Discoidin Domain Receptor 1 (DDR1) expression is associated with degree of immune exclusion across epithelial tumors

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Background

- Discoidin domain receptor 1 (DDR1) is highly expressed in epithelial cancers and has been implicated in tumor growth, invasion, and lack of response to therapy.
- DDR1 contributes to immune exclusion by promoting tumor collagen alignment in *in vivo* models. However, it is unclear how DDR1 expression impacts immune cell infiltration in human tumors.
- A first-in-human trial of PRTH-101, a DDR1-targeted therapeutic antibody, is underway.
- Establishing a correlation between DDR1 expression and immune infiltration in the tumor microenvironment (TME) will shed light on the role of DDR1 in the TME and inform indication and patient selection strategies for DDR1-targeted therapies.

Methods

- Adjacent formalin fixed paraffin embedded slides from colorectal, non-small cell lung (NSCLC), ovarian, pancreatic, and triple-negative breast cancers (TNBC) were stained by H&E and a multiplex immunofluorescence (mIF) panel containing DDR1 and immune cell markers CD8 and CD45.
- Tumor-stromal segmentation and cell identification were done from 40X H&E images using AI-powered models developed by PathAI (PathExplore™) or from mIF by image analysis using QuPath. *DDR1* mRNA expression was measured by bulk RNA-sequencing, while DDR1 protein expression was measured by mIF.
- Immune Exclusion Scores (IESs) were calculated for each tumor based on lymphocyte density (from H&E), CD8+ T cell density (from mIF), or CD45+ cell density (from mIF). Each IES is the orthogonal distance between the coordinate of immune cell density in tumor epithelium and stroma, and the regression line representing equal density in epithelium and stroma.

Conclusions

- Here we describe the development of a continuous scoring method which revealed widespread immune exclusion in tumors based on the spatial distribution of lymphocytes, CD8+ T cells, and CD45+ immune cells from H&E and mIF images.
- DDR1 mRNA and protein expression are correlated with immune exclusion at both the pan-cancer and specific indication level.
- This adds additional insight into the role of DDR1 in human cancers and may be useful in selecting indications and stratifying patients for DDR1-targeted therapies.

Results

- Cell density in tumor epithelium and stroma was plotted for lymphocytes (from H&E), CD8+ T cells, and CD45+ immune cell (from mIF). An immune exclusion score (IES) was determined for each sample by calculating the orthogonal distance between the coordinates for each sample and the y=x regression line, which represents a state of equal density of immune cells in the tumor epithelium and stroma, i.e. no immune exclusion (Figure 1, Table 1). More positive IESs represent greater immune exclusion within a sample while negative IESs represent immune infiltrated samples.

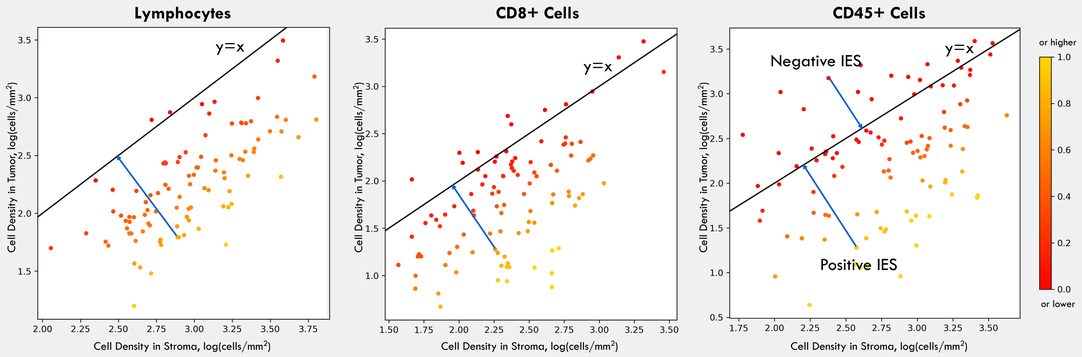

Figure 1: *Plots of immune cell density in tumor epithelium and tumor-associated stroma for lymphocytes, CD8+ cells, and CD45+ cells. The IES for each sample was calculated as the distance to the y=x regression line. Blue arrows are shown for a subset of samples to depict how the IES distance was calculated. Color scale depicts the immune exclusion score with yellow as more excluded (higher IES) and red as more infiltrated (lower IES).*

Immune Exclusion Score (IES) By Cell Type

	N	Mean	SD	Min	Max
Lymphocytes	99	0.51	0.22	-0.06	1.05
CD8+	102	0.40	0.33	-0.25	1.26
CD45+	102	0.36	0.45	-0.69	1.36

