MAGEA3, MAGEA4, and PDL1 Show Co-Expression in Colon, Lung, & Bladder Cancer

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Abstract

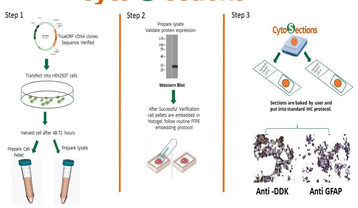
PD-L1 programmed death ligand 1/CD274 has been shown to be an excellent immune therapy target for many tumors. However new immune therapy targets that can work together with PD-L1 are constantly being evaluated. The cancer testis antigen family known as Melanoma Antigen Gene Family (MAGE-A) contains 12 family members shown to have limited expression in normal tissues and high expression in cancer which makes them excellent candidates for immunotherapy targets. Clinical trials have already begun targeting MAGE-A3 and MAGE-A4 proteins in multiple tumor types. Previously we presented CytoSections data showing highly specific MAGE-A3 clone OTIG9 and MAGE-A4 clone OTI2C1 antibodies for immunohistochemistry. In this study, we screened MAGE-A3 clone OTIG9 and MAGE-A4 clone OTI2C1 antibodies on lung, colon, and bladder cancers that have been previously evaluated with PD-L1 expression. The results show more than 70% of the cases express either or both MAGE-A3 and MAGE-A4 in lung, colon, and bladder cancers. The lung and bladder cancers had more than fifty percent of the tumors positive for either MAGE-A3 or MAGE-A4 with PD-L1 however in colon cancer MAGE-A4 was rarely expressed but PD-L1 and MAGE-A3 were frequently detected together.

Introduction

PD-L1 programmed death ligand 1/CD274 has a long history as an immune therapy target for many tumors. Additionally, combination PD-L1 immune therapy with other gene targets expressed in tumors has improved outcomes but the improved outcome is mostly limited to specific tumor types. Thus, additional screens for targets that could be co-expressed with PD-L1 in tumors should be evaluated. Melanoma Antigen Gene Family (MAGE-A) are part of the cancer-testis antigens whose limited expression in normal tissues and high expression in cancer make them excellent targets for immunotherapy. The MAGE-A family have twelve family members and this study looks at 2 members MAGE-A3 and MAGE-A4. Like PD-L1, MAGE-A3 and MAGE-A4 are expressed in many tumor types. Clinical trials have already begun targeting MAGE-A3 and MAGE-A4 proteins in some cancers. In this study, protein expression of MAGE-A3 and MAGE-A4 was done in three tumor types: bladder, colon, and lung cancer to see if these targets are expressed at higher levels. Bladder, colon, and lung tumor types are known to have positive expression of PD-L1 and these positive PD-L1 tumors are treated with PD-L1 immunotherapy. Screens with PD-L1, MAGE-A3, and MAGE-A4 were done by immunohistochemistry (IHC) and found that the MAGE-A3/-A4 proteins can be co-expressed. Previous study presented antibody specificity of MAGE-A3 clone OTIG9 and MAGE-A4 clone OTI2C1 antibodies using CytoSections. In this study, we screened MAGE-A3 clone OTIG9 and MAGE-A4 clone OTI2C1 antibodies on lung, colon, and bladder cancers with anti PD-L1 clone UMAB228 for co-expression. The results show more than 60% of the cases express either or both MAGE-A3 and MAGE-A4 in lung, colon, and bladder cancers. The lung and bladder cancers had more than fifty percent of the tumors positive for either MAGE-A3 or MAGE-A4 were positive for PD-L1. Finally, the CytoSections evaluation, suggest MAGE-A3 and MAGE-A4 are secreted.

Design & Methods

Fig 1 CytoSections



Immunocytochemistry

Tissues in this study are a part of the OriGene tissue bank collection. Tissues were collected from major US medical institutions under strict IRB and ethical consenting practices. Manual IHC staining of paraffin-embedded CytoSections and FFPE tissues using anti MAGE-A3 clone OTIG9, MAGE-A4 clone OTI2C1, and anti PD-l1 clone UMAB228 antibodies. All antibodies required heat induced epitope retrieval HIER using OriGene-Citrate pH6.0 or TEE buffer for MAGEA and PD-L1 antibodies, respectively. OriGene's Polink-1 a one-step anti- mouse polymer HRP detection (Cat# D12-100) and DAB chromogen was used according to manufacturer's protocol. Scoring was based on the percentage of positive cells and intensity.

