B-047

Serum MAGE-A3 Is a Potential Prognostic Marker for Prostate Cancer

Dehe Kong[1], Jina Yom[1], Tianli Qu[1], Zhaoying Guo[1], Xiaomin Hu[2], Qi Ren[2], Eden Zewdu[1], Anh-Thu Tran[1], Rachel Gonzalez[1], Wei Fu[1]

1) OriGene Technologies Inc.; 9620 Medical Center Drive, Suite 201, Rockville MD 20850

2) OriGene Wuxi Biotechnology Co., Ltd; 168 Mashan Meiliang Road, Binhu District, Wuxi, China

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Introduction

Melanoma-associated antigen 3 (MAGEA3) is a member of Melanoma Antigen Gene family. MAGEA3 protein has restricted expression to the testis and is aberrantly expressed in cancer cells. MAGEA3 has been found to be broadly expressed in a variety of malignancies, including melanoma, breast cancer, head and neck cancer, skin squamous cell carcinoma, gastric cancer, colorectal cancer, lung cancer, prostate cancer, etc. (1). Many researches indicated that MAGEA3 expression is associated with hallmarks of aggressive cancer (2). MAGEA3 expression in cancer cells has been shown to be associated with poor prognosis, for example, MAGEA3 expression in non-small cell lung cancer was found to be significantly correlated with decreased survival of patients, and MAGEA3 expression in breast cancer and prostate cancer is significantly associated with advanced tumor grade, and correlated with worse outcome (3-6). This study is to investigate whether serum MAGEA3 can be used as a biomarker in prostate cancer patient. Due to the high sequence similarity of MAGEA3 with other MAGEA family proteins (Table 1), antibodies with high specificity for MAGEA3 protein is difficult to generate. To identify antibodies with the least cross-reactivity to other MAGEA family proteins, 7 best antibodies from OriGene MAGEA3 antibody library that recognize epitopes on MAGEA3 protein were screened using cell lysates over-expressing different MAGEA family protein by western blot. A high sensitivity human MAGEA3 Sandwich ELISA was developed using one antibody pair with the least cross-reactivity with other MAGEA family proteins and MAGEA3 level was measured in 31 serum samples from prostate cancer patients.

Table 1.Homology of human MAGEA3 protein with other human MAGEA family proteins

Description	Max Score	Query Cover	Per. identity	Acc. Len	Accession #
MAGEA6	598	100%	95.86	314	P43360.1
MAGEA12	548	100%	85.35	314	NP_005358.2
MAGEA2	515	100%	84.39	314	NP_001373059.1
MAGEA4	416	97%	68.63	317	P43358.2
MAGEA1	377	96%	67.22	309	NP_004979.3
MAGEA8	363	99%	63.69	318	NP_001159873.1
MAGEA11	358	99%	59.87	429	P43364.2
MAGEA9	320	100%	59.62	315	NP_005356.1
MAGEA10	288	100%	47.79	369	P43363.2
MAGEA5	172	39%	71.77	124	P43359.1

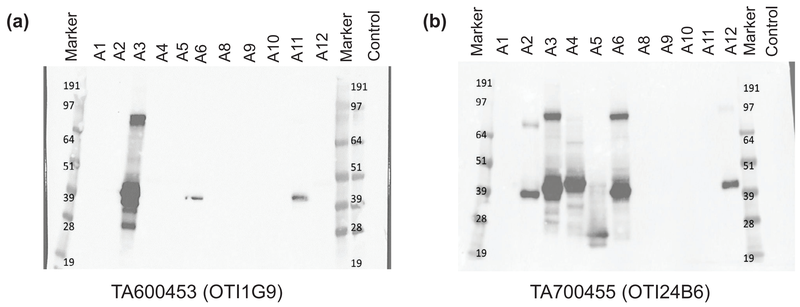
Methods

1. **Preparation of Cell Lysates over-expressing MAGEA family protein:** HEK293T cells in 10-cm dishes were transiently transfected with MegaTran Transfection Reagent (TT200002) and 5µg TrueORF cDNA plasmid. Transfected cells were cultured for 48hrs before collection. The cells were lysed in modified RIPA buffer (25mM Tris-HCl pH7.6, 150mM NaCl, 1% NP-40, 1mM EDTA, 1xProteinase inhibitor cocktail mix (Sigma), 1mM PMSF and 1mM Na3VO4), and then centrifuged to clarify the lysate. Overexpressed MAGEA family protein in cell lysates was confirmed by Western blot.
2. **MAGEA3 Antibody Screen:** 7 best mouse monoclonal antibodies from OriGene MAGEA3 antibody library that recognize epitopes on MAGEA3 protein were screened using cell lysates over-expressing different MAGEA family protein by Western blot.
3. **Sandwich ELISA Development:** Recombinant human MAGEA3 protein from OriGene and one MAGEA3 antibody pair with the least cross-reactivity with other MAGEA family proteins were used for Sandwich ELISA development. Assay characteristics such as LOD, LOQ, recovery, linearity and specificity were analyzed.
4. **Serum MAGEA3 measurement:** Serum samples from 31 prostate cancer patients and 24 non-cancer patient controls (BioIVT) were measured using the MAGEA3 Sandwich ELISA we developed. Data was analyzed using Student's t-Test.

