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

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Abstract

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. Clinical trials have already begun targeting MAGE-A3 and MAGE-A4 proteins in tumors. Should these trials lead to new treatment protocols, it is important to develop a diagnostic immunohistochemistry (IHC) tool for pre-screening patients who would benefit. The MAGE-A family consists of 12 members that share up to 80% sequence homology presenting a challenge for finding highly specific antibodies for IHC. Using CytoSections, a new screening control tool for IHC, ICC, IF, and in-situ hybridization, highly specific IHC MAGE-A3 and MAGE-A4 antibodies were developed and screened. The MAGE-A3 and MAGE-A4 antibodies were assessed on twenty-two lung cancers, twenty-one colon cancer, and more than thirty bladder cancers which resulted in cases that co-express MAGE-A3 and MAGE-A4 proteins in all three types of tumors. Using immunofluorescence, tumors positive for both proteins were double stained for MAGE-A3 and MAGE-A4 to show the proteins were co-expressed in the same tumor cells.
Immunohistochemistry continues to be a rapid and reproducible method for the detection of proteins in tumors. Antibodies specific to MAGE-A3 and A4 proteins for IHC may be a useful tool in predicting outcomes or benefits for patients.

Introduction

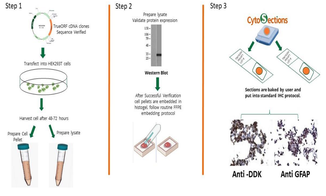
Melanoma associated antigen 3 and 4 (MAGEA3 and MAGEA4) belong to a group of cancer testis antigens (CTA's) whose expression is restricted to germ cells and are not expressed in normal tissues. MAGEA3 and MAGEA4 both have tumor promoting mechanisms. MAGEA3 is known to reduce macro autophagy, alter E3 ubiquitin ligase activity, and cause secretion of proteins like survivin all key for metabolic reprograming and protein expression in tumor cells. While MAGEA4 tumor promoting activity is suggested with the co expression of secreted phosphoprotein-1 (SPP1) regulation of apoptosis and interaction with p53 protein. Several studies have looked at either MAGAE3 or MAGEA4 protein expression in bladder, lung and colon cancers however RNA studies suggest that both proteins may be present in the tumors at the same time. In this study, using highly specific antibodies to MAGEA3 and MAGEA4, as verified by cDNA generated CytoSections co-expression was found in all three cancer types. Additionally, overexpression of MAGEA3 and MAGEA4 in the cDNA CytoSections suggest both these proteins are secreted.

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

Design & Methods

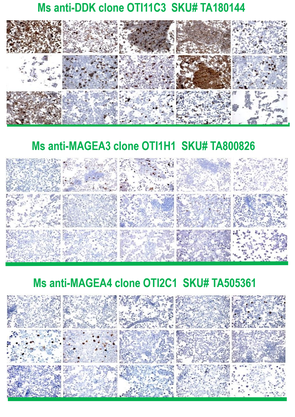
Fig 1



Immunocytochemistry

Manual IHC staining of paraffin-embedded CytoSections and FFPE tissues using anti MAGEA3 and 4 antibodies. Tissues are banked under strict collection protocols and undergo rigorous quality control to ensure each source block's unparalleled quality. All tissues were collected from major US institutions under strict IRB and ethical consenting practices
All antibodies required heat induced epitope retrieval HIER using OriGene-Citrate pH6.0 buffer for all MAGEA antibodies. 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. Tissues were sourced from OriGene Technology's tissue collection. Scoring was based on the percentage of positive cells and not the intensity.

