Gene expression correlation of immune checkpoint molecules Siglec-15 and PD-L1 varies widely by cancer indication

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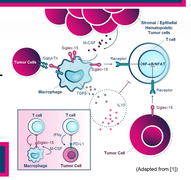
Abstract # 1373

PYXIS ONCOLOGY

Background

Siglec-15 is a novel target for immunotherapy in cancer and exhibits low mRNA expression in normal tissues but broad expression across cancer indications, specifically on tumor-associated macrophages and tumor cells [1]. Expression of Siglec-15 and the immune checkpoint molecule PD-L1 have previously been reported to be mutually exclusive [1][2]. However, a large-scale meta-analysis of this claim has not been undertaken across cancer indications. Here, in silico RNA-Seq analyses were performed across bulk tumor samples, cell lines and single cells for Siglec-15 and PD-L1 mRNA expression.

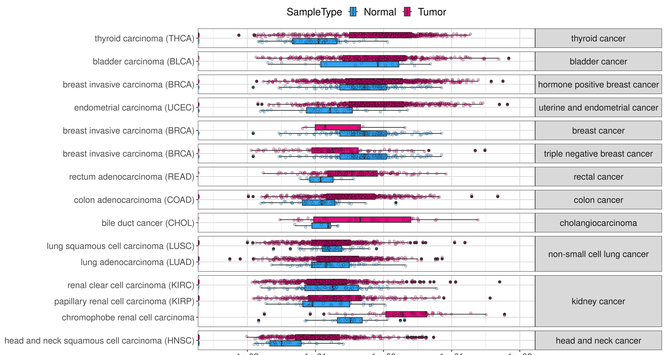


(Adapted from [1])

Methods

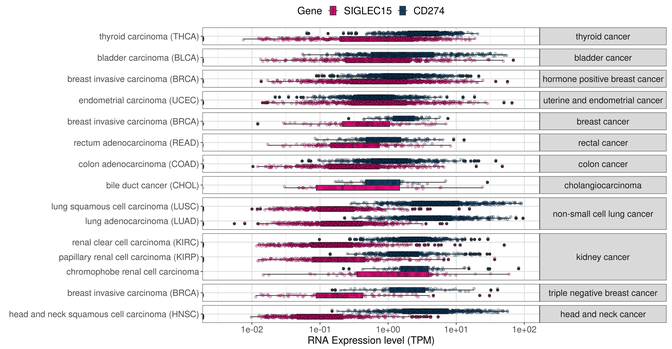
Data and meta-data from The Cancer Genome Atlas (TCGA), Cancer Cell Line Encyclopedia (CCLE), and primary human tumor samples from 62 studies were obtained through QIAGEN OmicSoft, OncoLand and single cell analysis was performed on the BioTuring Talk2Data platform. Analyses were limited to squamous and adenocarcinoma non-small cell lung cancer (NSCLC), cholangiocarcinoma, breast, thyroid, head and neck, colon, rectal, bladder, kidney, uterine and endometrial cancers. Breast cancer was subdivided into triple negative breast cancer, hormone receptor positive breast cancer, or breast cancer that are neither triple negative or hormone receptor positive. Bulk primary tumor samples used in these analyses had been dissociated and consist of multiple cells types including tumor, immune, stromal, and other cells.