Results

Human MAGEA3 antibody screen results:

Figure 1. Binding activity of MAGEA3 antibody pair with MAGEA family proteins on Western Blot



TA600453 (OTI1G9) TA700455 (OTI24B6)

Table 2 Summary of Binding Activity of MAGEA3 Antibodies with MAGEA Family Proteins

MAGEA3 Ab	MAGEA1	MAGEA2	MAGEA3	MAGEA4	MAGEA5	MAGEA6	MAGEA8	MAGEA9	MAGEA10	MAGEA11	MAGEA12
TA600452 (OTI1A10)	-	++	+++++	++++	+	+++	-	-	-	-	+++
TA700452 (OTI2B1)	-	++	++++	++++	+	++++	-	-	-	-	++++
TA600453 (OTI1G9)*	-	-	++++++	-	-	+	-	-	-	++	-
TA700453 (OTI2F10)	-	++	++++++	++++	+++	++++	-	-	-	-	++
TA700454 (OTI1H1)	-	++	++++++	-	-	+++	-	-	-	-	-
TA600455 (OTI4E2)	-	++	++++++	++++	+++	++++	-	-	-	-	+
TA700455 (OTI24B6)*	-	++	++++++	++++	+	++++	-	-	-	-	++

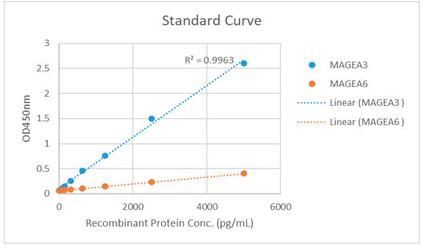
* Antibody pair TA600453 and TA700455 were chosen for ELISA development.

Performance of Human MAGEA3 Sandwich ELISA

Table 3 Example Data at 450nm.

Standards	450nm absorbance	
	MAGEA3	MAGEA6
5000 pg/mL	2.606	0.404
2500 pg/mL	1.494	0.234
1250 pg/mL	0.758	0.146
625 pg/mL	0.454	0.106
312.5 pg/mL	0.256	0.079
156 pg/mL	0.151	0.070
78 pg/mL	0.108	0.070
0 pg/mL	0.062	0.066

Figure 2. Standard Curve



Assay Sensitivity: 12.86pg/mL

Specificity: This assay recognizes recombinant human MAGEA3 and MAGEA6. Recombinant MAGEA6 cross-reacts approximately 11% in this assay. No significant cross-reactivity was observed in other MAGEA family proteins.

Table 4. Assay Recovery

Sample	Average Recovery	Range
Hu serum	103%	102-103%
Hu EDTA Plasma	101%	100-101%
Culture Media	107%	105%-109%

Table 5. Assay Linearity

	Cell culture media	EDTA-plasma	Serum
%Expected at 1:2 dilution	107.82	103.72	103.26
%Expected at 1:4 dilution	109.53	95.79	103.4
%Expected at 1:8 dilution	110.55	92.16	89.59

Table 6. Intra-assay Precision

Sample	%CV in Assay 1	%CV in Assay 2	%CV in Assay 3	Ave %CV
Serum (n=10)	3.14	2.69	7.19	4.34
EDTA-Plasma (n=10)	2.14	2.11	3.13	2.46
Culture Media (n=10)	6.57	5.52	10.91	7.67

Table 7. Inter-assay Precision

Sample	Mean (pg/ml) assay1	Mean (pg/ml) assay2	Mean (pg/ml) assay3	Ave (pg/ml)	SD	%CV
Serum (n=10)	1151.50	1186.26	1173.70	1170.49	17.60	1.50
EDTA-Plasma (n=10)	927.24	972.47	956.96	952.22	22.99	2.41
Culture Media (n=10)	358.09	402.78	419.52	393.46	31.75	8.07

Results

Serum MAGEA3 measurement results:

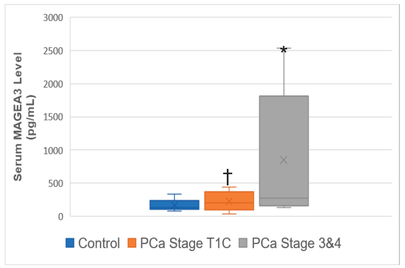


Figure 3. Serum MAGEA3 concentration in control and prostate cancer patients.
*: p<0.05 compared to Stage T1C and control groups
†: p>0.05 compared to control group

Table 8. Summary of serum MAGEA3 levels in prostate cancer patients and controls

		Serum MAGEA3 Levels				Student's t-test
Sample Type	Sample No.	Range	Median	Mean	SD	p value
PCa	31	36-2532 pg/mL	247	447	616	<0.05 ‡
PCa Stage 3&4	11	130-2532 pg/mL	270	850	909	<0.05*
PCa Stage T1C	20	36-444 pg/mL	205	225	137	>0.05†
Control	24	84-337 pg/mL	136	168	76	

‡: p<0.05 compare to Control group; †: p>0.05 compare to control group; *: p<0.05 compare to Stage T1C and control groups.

Conclusions

1. The Human MAGEA3 Sandwich ELISA we developed is highly sensitive (assay sensitivity < 13 pg/mL).
2. The assay has no significant cross-reactivity with other MAGEA family proteins except about 11% cross-reactivity with recombinant human MAGEA6 protein.
3. The serum MAGEA3 levels in prostate cancer patients, especially in late-stage prostate cancer patients were significantly elevated compared with those of the controls.
4. The serum MAGE-A3 levels in early-stage prostate cancer patients have no significant change compared to those of the controls.
5. Our results suggest that serum MAGEA3 is a promising prognostic biomarker for prostate cancer.
6. Future studies should investigate more serum samples from different stages especially late stage prostate cancer patients and whether serum MAGEA3 could be used to monitor therapeutic effects.

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