Expression Pattern of MAGEA3 and MAGEA4 in Lung Cancer

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Abstract

Lung cancer represents 25% of all cancer deaths in the world. Through time, treatments have improved 5-year survival rates for early-stage lung cancer. New treatment targets are needed for advanced stage lung cancers. Melanoma-associated antigen gene A (MAGEA) family proteins expressed in a variety of tumors with each MAGEA protein having unique roles in cancer pathogenesis. One advantage of targeting MAGEA family members is the lack of expression in normal tissues which makes them well suited for targeted cancer immunotherapy for advanced stage cancers. The challenge in screening MAGEA family is to find a specific antibody since the 12-member family has over 60% homology in sequence. In this study, multiple MAGEA3 and MAGEA4 antibodies were evaluated using CytoSections. A specific antibody to each MAGEA3 and MAGEA4 protein was identified, which were used to screen twenty-two non-small cell lung cancer (NSCLC) tissue samples. The screen results showed that MAGEA3 was present in 14 of the 22 lung cancers, while MAGEA4 was present in 10 of the 22 lung cancers evaluated. 3 cancers were strong positives and 4 cancers were weak positives for both targets. Another 4 tumors had positivity for both targets, but the area of positivity did not overlap. These findings show that MAGEA3 was frequently present in the immune cells adjacent to lung cancer while MAGEA4 was only detected 3 times in the immune cells surrounding the tumor.

Introduction

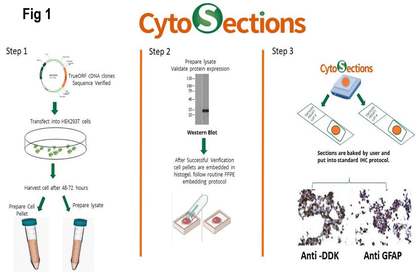
Recently clinical trials have started targeting melanoma associated antigen 3 and 4 (MAGEA3 and MAGEA4) in lung cancer. If the trials are successful, having good diagnostic antibodies for MAGEA3 or MAGEA4 proteins will be needed for determining patients' therapy with these targets. Establishing specificity is challenging for genes in the same family, when there are significant overlapping sequences. It has been shown that MAGEA family members have 60 to 90% sequence overlap. Here we show how testing the specificity of antibody against this large gene family is doable with CytoSections.

Instead of finding unique tissues that express each of the twelve MAGEA 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 MAGEA3 and MAGEA4 antibodies were assessed using CytoSections for specificity to their targets, which resulted in highly specific antibodies for MAGEA3 and MAGEA4, respectively, to screened 22 lung cancers. The results showed MAGEA3 and MAGEA4 can both be present in the lung cancer at the same time. However, many tumors expressed only one protein. Expression of both MAGEA3 and MAGEA4 in the same tumors did not always overlap within the same cells. Greater than 60% of the tumors had MAGEA3 expression in the infiltrating immune cells. This study reveals the complexity of the MAGEA3 and MAGEA4 protein expression in lung cancer.

Design & Methods

Fig 1



Immunocytochemistry

Manual IHC staining of paraffin-embedded CytoSections and tissues using anti MAGEA3 and 4 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, 4, & 9 Antibodies

MAGEA3	MAGEA3	MAGEA4	MAGEA4
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 3 MAGEA3 and 4 Antibody Detection Pattern on MAGEA1-12 CytoSections

Antibody SKU #	OTI11C3	OTI1H1	OTI1G9	OTI2F10	OTI1A9	OTI1F9	OTI2C1	OTI5E8	OTI1F12
Antibody Target	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