Siglec-15 is overexpressed in multiple primary tumor types



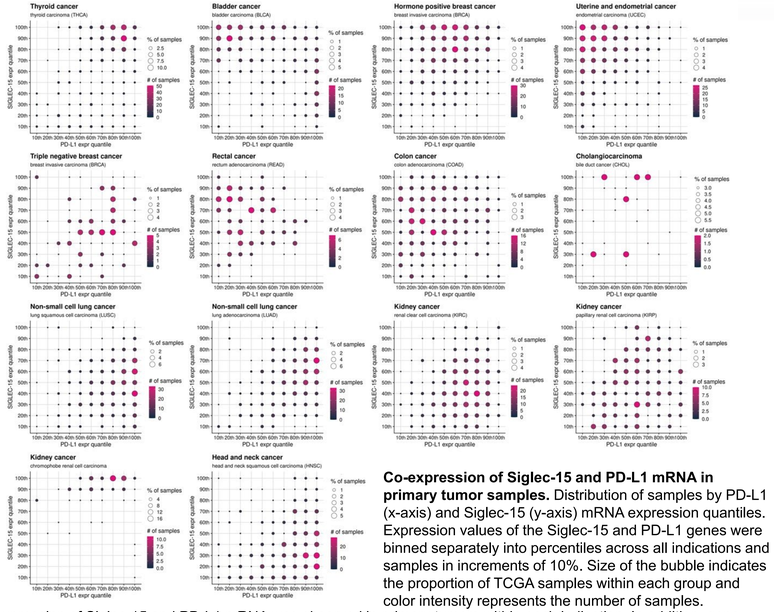
Expression of Siglec-15 mRNA in primary bulk tumors and matched normal tissue. RNA seq data from primary bulk human tumor and matched normal tissue samples from the TCGA database are plotted as transcripts per million (TPM). Siglec-15 is overexpressed in multiple tumor types (shown in pink) compared to normal tissue expression (shown in blue).

Siglec-15 and PD-L1 mRNA expression vary within tumor indications



Expression of Siglec-15 and PD-L1 mRNA in primary bulk tumor samples from the TCGA database. RNA seq data for Siglec-15 (SIGLEC15, shown in pink) and PD-L1 (CD274, shown in blue) genes from tumor samples are plotted as TPM. Individual mRNA expression levels of Siglec-15 and PD-L1 in bulk tumor samples varied substantially within indications.

Siglec-15 and PD-L1 are co-expressed in primary bulk tumors from TCGA



Co-expression of Siglec-15 and PD-L1 mRNA in primary tumor samples. Distribution of samples by PD-L1 (x-axis) and Siglec-15 (y-axis) mRNA expression quantiles. Expression values of the Siglec-15 and PD-L1 genes were binned separately into percentiles across all indications and samples in increments of 10%. Size of the bubble indicates the proportion of TCGA samples within each group and color intensity represents the number of samples.

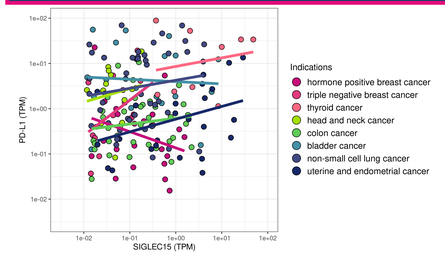
Co-expression of Siglec-15 and PD-L1 mRNA was observed in primary tumors within each indication. In addition, gene expression of Siglec-15 and PD-L1 showed a positive trend in several indications including NSCLC, head and neck, kidney, and thyroid cancers.

Siglec-15 and PD-L1 are co-expressed in primary bulk tumors from OncoLand

Meta-analysis of publicly available data from the OncoLand database (only indications with ≥100 samples shown).
A: Expression of Siglec-15 and PD-L1 mRNA in primary tumor samples. RNA seq data for Siglec-15 (SIGLEC15, shown in pink) and PD-L1 (CD274, shown in blue) genes from tumor samples are plotted as TPM. Individual gene mRNA expression levels of Siglec-15 and PD-L1 in bulk tumor samples varied substantially within indications. **B: Co-expression of Siglec-15 and PD-L1 mRNA in primary tumor samples.** Distribution of samples by PD-L1 (x-axis) and Siglec-15 (y-axis) expression quantiles.