Fig 2 Specificity Screens of MAGEA3 & MAGEA4 Ab's on MAGEA CytoSections

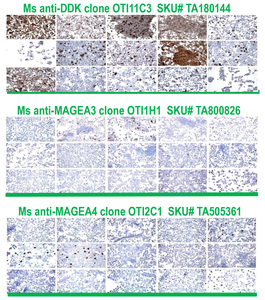


Table 1 MAGEA CytoSections Images Map

MAGEA1-12 CytoSection Map				
MAGE-A1 TS402134	MAGE-A2 TS423561	MAGE-A3 TS403288	MAGE-A4v1 TS418952	MAGE-4v2 TS423938
MAGE-A4v3 TS404482	MAGE-4v4 TS423561	MAGE-A5 TS418575	MAGE-A6 TS423578	MAGE-A8 TS429878
MAGE-A9 TS401760	MAGE-A10 TS402501	MAGE-A11 TS402471	MAGE-A12 TS429868	HEK293T CONTROL

Results

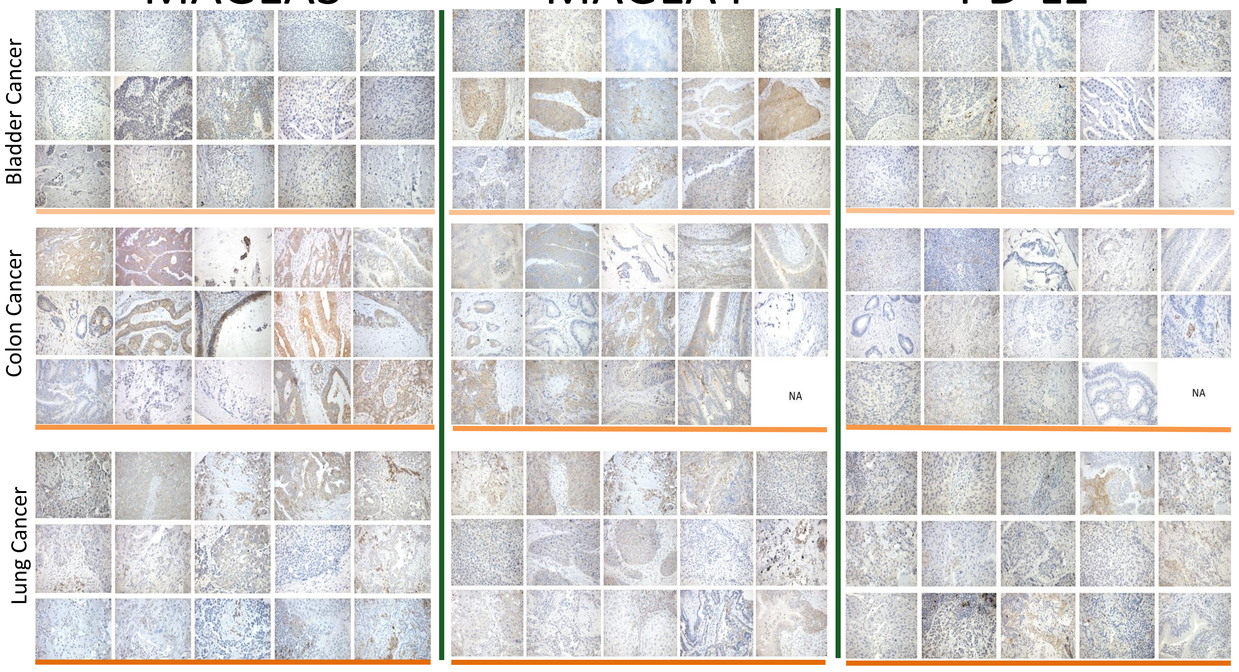
Table-2 IHC Scores

Table-2A Bladder Cancer	MAGEA3 Clone OTI1G9	MAGEA4 Clone OTI2C1	PD-L1 Clone UMAB228
S1	NEG	WEAK POS 70%	POS T & IC
S2	NEG	POS	NEG
S3	NEG	POS	NEG with Rare Pos T
S4	NEG	POS	POS T (LOW)
S5	NEG	POS	POS T
S6	NEG	POS	NEG with Rare Pos T
S7	WEAK POS 70%	POS	POS T & IC
S8	POS	POS	POS T & IC
S9	WEAK POS 25%	POS	NEG with Rare Pos T
S10	NEG	POS	NEG with Rare Pos T
S11	NEG	POS	NEG
S12	WEAK POS 10%	POS	POS T & IC
S13	POS	POS	POS T & IC
S14	POS	POS T & NI adj	POS T & IC
S15	NEG	POS T & NI adj	NEG
S16	NEG	POS T & NI adj	NEG
S17	NEG	WEAK POS 70%	Neg with Rare Pos T
S18	NEG	Neg	NEG
S19	WEAK POS 70%	Neg	POS T & IC
S20	NEG	POS	NEG T & IC POS
S21	NEG	WEAK POS 70%	NEG
S22	NEG	POS	NEG T & IC POS
S23	WEAK POS 50%	WEAK POS 25%	NEG T & IC POS
S24	WEAK POS 50%	POS	NEG T & IC POS