Fig 2 DDK, MAGEA3, and MAGEA4 antibodies on MAGEA1-12 CytoSections

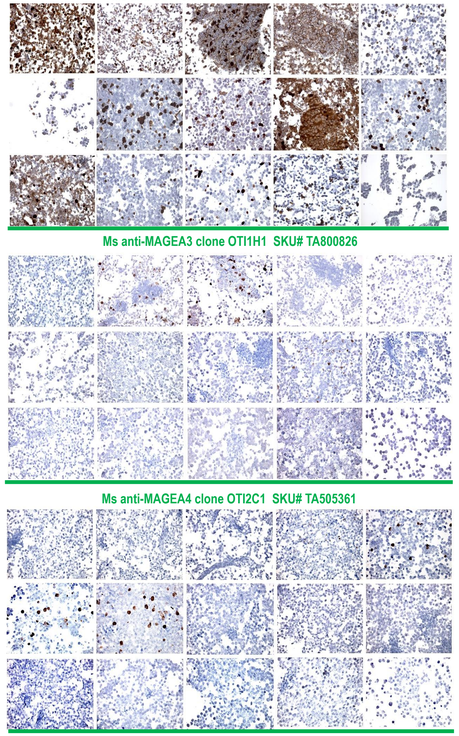


Table 2 MAGEA Family Member 1-12 CytoSections Images Map

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

Fig 3 MAGEA3 and MAGEA4 antibodies on Lung Cancer Tissues

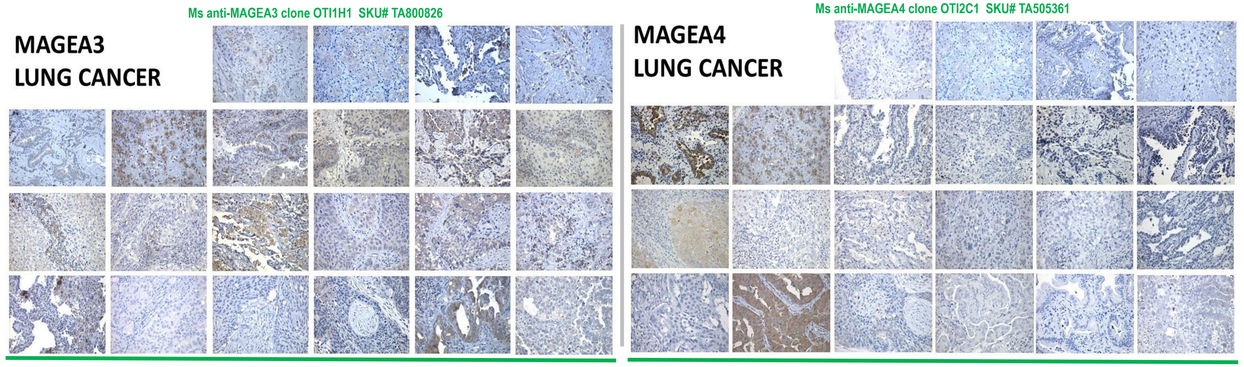
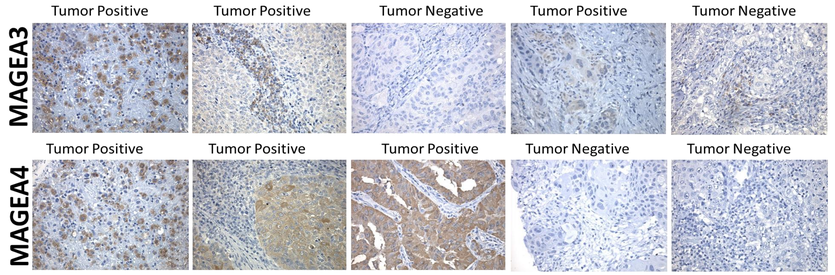


Table 4 Western Anti DDK 1:3000 on 10ug of MAGEA1-12 Lysate

TISSUE	TUMOR	TUMOR	IMMUNE CELLS	IMMUNE CELLS	Tumor Overlap
	MAGEA3	MAGEA4	MAGEA3	MAGEA4	MAGEA3w4
1	POS	NEG	NEG	NEG	DIFFERENT
2	POS	POS	NEG	NEG	YES
3	NEG	POS	NEG	POS	DIFFERENT
4	POS	POS	POS	NEG	YES
5	POS	POS	POS	NEG	DIFFERENT
6	NEG	NEG	POS	NEG	YES
7	NEG	NEG	NEG	POS	DIFFERENT
8	POS	NEG	NEG	NEG	DIFFERENT
9	POS	POS	POS	NEG	DIFFERENT
10	NEG	NEG	POS	NEG	DIFFERENT
11	POS	POS	POS	NEG	YES
12	NEG	NEG	NEG	NEG	DIFFERENT
13	NEG	NEG	POS	NEG	DIFFERENT
14	POS	NEG	POS	NEG	DIFFERENT
15	POS	NEG	POS	NEG	DIFFERENT
16	POS	NEG	POS	NEG	DIFFERENT
17	POS	POS	POS	POS	YES
18	NEG	NEG	NEG	NEG	DIFFERENT
19	NEG	POS	NEG	NEG	DIFFERENT
20	POS	POS	POS	NEG	YES
21	POS	POS	POS	NEG	YES
22	POS	NEG	POS	NEG	DIFFERENT

Fig 4 Examples of Positive and Negative staining in Lung Cancer Tissue for MAGEA3 and MAGEA4



Conclusion

- Specific antibodies were identified for both MAGEA3 and MAGEA4 for screening in tissue
- MAGEA3 and MAGEA4 can be co-expressed in lung cancer, but it is not always expressed in the same cell
- MAGEA3 is frequently expressed in infiltrating immune cells even when the tumor is negative for MAGEA3
- MAGEA4 is rarely expressed in infiltrating immune cells
- CytoSections can reduce the time required to find the right tissue and mitigate the use of rare and less stable FFPE tissues

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