Integrative genomic and proteomic analysis identifies cancer subtypes and signaling networks associated with aberrant tumor expression of VISTA

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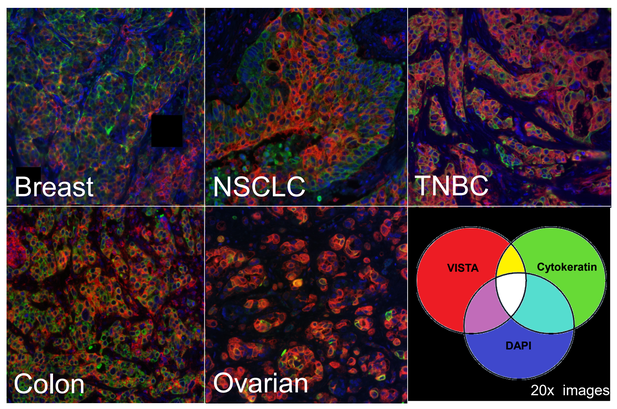
Introduction

VISTA is a negative regulator of T-cell and myeloid cell function and is a target for cancer immunotherapy [1-3]. VISTA is highly expressed in tumor-infiltrating leucocytes, particularly within the myeloid lineage. Recent evidence suggests that tumor cells themselves also express VISTA, exacerbating the immunosuppressive milieu within the tumor microenvironment [4,5]. Determining tumor subtypes that overexpress VISTA can inform the indication selection for VISTA-targeting agents and design of clinical trials in specific patient populations.

Identification of VISTA protein expression in tumor cells by IHC

A.



B.

Tumor Type	Percentage of Cores with Tumor Expression of VISTA
Breast Cancer	4% (15/384)
Colon Cancer	15% (91/597)
Non-Small Cell Lung Cancer	7% (19/254)
Ovarian Cancer	10% (27/275)
Triple Negative Breast Cancer	11% (22/196)

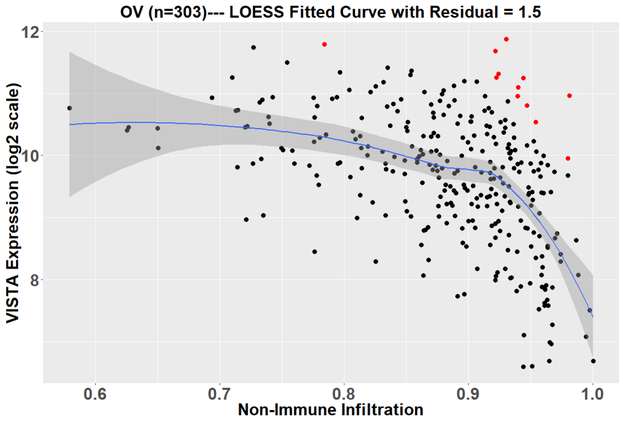
C.

Tumor Type	Percentage of Samples with VISTA Expressing Tumor Cells
Gastric Cancer	8.8% (41/464)[4]
Mesothelioma	96% (25/26)[6]

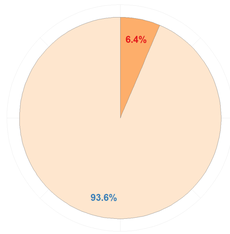
Quantitative immunofluorescence (QIF) of VISTA protein expression was run on cancer tissue microarrays (TMA): breast (n=384), colon (n=597), lung (n=254), ovarian (n=275) and triple negative breast (n=196). Tumor compartment was defined by cytokeratin and DAPI staining. Localization of VISTA protein expression within the tumor compartment was seen in cores across different tumor types tested (Figure A). Percentage of cores demonstrating VISTA expression in tumor cells range from 4-15%, with highest frequency in colon cancer (Table B). Work conducted by Boger et. al. [4] and Zauderer et. al. [6] also show tumor expression of VISTA in gastric cancer and mesothelioma, respectively (Table C).

Genomic approach to identification of tumor cells expressing VISTA

A.



B.



Common tumor types within the 6.4%:
Glioblastoma Multiforme (GBM)
Head and Neck Squamous Cell Carcinoma (HNSC)
Kidney Renal clear cell Carcinoma (KIRC)
Lower Grade Glioma (LGG)
Mesothelioma (MESO)
Sarcoma (SARC)

C.

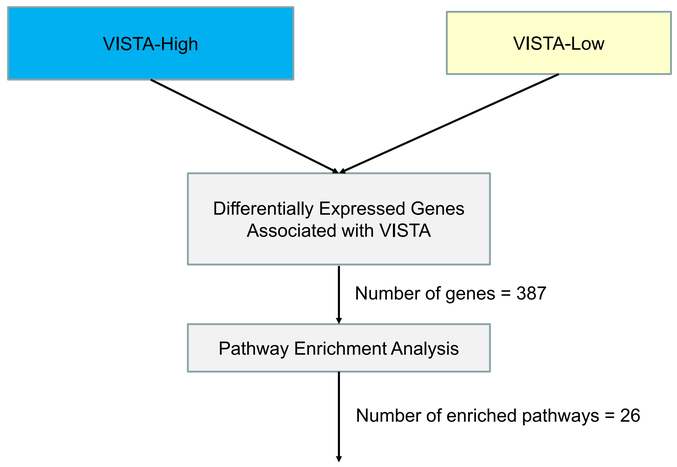
Tumor Type	Percentage of Samples with Tumor VISTA Expression
Colon adenocarcinoma (COAD)	4.03% (17/442)
Breast invasive carcinoma (BRCA)	4.49% (48/1069)
Lung adenocarcinoma (LUAD)	5.10% (26/509)
Ovarian serous cystadenocarcinoma (OV)	3.96% (12/303)

