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Abstract # 2526

Altered pan-Ras pathway and activating mutations in EGFR result in elevated CD73 in multiple cancers

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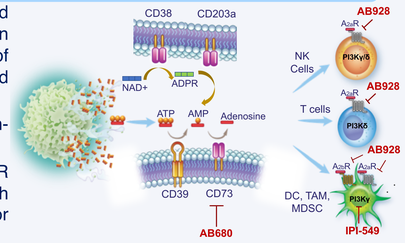
Introduction

- AB928, a dual $A_{2a}R/A_{2b}R$ antagonist, and AB680, a potent selective inhibitor of CD73, can rescue the immunosuppressive effects of adenosine in experimental cell culture and tumor models.
- Oncogene-driven cancers tend to be non-responsive to PD(L)-1 inhibition.
- Here we show that pan-RAS, BRAF and EGFR alterations drive the expression of CD73, which may contribute to suppressed anti-tumor immunity.



Materials and Methods

- **Regression analysis:** We used linear models adjusted for individual tumor types to assess if the expression of CD73 and other genes in the adenosine fingerprint can be predicted by alterations in 299 consensus cancer driver genes (Bailey *et.al* Cell (2018)) in pan-cancer TCGA dataset (GDC Commons). For regression models, multiple testing correction was performed using Benjamini-Hochberg method.
- **Survival analysis:** Kaplan-Meier curves were plotted in R showing log-rank p-values. Cox-regression analysis were used to compute hazard ratios and p-values.
- **Histology:** CD73 (Cell Signaling, D7F9A) immuno-histochemistry (IHC) was performed on sections of formalin fixed paraffin embedded (FFPE) tumor tissue and quantified using QuPath software.
- **Cancer cell lines:** RNAseq data was obtained from Broad's Cancer Cell Line Encyclopedia (CCLE) database. RNA from human cancer cell lines was extracted and converted into cDNA. Real-time qPCR was performed using Taqman probes. HPRT1 was used as the housekeeping gene and raw data was analyzed using the 2-ΔCT method. CD73, CD39 and CD26 protein expression on human cancer cell lines was determined using flow cytometry.

Results

KRAS, BRAF and EGFR strongly predict adenosine fingerprint expression in pan-cancer TCGA

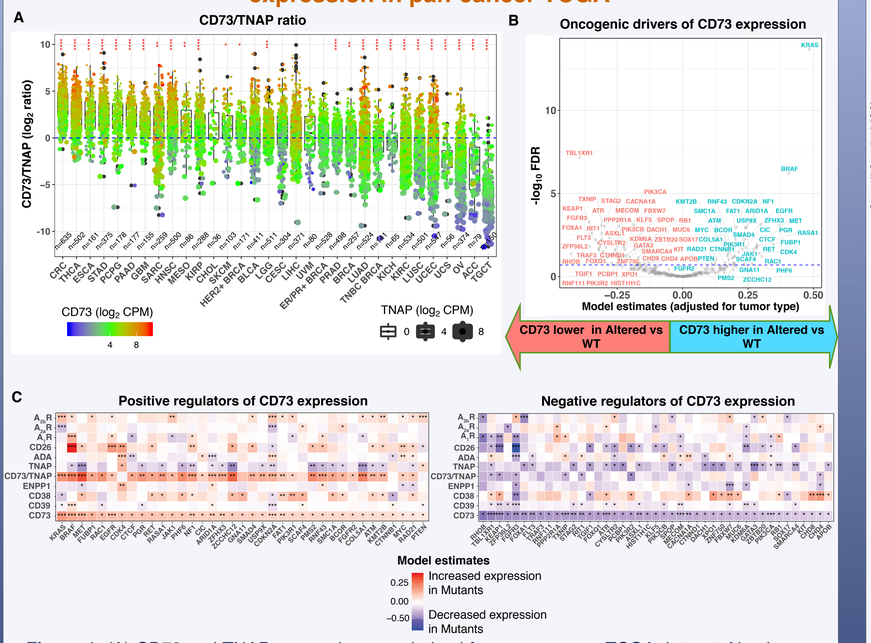
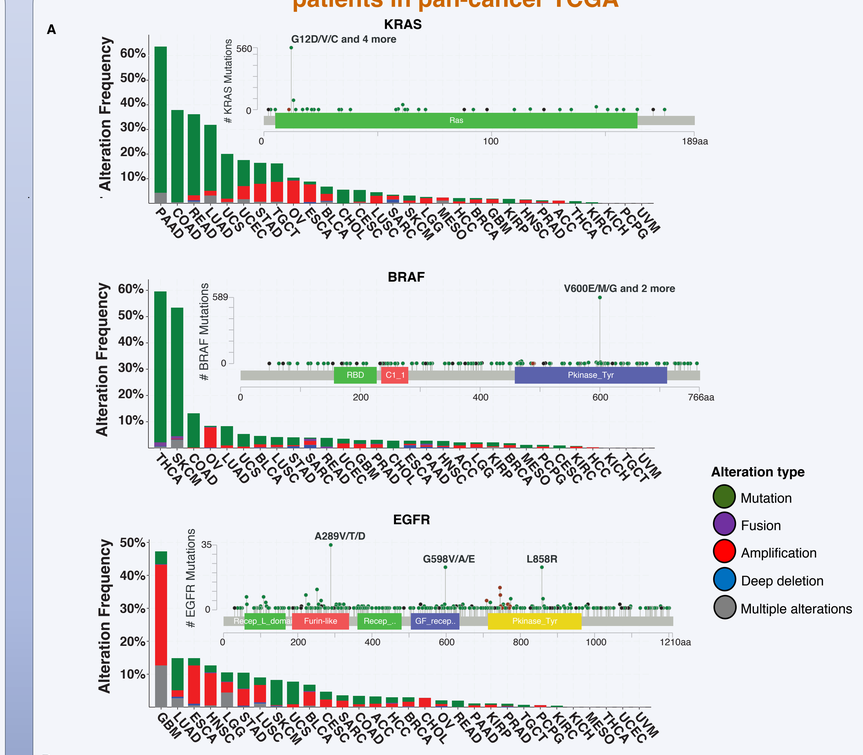