Table 1: *Quantification of the immune exclusion score distributions for lymphocyte, CD8+ cells, and CD45+ cells. Mean, standard deviation, minimum, and maximum IES values for each cell type are reported.*

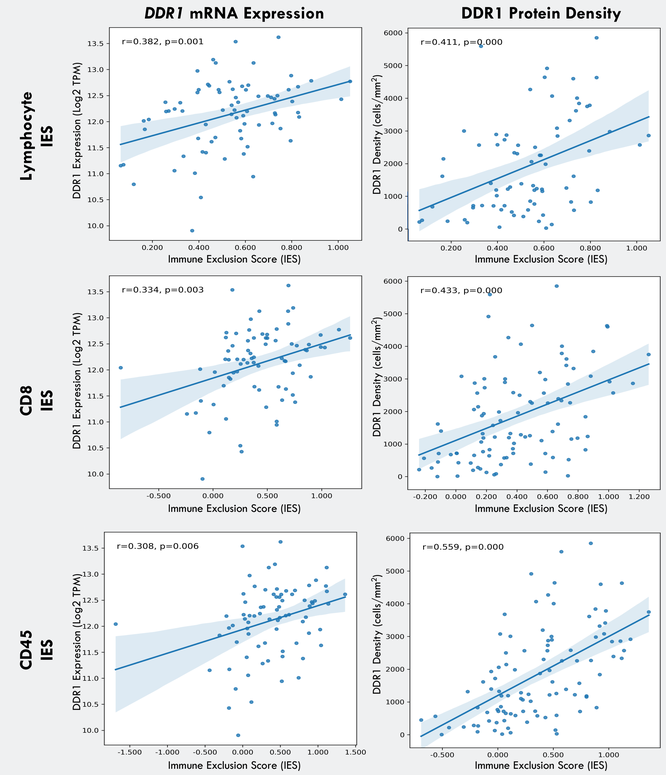

Figure 2: *Correlation between IES scores for Lymphocytes, CD8+ cells, and CD45+ cells and DDR1 mRNA expression or DDR1 protein density. Each dot represents a tumor sample.*

- Immune exclusion (positive IES) was observed in 98%, 90%, and 80% of samples as assessed by distribution of lymphocytes, CD8+ cells, and CD45+ cells, respectively, with lymphocytes showing a higher average IES than CD8+ or CD45+ cells (0.51 vs 0.40 and 0.36, respectively).
- IESs for lymphocytes, CD8+ cells, and CD45+ cells were correlated with DDR1 mRNA and protein expression. Significant correlations were observed across indications for all cell types and both DDR1 mRNA and DDR1 protein (R: 0.31-0.56, adjusted p: <0.001-<0.01) (Figure 2).
- While the degree of correlation varied between tumor indications by IES immune cell type, pancreatic cancer exhibited the strongest correlation between the lymphocyte-based IES and DDR1 mRNA and protein expression (R: 0.48-0.54, adjusted p:0.04-0.07). *DDR1* mRNA was also moderately correlated with lymphocyte IES in NSCLC and TNBC (R: 0.37-0.48, adjusted p: 0.12-0.14).

DDR1-IES Correlations

	Lymphocyte (H&E) IES				CD8+ (mIF) IES				CD45+ (mIF) IES			
	DDR1 mRNA (Log2 TPM)		DDR1 Protein (cells/mm²)		*DDR1* mRNA (Log2 TPM)		DDR1 Protein (cells/mm²)		DDR1 mRNA (Log2 TPM)		DDR1 Protein (cells/mm²)	
	R value	Adj. p	R value	Adj. p	R value	Adj. p	R value	Adj. p	R value	Adj. p	R value	Adj. p
All Indications	0.38	0.000	0.41	0.000	0.33	0.003	0.43	0.000	0.31	0.006	0.56	0.000
Colorectal	0.01	0.970	0.38	0.135	0.27	0.298	0.20	0.436	0.23	0.376	0.07	0.799
NSCLC	0.48	0.120	0.42	0.180	0.43	0.141	0.12	0.702	0.54	0.056	0.43	0.140
Ovarian	0.33	0.230	0.07	0.799	0.10	0.734	-0.05	0.859	0.01	0.972	0.06	0.827
Pancreatic	0.48	0.070	0.54	0.040	0.51	0.051	0.35	0.203	0.37	0.173	0.37	0.176
TNBC	0.37	0.140	0.12	0.645	0.08	0.773	0.18	0.482	0.00	0.993	0.24	0.349

Table 2: *Correlation between DDR1 expression (mRNA and protein) and Immune Exclusion Score (IES) based on immune cell distribution in tumor epithelium and stroma.*

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