Secretion of MAGE-A3 and MAGE-A4 in cell lines and lung cancer

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Abstract

Melanoma-associated antigen gene A (MAGE-A) family of proteins have previously been shown to express in a variety of tumors, having different roles in cancer pathogenesis. MAGE-A3 and MAGE-A4 have both been shown to inhibit the p53 pathway which leads increased cell growth by stopping apoptosis. Both of these proteins have been associated with cancer progression and poor prognosis. In our studies, we have evaluated multiple MAGE-A3 and MAGE-A4 antibodies using CytoSections. While previous research had only pointed to these proteins being intracellular, our overexpression studies in the development of CytoSections indicated that some MAGE-A proteins were secreted. In this study, ELISA binding assays corroborated that MAGE-A3 and MAGE-A4 were secreted from cells overexpressing these proteins, and serum MAGE-A4 was detected in 24% of lung cancer patients. Flow cytometry data was then collected to see that exogenous MAGE-A proteins were binding to cells in a pattern consistent with secretion data from IHC experiments. Variants of MAGE-A4, differing by only a handful of amino acids, showed very different levels of secretion from one another. MAGE-A3 and MAGE-A6 share 95% homology and were also extremely different in

Introduction

Clinical trials have started targeting melanoma associated antigen 3 and 4 (MAGE-A3 and MAGE-A4) in lung 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, where 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.

The MAGE-A protein family has been shown to have a variety of roles in cancer pathogenesis, with a large focus on its affect on the p53 pathway in inhibiting apoptosis. This has made these proteins a popular target for vaccine therapy, although the underlying biological mechanisms have not been fully studied.

Elevated levels of MAGE-A4 in lung cancer have been reported in the past, but MAGE-A3 had not been studied or detected in lung cancer patients previously. By using OriGene's MAGE-A3 and MAGE-A4 antibodies, we were able to detect the presence of secreted protein in both over-expressing cells and in patient tumor samples, through IHC and ELISA.

Flow cytometry is another powerful tool that can be used to detect the presence of a protein in a cell. Cells that were treated with MAGE-A proteins were tested to evaluate the ability of those cells to bind to the proteins or allow them to pass through the membrane.

Design & Methods

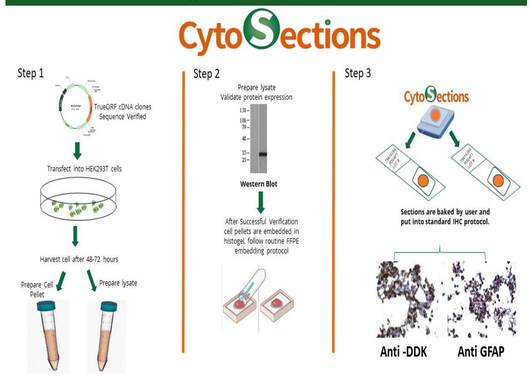


Figure 1 CytoSections production

Immunocytochemistry

Manual IHC staining of paraffin-embedded CytoSections and tissues using anti DDK antibody (TA180144). All antibodies required heat induced epitope retrieval HIER using OriGene-Citrate pH6.0 buffer. 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. CytoSections were generated using the process shown in Figure 1.

ELISA

HEK293T cells in a 10-cm cell culture dish were transiently transfected using PEI and MAGE-A3 or MAGE-A4 TrueORF cDNA plasmid. Supernatants of transient transfections were collected after 12hrs, 24hrs, and 48hrs. MAGE-A3 and MAGE-A4 as well as serum samples from 21 lung patients and 30 non-cancer patient controls (BiolVT) were measured using the MAGE-A3 and MAGE-A4 Sandwich ELISA we developed, picking the antibodies with the least cross-reactivity. Data was analyzed using Student's t-test.

Flow Cytometry

HEK293T cells in a 10-cm cell culture dish were transiently transfected using PEI and MAGE-A3 or MAGE-A4 TrueORF cDNA plasmid. Media from the transfection was collected and concentrated before adding to untransfected cells, to treat the cells with secreted MAGE-A3 or MAGE-A4 proteins. After 48hrs, cells were harvested and then prepared by fixing in 10% NBF for 2 hours, before blocking with FACS blocking buffer (1% BSA, 2% FBS, 0.002% NaN_3, 1X PBS) Flow cytometry was run using FITC conjugated Anti-DDK antibody at a 1:1000 dilution in FACS dilution buffer (0.1% NaN_3, 3% BSA, 1X PBS). Samples were gated using data from the negative control and absorbance in fluorescence channel 1 was detected.