Table-2B Colon Cancer	MAGEA3 Clone OTI1G9	MAGEA4 Clone OTI2C1	PD-L1 Clone UMAB228
S1	POS	NEG	POS T & IC
S2	POS	POS	POS T & IC
S3	POS	NEG	NEG
S4	POS	POS	POS T
S5	WEAK POS 70%	POS	NEG
S6	POS	POS	NEG
S7	POS	NEG	NEG T & IC POS
S8	POS	POS	NEG
S9	POS	POS	NEG
S10	NA	NA	NEG
S11	POS	POS	NEG T & IC POS
S12	NEG	POS	NEG T & IC POS
S13	POS	Weak Pos 70%	POS T & IC
CCTA-2A	POS	NEG	POS T
CCTA-2B	POS	NEG	NA
CCTA-2C	POS	NA	NEG T & IC POS
CCTA-3A	NA	NA	NA
CCTA-3B	POS	NEG	POS T & IC
CCTA-3C	NA	NA	NA
CCTA-4A	POS	POS	POS T & IC
CCTA-4B	POS	Weak Pos 70%	POS T & IC
CCTA-4C	POS	Weak Pos 70%	POS T & IC
CCTA-5A	NA	POS	POS T & IC
CCTA-5C	POS	NA	NA

Table-2C Lung Cancer	MAGEA3 Clone OTI1G9	MAGEA4 Clone OTI2C1	PD-L1 Clone UMAB228
S1	POS	POS	POS T & IC
S2	POS	POS	POS T & IC
S3	POS	POS	POS T & IC
S4	POS	NEG	WEAK T & 3+ IC
S5	POS	NEG	NEG T & IC POS
S6	WEAK POS 70%	NEG	POS T & IC
S7	WEAK POS 70%	NEG	POS T & IC
S8	WEAK POS 70%	POS	POS T & IC
S9	POS	POS	POS T & IC
S10	NEG	NEG	NEG T & IC POS
S11	WEAK POS 70%	POS	POS T & IC
S12	POS	POS	POS T & IC
S13	NEG	WEAK POS 70%	NEG
S14	POS	WEAK POS 70%	NEG
S15	POS	RARE POS T /C POS	NEG T & IC POS
S16	WEAK POS 70%	POS	NEG T & IC POS
S17	WEAK POS 70%	POS	NEG T & IC POS
S18	WEAK POS 70%	POS	NEG T & IC POS
S19	WEAK POS 70%	POS	NEG T & IC POS
S20	NA	WEAK POS 70%	NEG T & IC POS
S21	WEAK POS 70%	WEAK POS 70%	NEG T & IC POS
S22	NEG	POS	POS T & IC
S23	POS	NEG	NEG T & IC POS
S24	NEG	NEG	NEG T & IC POS

Fig 3 IHC images



Conclusion

Table-3 PD-L1 Positive Overlap with MAGE-A3 and MAGE-A4

TOTALS Bladder Cancer	MAGEA3 Clone OTI1G9	MAGEA4 Clone OTI2C1	PD-L1 Clone UMAB228	TOTALS Colon Cancer	MAGEA3 Clone OTI1G9	MAGEA4 Clone OTI2C1	PD-L1 Clone UMAB228	TOTALS Lung Cancer	MAGEA3 Clone OTI1G9	MAGEA4 Clone OTI2C1	PD-L1 Clone UMAB228
NEG	15	2	6	NEG	1	7	5	NEG	4	6	2
WEAK POS or NEG T & IC POS	6	4	9	WEAK POS or NEG T & IC POS	1	3	4	WEAK POS or NEG T & IC POS	9	5	11
POS	3	18	9	POS	18	10	10	POS	10	13	11
POS with PD-L1 POS Tumor	3	8	9	POS with PD-L1 POS Tumor	9	4	10	POS with PD-L1 POS Tumor	6	7	11
POS with PD-L1 POS Immune cell	3	10	10	POS with PD-L1 POS Immune cell	11	5	12	POS with PD-L1 POS Immune cell	9	13	11
POS with PD-L1 T and/or IC	3	13	18	POS with PD-L1 T and/or IC	12	6	14	POS with PD-L1 T and/or IC	9	13	22

- Tumors positive for MAGEA3 and MAGEA4 overlapped with positive PD-L1 tumors.
- CytoSections suggest the many of the MAGE-A family member are secreted proteins.
- MAGE-A3 and MAGE-A4 was detected in adjacent normal tissue. However, MAGE-A3/A4 was not present in tissues negative for MAGEA proteins
- Secretion of MAGE-A3 and MAGE-A4 is consistent with another poster presented by OriGene: **Secretion of MAGE-A3 and MAGE-A4 in cell lines and lung cancer.**