Figure 1. (A) CD73 and TNAP expression was derived from pan-cancer TCGA dataset. Numbers on Y-axis indicate ratio of log_2 CPM values for CD73 and TNAP. Tumors on left are high in CD73 and low in TNAP whereas tumors on right are high in TNAP and low in CD73. (B) Linear model estimates adjusted for tumor type of alterations in cancer driver genes that predict CD73 expression. (C) X-axis denotes the positive (left) and negative (right) regulators of CD73 from panel B. Y-axis shows linear model estimates adjusted for tumor type for each gene in the adenosine fingerprint. Stars indicate significant FDR (*** < 0.001,** <0.01,* < 0.2).

CD73 expression is prognostic in KRAS, BRAF and EGFR mutant patients in pan-cancer TCGA



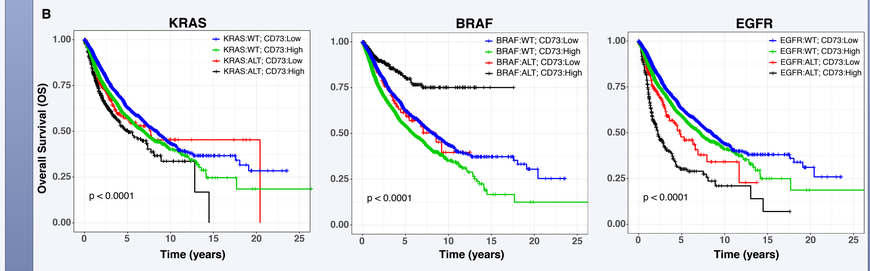


Figure 2. (A) shows frequency and type of alterations in KRAS, BRAF and EGFR from cBioPortal. Inset shows number of mutations in a given gene. (B) Kaplan-Meier curves show impact of CD73 expression on the individual cancer drivers with wild-type and altered status on overall survival.

High protein expression of CD73 in KRAS mutated patients compared to wild-type in CRC and NSCLC

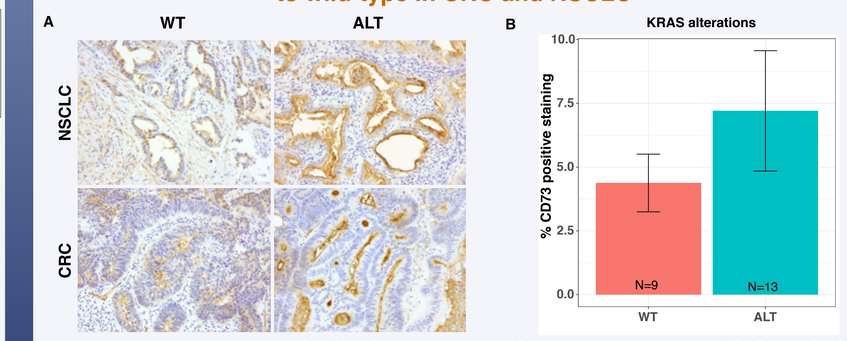


Figure 3. (A) Representative CD73 IHC images for KRAS wild-type and altered NSCLC and CRC patients. (B) Quantification of percent CD73 positive area in CRC and NSCLC patients.

