Colocalization of MAGE-A3 and MAGE-A4 in bladder cancer

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Abstract

The treatment of bladder cancers has improved with new immunotherapy such as PD-L1. However, not all bladder cancers respond to PD-L1 immunotherapy which is why new targets are being assessed. Melanoma-associated antigen gene A (MAGE-A) family proteins are expressed in a variety of tumors, with each MAGE-A protein having unique roles in cancer pathogenesis. One advantage of targeting MAGE-A family members is the lack of expression in normal tissues which makes them well-suited for targeted cancer immunotherapy. The challenge with screening the MAGE-A family is to find a specific antibody since the 12-member family has over 60% sequence in homology. In this study, we evaluate multiple MAGE-A3 and MAGE-A4 antibodies using CytoSections, a reproducible alternative to control tissue with an expression of target biomarkers. A series of antibodies specific to each MAGE-A3 and MAGE-A4 protein were discovered and used to screen twenty-five bladder cancer tissues. The results showed that MAGE-A3 was present in 9 of the 25 bladder cancers, while MAGE-A4 was present in 10 of the 25 bladder cancers evaluated; in the meantime, 7 bladder cancers were positive for both targets after double immunofluorescence staining with MAGE-A3 or A4 specific antibodies. The data presented here could be an important development in treatment protocols for pre-screening patients who are at risk. Our findings show that MAGE-A3 and MAGE-A4 can be both present in bladder cancer, suggesting that targeting both genes may be necessary for the success of immunotherapy treatment.

Introduction

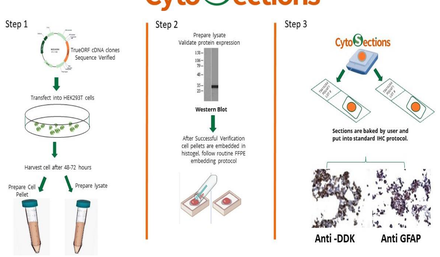
Recently clinical trials have started targeting melanoma associated antigen 3 and 4 (MAGE-A3 and MAGE-A4) in bladder cancer. If the trials are successful, having good diagnostic antibodies for MAGE-A3 or MAGE-A4 proteins will be needed for determining patients' therapy with these targets. Establishing specificity is challenging for genes in the same family, when there are significant overlapping sequences. It has been shown that MAGE-A family members have 50% to 80% sequence overlap. Here we show how testing the specificity of antibody against this large gene family is possible with CytoSections.

Instead of finding unique tissues that express each of the twelve MAGE-A family members individually, CytoSections are used to express each target. Unlike cell lines, which often have protein expression change in culture, CytoSections will remain as a uniform control with set expression levels. Production of CytoSections is illustrated in the cartoon of Figure 1.

Eight different MAGE-A3 and MAGE-A4 antibodies were assessed using CytoSections for specificity to their targets. This resulted in highly specific antibodies for MAGE-A3 and MAGE-A4 being used to screen 17 bladder cancers. The results showed that both proteins can be expressed within the same tumors and did not always overlap in the same cells. Multi-staining was done to evaluate the tumor cell and immune cell expression of MAGE-A3 and MAGE-A4 on the same tissue.

Design & Methods

Figure 1



Immunocytochemistry

Manual IHC staining of paraffin-embedded CytoSections and tissues using anti MAGE-A3 and MAGE-A4 antibodies (Table 1). 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.

Table 1 MAGEA-3, MAGEA-4 Antibodies

MAGE-A3		MAGE-A4	
Ab Clone #	**Ab SKU #**	**Ab Clone #**	**Ab SKU #**
OTI1H1	TA800826	OTI1F9	TA505362
OTI1G9	TA800804	OTI2C1	TA505361
OTIF210	TA800802	OTI5E8	TA505423
OTI1A9	TA800828	OTI1F12	TA505396

Table 2 MAGE-A3 and MAGE-A4 Antibody Detection Pattern on MAGEA1-12 CytoSections

Antibody sku #	OTI1C3	OTIH1	OTI1G9	OTI2F10	OTI1A9	OTI1F9	OTI2C1	OTI5E8	OTI1F12
AntibodyTarget	DDK -	MAGE-A3	MAGE-A3	MAGE-A3	MAGE-A3	MAGE-A4	MAGE-A4	MAGE-A4	MAGE-A4
CytoSection	Dilution = 1:600	Dilution = 1:10000	Dilution = 1:2000	Dilution = 1:2000	Dilution = 1:2000	Dilution = 1:1000	Dilution = 1:10000	Dilution = 1:1000	Dilution = 1:1000
MAGE-A1	95	0	0	5	0	95	0	95	0
MAGE-A2	95	5	0	95	5	0	0	0	0
MAGE-A3	100	10	100	95	95	0	0	95	95
MAGE-A4 v1	100	0	0	95	0	95	0.1	95	95
MAGE-A4 v2	10	0	0	95	0	10	10	10	10
MAGE-A4 v3	10	0.01	0	na	0	10	10	10	10
MAGE-A4 v4	10	0	0	10	0	10	10	10	10
MAGE-A5	10	0	0	95	0	0	0	90	10
MAGE-A6	100	10	10	95	10	0	0	95	10
MAGE-A8	10	0	0	0	0	10	0	10	10
MAGE-A9	10	0	0.1	0	0	5	0	0	0
MAGE-A10	10	0	0	0	0	5	0	0	0
MAGE-A11	10	0.01	0	0.1	0	0	0	5	5
MAGE-A12	10	0	10	3	0	0.1	0	5	5
NEG CONTROL	0	0	0	0	0	0	0	0	0

