis and the measured PD-L1 expression in tumor cells is on the y-axis; and B. Model 2 (CD274 + group); C. Model 3 (CD274 + group + CD274 + PLAU + RAC1); and D. Model 4 (CD274 + group + CD274 + PLAU + RAC1 + 2 components).

TCGA Independent RNA Expression Predictive of PD-L1 Protein Expression by Tumor Type
A. PLAU vs tumor type; B. RAC1 vs tumor type

Positive and Negative Correlations of 21 Genes
Correlations between transcription levels of 21 genes are shown as the color on the heat map on a scale of -1 (red; negative) to +1 (white; positive).

PD-L1 Expression as a Function of Tumor Type
A. Solid tumor type vs. % of PD-L1 protein in tumor samples; B. Solid tumor type vs. CD274 (PD-L1) transcript in tumor samples; and C. Solid tumor type vs. % of PD-L1 protein in immune cells.