High expression of adenosine fingerprint in KRAS altered versus wild-type in CRC and NSCLC cell lines

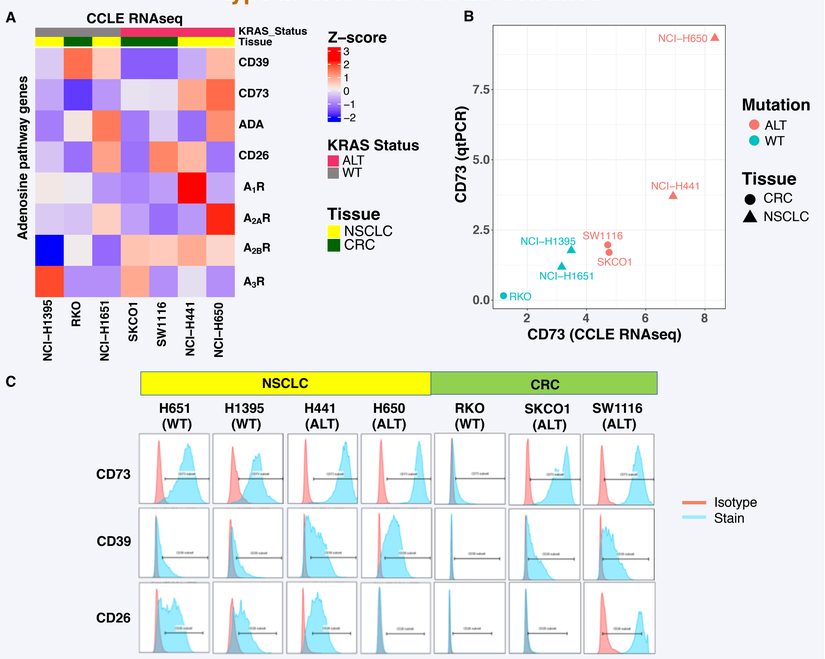


Figure 4. (A) Heatmap of adenosine fingerprint in Broad's CCLE RNAseq data for representative CRC and NSCLC cell lines. (B) Correlation scatterplot of CD73 expression in cancer cell lines from panel A and qPCR. (C) Flow cytometry proteomic validation of CD73, CD39 and CD26 expression in KRAS WT and ALT CRC and NSCLC cell lines.

High expression of adenosine fingerprint and PD1 in T cells correlates with resistance to anti-PD1 in KRAS and EGFR mutant NSCLC

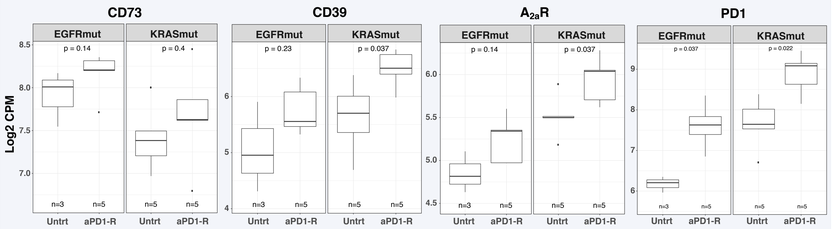


Figure 5. Left to right – RNAseq gene expression (dataset from Koyama *et.al* Nat. Comm. (2015)) of CD73 (Nt5e), CD39 (Entpd1), $A_{2a}R$ (Adora2a) and PD1 (Pdcd1) in T cells derived from either EGFR L858R/T790M mutant or KRAS G12D mutant mouse tumors that were either untreated (Untrt) or resistant to anti-PD1 (aPD1-R). P-values denote significance by Wilcoxon ranked-sum test.

Conclusions

- Alterations in KRAS, BRAF and EGFR as well as pan-Ras classifier score (data not shown) strongly predict high expression of the adenosine fingerprint in pan-cancer TCGA.
- In KRAS, BRAF and EGFR wild-type patients, high CD73 expression is significantly associated with poor prognosis. High CD73 expression is strongly associated with poor prognosis in EGFR and KRAS mutant patients, and good prognosis in BRAF mutant patients.
- Adenosine fingerprint exhibits higher expression in KRAS mutant versus wild-type in CRC and NSCLC patients as well as cell lines at the gene and protein level.
- EGFR mutant NSCLC patients have significantly poor prognosis associated with lower rate of durable clinical benefit with pembrolizumab treatment (data not shown).
- High expression of adenosine fingerprint and PD1 in T cells is associated with resistance to anti-PD1 therapy in mutant KRAS and EGFR murine NSCLC.

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