Results

Figure 2 DDK, MAGE-A3, and MAGE-A4 antibodies on MAGEA1-12 CytoSections

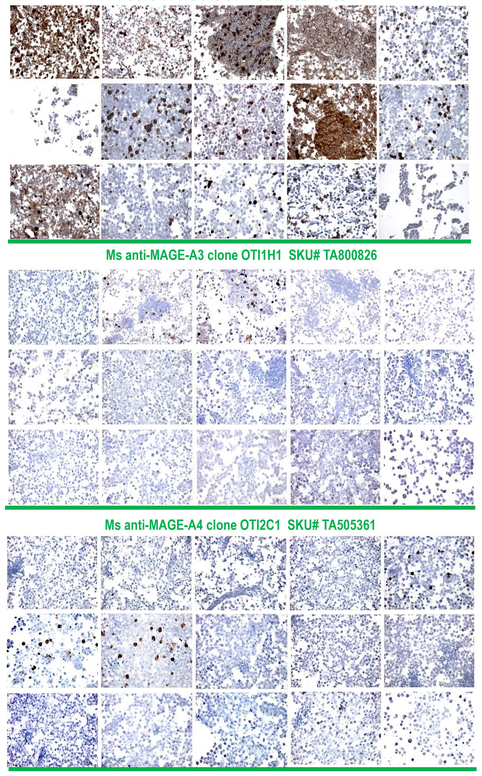
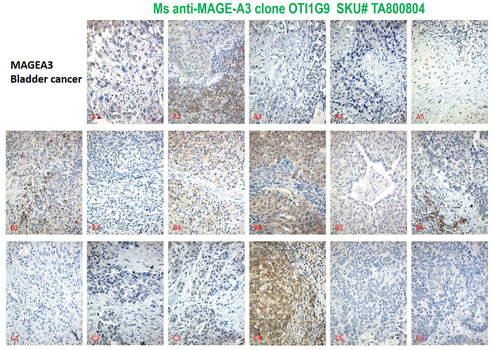


Table 3 MAGE-A Family Member 1-12 CytoSections Images Map for Figure 2

MAGE-A1-12 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

Figure 3 MAGE-A3 and MAGE-A4 antibodies on Bladder Cancer Tissues



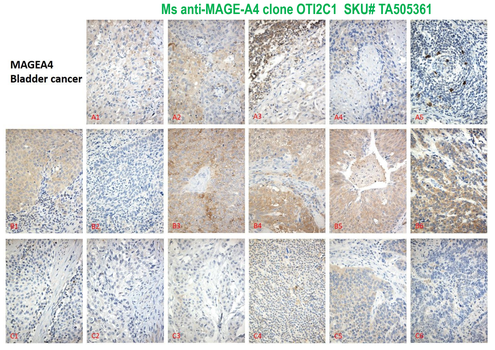
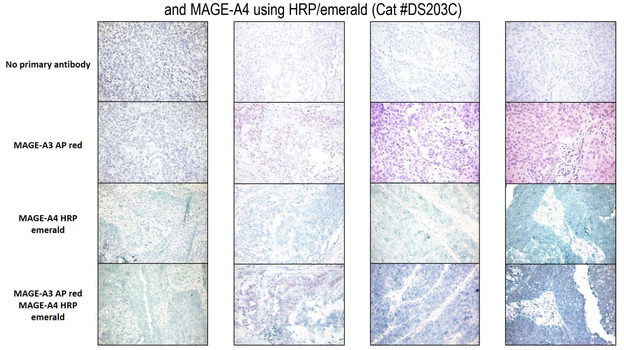


Table 4 MAGE-A3 and MAGE-A4 scoring results for Figure 3

Tumor #	MAGE-A3 Tumor Cells	MAGE-A3 Immune Cells	MAGE-A4 Tumor Cells	MAGE-A4 Immune Cells
A1	Weak Pos	Pos	Pos	Pos*
A2	Weak Pos	Pos*	Pos	Pos
A3	Neg	Pos*	Pos	Pos
A4	Pos	Pos*	Pos	Pos*
A5	Neg	Pos*	Pos	Pos*
B1	Weak Pos	Pos*	Pos	Pos*
B2	Pos	Pos*	Neg	Pos
B3	Pos	Neg	Neg	Pos*
B4	Pos	Pos*	Pos	Pos*
B5	Weak Pos	Pos*	Pos	Pos*
B6	Pos	Pos*	Pos	Pos*
C1	Neg	Neg	Pos	Pos
C2	Weak Pos	Pos*	Pos	Pos*
C3	Pos	Neg	Pos	Pos*
C4	Pos	Pos	Pos	Pos*
C5	Pos	Pos*	Pos	Pos*
C6	Pos	Neg	Pos	Pos

Figure 4 Multi-staining in Bladder Cancer Tissue for MAGE-A3 using AP/red and MAGE-A4 using HRP/emerald (Cat #DS203C)



Conclusion

- Specific antibodies were identified for both MAGE-A3 and MAGE-A4 for screening in tissue
- MAGE-A3 and MAGE-A4 can be co-expressed in bladder cancer, but it is not always expressed in the same cells
- MAGE-A3 and MAGE-A4 are frequently expressed in infiltrating immune cells even when the tumor is negative
- CytoSections can reduce the time required to find the right tissue and mitigate the use of rare and less stable FFPE tissues
- Multi-staining allows for cell by cell differentiation in staining on the same tissue