Fig 2 DDK, MAGEA3 & MAGEA4 Ab's on MAGEA CytoSections



Results

Fig 3

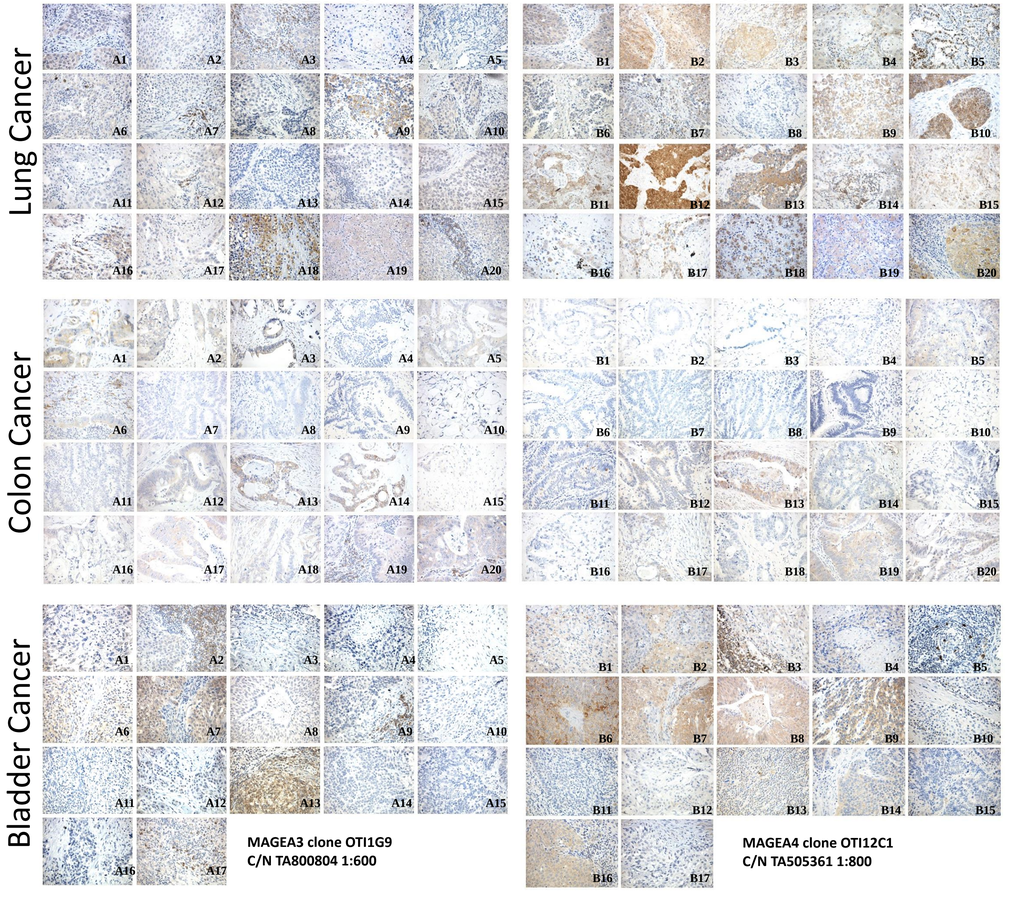
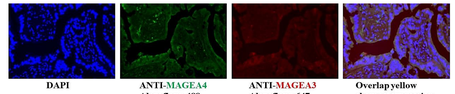


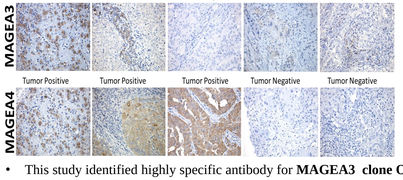
Table 2 MAGEA3 & MAGEA4 Tumor Expression Score

Lung Cancer				Colon Cancer				Bladder Cancer			
	MAGEA3		MAGEA4		MAGEA3		MAGEA4		MAGEA3		MAGEA4
A1	POS	B1	POS	A1	POS	B1	NEG	A1	Weak POS	B1	POS
A2	NEG	B2	POS	A2	POS	B2	NEG	A2	Weak POS	B2	POS
A3	Weak POS	B3	POS	A3	POS	B3	NEG	A3	NEG	B3	POS
A4	NEG	B4	POS	A4	NEG	B4	NEG	A4	Weak POS	B4	POS
A5	NEG	B5	POS	A5	POS	B5	POS	A5	NEG	B5	NEG
A6	Weak POS	B6	NEG	A6	POS	B6	NEG	A6	POS	B6	POS
A7	NEG	B7	POS	A7	NEG	B7	NEG	A7	POS	B7	POS
A8	NEG	B8	NEG	A8	NEG	B8	NEG	A8	Weak POS	B8	POS
A9	POS	B9	POS	A9	NEG	B9	POS	A9	NEG	B9	POS
A10	Weak POS	B10	POS	A10	NEG R+	B10	NEG	A10	NEG	B10	NEG
A11	NEG	B11	POS	A11	NEG	B11	NEG R+	A11	NEG	B11	NEG
A12	Weak POS	B12	POS	A12	POS	B12	Weak POS	A12	Weak POS	B12	Weak POS
A13	NEG	B13	POS	A13	POS	B13	POS	A13	POS	B13	POS
A14	Weak POS	B14	POS	A14	POS	B14	Weak POS	A14	NEG	B14	POS
A15	POS	B15	Weak POS	A15	Weak POS	B15	NEG	A15	NEG	B15	NEG
A16	POS	B16	Weak POS	A16	Weak POS	B16	NEG	A16	NEG	B16	POS
A17	NEG	B17	POS	A17	POS	B17	POS	A17	Weak POS	B17	NEG
A18	POS	B18	POS	A18	POS	B18	NEG	*Note Scores only represent image*			
A19	POS	B19	POS	A19	POS	B19	POS				
A20	Weak POS	B20	POS	A20	POS	B20	POS				

Fig 4 Colon Cancer (A19&B19) using immunofluorescence to co-expression



Conclusion



MAGEA3 clone OTI1G9
C/N TA800804

MAGEA4 clone OTI12C1
C/N TA505361

- This study identified highly specific antibody for **MAGEA3 clone OTI1G9 C/N TA800804** and for **MAGEA4 clone OTI12C1 C/N TA505361** protein screening in FFPE tissues.
- CytoSections cDNA generated targets to all 12 members of the MAGEA Family reduced the time required to find the right tissue and mitigate the use of rare FFPE tissues. CytoSections showed specificity of the MAGEA3 and MAGEA4 antibodies.
- MAGEA3 & MAGEA4 are sometimes co-expressed in lung, colon, and bladder cancer.
- MAGEA3 & MAGEA4 are detected in the infiltrating immune cells, even in areas the tumor is negative for MAGEA3 & MAGEA4.