Genome-scale multi-omics data was obtained from the TCGA Pan-Cancer Atlas dataset. Previously published molecular signature was utilized to establish a leukocyte infiltration score for each individual tumor samples [7]. The immune cell component of VISTA expression was modeled using a nonlinear non-parametric regression model (LOESS). Samples which are 1.5 residuals away from the regression curve demonstrating high VISTA expression independent of leukocyte infiltration were selected as likely demonstrating VISTA expression on tumor cells (Figure A). Utilizing this analysis, 703 of the 10,984 tumor samples (6.4%) in the TCGA were identified. VISTA expressing tumors were significantly enriched (hyper-geometric test, P<< 0.01) in several of the 33 cancer types (Figure B). Additionally, subpopulations of other cancers also exhibit VISTA expression in the tumor compartment (Table C).

Summary

Detection of VISTA expressing tumors via two different methods presented here, show similar prevalence in overlapping tumor types in two independent datasets. These methods can be used to provide personalized therapy in the growing immuno-oncology era. For tumor types, such as mesothelioma, where prevalence of VISTA positive tumors are high selection may not be necessary to identify those sensitive to VISTA-targeting agents. In addition, a better understanding of associated pathways may drive better combination strategies

Pathways associated with VISTA expressing tumors



Top VISTA associated pathways	
Pathway	**Hyper-Geometric p-Value**
Arf6 trafficking events	0.013
Effects of Botulinum toxin	0.003
VEGFR1 specific signals	0.014
Lissencephaly gene (LIS1) in neuronal migration and development	0.018
Regulation of Ras family activation	0.020
FAS (CD95) signaling pathway	0.034
EPHB forward signaling	0.039
FOXA1 transcription factor network	0.049
ErbB2/ErbB3 signaling events	0.049

TCGA Pan-Cancer Atlas dataset was further utilized to identify pathways associated VISTA expression on tumor cells. Three hundred eighty-seven genes were selected based on differential gene analysis conducted between sample which either had high or low VISTA expression independent of leukocyte infiltration. These genes were enriched in 26 pathways.

References

1. Lines, J. L., L. F. Sempere, T. Broughton, L. Wang, and R. Noelle. 2014. 'VISTA is a novel broad-spectrum negative checkpoint regulator for cancer immunotherapy', Cancer Immunol Res, 2: 510-7.
2. Nowak, E. C., J. L. Lines, F. S. Varn, J. Deng, A. Sarde, R. Mabaera, A. Kuta, I. Le Mercier, C. Cheng, and R. J. Noelle. 2017. 'Immunoregulatory functions of VISTA', Immunol Rev, 276: 66-79.
3. Gao, J., Ward, J.F., Pettaway, C.A., Shi, L.Z., Subudhi, S.K., Vence, L.M., Zhao, H., Chen, J., Chen, H., Efstathiou, E. and Troncoso, P., 2017. 'VISTA is an inhibitory immune checkpoint that is increased after ipilimumab therapy in patients with prostate cancer'. Nature medicine, 23(5): 551.
4. Boger, C., H. M. Behrens, S. Kruger, and C. Rocken. 2017. 'The novel negative checkpoint regulator VISTA is expressed in gastric carcinoma and associated with PD-L1/PD-1: A future perspective for a combined gastric cancer therapy?', Oncoimmunology
5. Villarroel-Espindolaa, F., Yu, X., Datara, I., Mani, N., Sanmamedc, M., Velcheti, V., Syrigos, K., Toki, M., Zhao, H., Chen, L., Herbst, R.S., and Schalper, K.A. 2018. 'Spatially resolved and quantitative analysis of VISTA/1 PD-1H as a novel immunotherapy target in human non-small cell lung cancer', Clin Cancer Res, 24(7): 1562-1573.
6. Zauderer, M.G., Muller, S., Lai, W.V., Ni, A., Jungbluth, A., Ginsberg, M.S., Daly, R.M., Hellmann, M.D., Ladanyi, M., and Sauter, J.L. VISTA is highly expressed in malignant pleural mesothelioma (MPM) and independent of PD-L1 expression. Poster presented at: International Association for the Study of Lung Cancer. 19th World Conference on Lung Cancer; 2018 Sep 23-26 ;Toronto, Canada.
7. Hoadley, K.A., Yau, C., Hinoue, T., Wolf, D.M., Lazar, A.J., Drill, E., Shen, R., Taylor, A.M., Cherniack, A.D., Thorsson, V., Akbani, R., Bowlby, R., Wong, C.K., Wiznerowicz, M., Sanchez-Vega, F., Robertson, A.G., Schneider, B.G., Lawrence, M.S., Noushmehr, H., Malta, T.M., Cancer Genome Atlas Network, Stuart, J.M., Benz, C.C., and Laird, P.W. 2018. 'Cell-of-origin patterns dominate the molecular classification of 10,000 tumors from 33 types of cancer'. Cell, 173(2): 291-304.

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