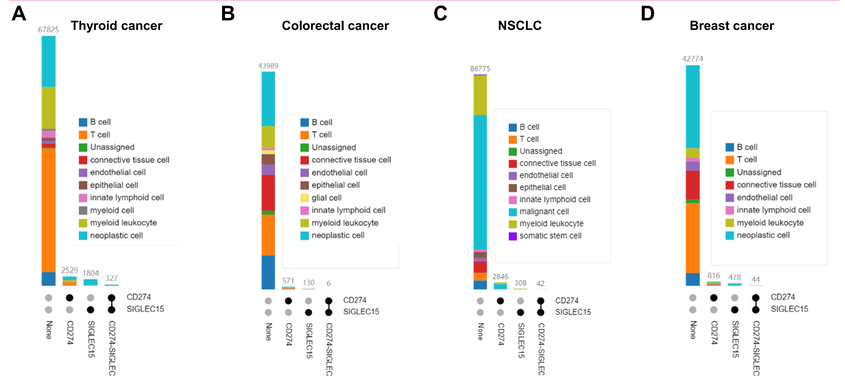
Expression values of Siglec-15 and PD-L1 genes were binned separately into percentiles across all indications and samples in increments of 10%. Size of the bubble indicates the proportion of OncoLand samples within each group and color intensity represents the number of samples. Analyses from additional studies in primary bulk tumor samples confirmed co-expression of Siglec-15 and PD-L1 mRNA in NSCLC and head and neck cancer.

Co-expression of Siglec-15 and PD-L1 mRNA positively correlate in some cancer cell lines



Co-expression of Siglec-15 and PD-L1 mRNA in cancer cell lines. RNA seq data from 183 cell lines from the CCLE. Cell lines were filtered out from the analysis if they had ≤3.162e-03 Siglec-15 TPM. Gene expression of Siglec-15 is plotted on the x axis and PD-L1 on the y-axis. A positive correlation between Siglec-15 and PD-L1 mRNA expression is observed across thyroid, triple negative breast, uterine and endometrial cancer cell lines. Unlike the bulk tumor samples which consist of tumor, immune, stromal and other cells, cell lines consist of exclusively cancer cells.

Single cell analysis reveals that Siglec-15 and PD-L1 are rarely co-expressed on the same cell in bulk tumor samples



Single cell analysis on the BioTuring Talk2Data platform. Gene expression of Siglec-15 and PD-L1 (CD274) are shown from 4 independent studies. Each figure depicts the number of cells from dissociated tumors with the following mRNA expression within the same cell; 1) no Siglec-15 or PD-L1 (○ ○), 2) PD-L1 only (● ○), 3) Siglec-15 only (○ ●), or 4) both PD-L1 and Siglec-15 (● ●). **A:** 71,831 cells from 20 thyroid cancer subjects were analyzed on the 10x3' (using v3 chemistry) platform and 0.46% expressed both Siglec-15 and PD-L1, the majority were tumor cells [3]. **B:** 44,684 cells from 7 colorectal cancer subjects were analyzed on the Chromium (v2) platform and 0.01% expressed both Siglec-15 and PD-L1, the majority were macrophages [4]. **C:** 89,887 cells from 42 NSCLC subjects were analyzed on the GEXSCOPE scRNA platform and 0.05% expressed both Siglec-15 and PD-L1, the majority were tumor cells [5]. **D:** 44,024 cells from 14 breast cancer subjects were analyzed on the 10x5' (v2) platform and 0.10% expressed both Siglec-15 and PD-L1, the majority were tumor cells [6]. These studies show that Siglec-15 and PD-L1 are rarely expressed on the same cell within a bulk tumor sample.

Conclusions

- At the bulk tumor level, expression of Siglec-15 and PD-L1 genes are not mutually exclusive across cancer indications but instead the expression of each gene varies broadly within a given tumor type.
- Siglec-15 and the PD-L1 mRNA expression are frequently positively correlated, including in NSCLC, head and neck, kidney, and thyroid cancers.
- Siglec-15 and the PD-L1 mRNA are rarely found to be co-expressed on the same cell.
- These associations enable a better understanding of the landscape of target expression in patients across a wide variety of cancer indications and can inform combination strategies with anti-Siglec-15 therapies.

References

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[5] Wu, F., et al. Nat Commun 2021;12(1):2540.
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