Results

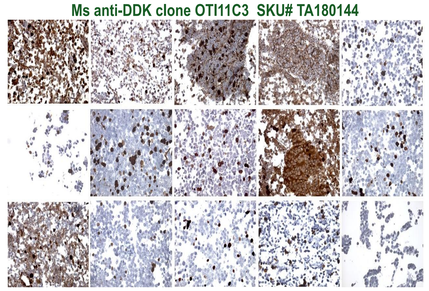


Figure 2 DDK antibody on MAGEA1-12 CytoSections

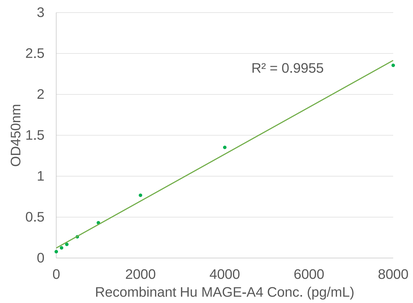


Figure 3 Standard curve for Table 2 and Table 3

MAGE-A4 Standards	450nm absorbance
Standard 1 (8000 pg/mL)	2.355
Standard 2 (4000 pg/mL)	1.352
Standard 3 (2000 pg/mL)	0.767
Standard 4 (1000 pg/mL)	0.431
Standard 5 (500 pg/mL)	0.259
Standard 6 (250 pg/mL)	0.167
Standard 7 (125 pg/mL)	0.124
Standard 8 (0 pg/mL)	0.078

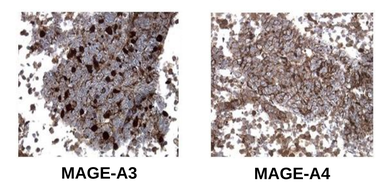
Table 2 Example ELISA data at 450nm

MAGE-A1-12 CytoSection Map				
MAGE-A1 (TS402134)	MAGE-A2 (TS423561)	MAGE-A3 (TS403288)	MAGE-A4v1 (TS418952)	MAGE-A4v2 (TS423938)
MAGE-A4v3 (TS404482)	MAGE-A4v4 (TS423561)	MAGE-A5 (TS418575)	MAGE-A6 (TS423578)	MAGE-A8 (TS429878)
MAGE-A9 (TS401760)	MAGE-A10 (TS402501)	MAGE-A11 (TS402471)	MAGE-A12 (TS429868)	HEK293T CONTROL (TC400001)

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

Sample	MAGE-A3 (pg/uL)	MAGE-A4 (pg/uL)
12hr Culture Supernatant	ND	ND
24hr Culture Supernatant	25	ND
48hr Culture Supernatant	570	1153

Table 3 MAGE-A3, and MAGE-A4 supernatant ELISA results using MAGE-A3 and MAGE-A4 Sandwich ELISA, respectively



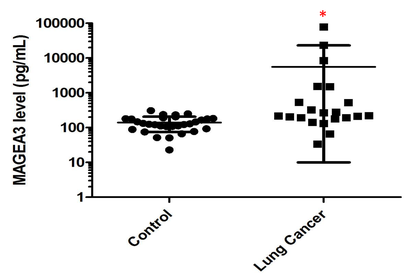
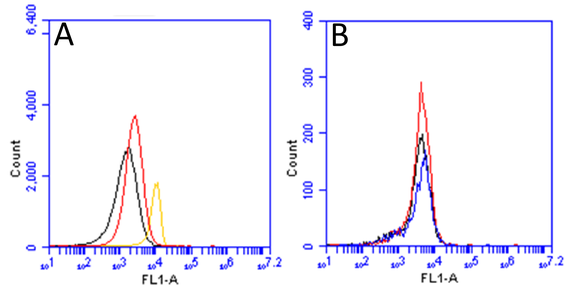


Figure 4 Serum MAGE-A3 concentration in control and lung cancer patients

* p<0.05 compared to control group



	Median
Negative control	1,402.0
MAGE-A3 media	2,406.0
MAGE-A3 lysate	9,780.0

	Median
Negative control	3,752.0
MAGE-A4v1 media	4,097.0
MAGE-A4v2 media	4,057.0

Figure 5 MAGE-A3, and MAGE-A4 flow cytometry results using FITC and median fluorescence channel 1 absorbance (FL1-A).
A. Comparison of FL1-A of cells in media containing secreted MAGE-A3 from transfection to negative control and MAGE-A3 over-expression protein lysate.
B. Comparison of FL1-A of cells in media containing secreted MAGE-A4 variant 1 and MAGE-A4 variant 2 to negative control

Conclusion

- Specific antibodies were identified for both MAGE-A3 and MAGE-A4 for screening in tissue
- CytoSections can reduce the time required to find the right tissue and mitigate the use of rare and less stable FFPE tissues
- MAGE-A3 and MAGE-A4 are secreted from over-expressed cells and serum of lung cancer patients
- Serum MAGE-A3 levels were significantly elevated in lung cancer patients
- MAGE-A3 and MAGE-A4 infiltrate cells and can be detected in them using flow cytometry
- MAGE-A3 and MAGE-A4 may serve as potential prognostic biomarkers for lung cancer
- Future studies expanding on the amount of MAGE-A3 and MAGE-A4 secreted from cells should be done to quantify the peak shift in flow cytometry, as well as using different fluorescent channels