MAGE-A3 expression with MMR-MSH6 screening suggests MAGE-A3 as a potential target for microsatellite-stable colorectal cancer

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Abstract

Colorectal Cancer (CRC) is the second leading cause of death worldwide. Recent developments in cancer immunotherapy have obtained encouraging clinical responses, including PD-1/PD-L1 blockade therapy, although response rates vary depending on tumor type. For example, despite the lack of response by microsatellite-stable CRC, a subset of CRC with deficient mismatch repair (dMMR) and high microsatellite instability (MSI-H) responds especially well to the PD-1/PD-L1 blockade therapy. For other types of CRC which do not respond to PD-L1 therapy, new targets needs to be investigated. One family of targets is the cancer testis antigen MAGE-A whose expression is restricted to germline cells in normal tissue but is overexpressed in many cancer cells. In this study, immunohistochemical analysis of MAGE-A3 expression in colon cancer along with IHC screening of mismatch protein MSH6 was performed. Many of the cases in this study observed a co-expression in the tissue. In the tumors, MAGE-A3 was observed to have weak cytoplasmic expression while MSH6 had strong nuclear expression in the epithelial cells. The immune cells adjacent to the epithelial cells were also positive for MAGE-A3. This result suggests that MAGE-A3 may be a promising target for microsatellite-stable colorectal cancer immunotherapy.

Introduction

Recent reports have shown that colorectal cancer with mismatch repair (MMR) mutations can benefit from PD-1/PD-L1 blockade therapy. The four main mismatch repair proteins (MSH2, MSH6, PMS2, MLH1) play an essential role in maintaining genome integrity during DNA replication by repairing mismatched DNA bases. Deficient MMR (dMMR) will lead to carcinogenetic pathway called microsatellite instability (MSI) due to DNA polymerase slippage and is defined by a lack of detectable MMR expression by immunohistochemistry. Despite favorable results with immunotherapy in patients with MSI/dMMR tumor, the 85% of colorectal cancer patients without dMMR did not have much success with PD-1/PD-L1 blockade therapy.

Here we look at Melanoma-associated antigen gene A (MAGEA3) as a potential target for MSH6 microsatellite-stable tumors. MAGEA family of proteins are expressed in variety of tumors with each MAGEA protein having unique roles in cancer pathogenesis. The challenge in looking at a specific MAGEA protein arises in the 12-member family having over 60% sequence homology. Instead of finding unique tissue that express each of the twelve MAGEA family member individually, a specific antibody for MAGEA3 was found using CytoSections (Figure 2), which was then used along with an anti-MSH6 antibody to show the overlap of MAGEA3 and MSH6 expression in 23 colon cancers.

Design & Methods

Immunocytochemistry

Manual IHC staining of paraffin-embedded CytoSections and tissues using anti MAGEA3 (Table 1). All antibodies required heat induced epitope retrieval HIER using OriGene-Accel pH9.0 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. Scoring was based on the percentage of positive cells and not the intensity.

Manual IHC staining of paraffin-embedded colon cancer tissues using anti MAGEA3 and MSH6 antibodies (Figure 5). All antibodies required heat induced epitope retrieval HIER using OriGene-Accel buffer (Cat# B22C-1L). Anti-MAGEA3 was incubated for 3 hours at 1:300 in room temperature and anti-MSH6 was incubated for overnight at 1:50 in 4C. OriGene Double Staining Kit for 2 mouse primary antibodies on human tissue (Cat# DS203A) according to manufacturer's protocol. Tissues were sourced from OriGene Technology's tissue collection.

Results

Fig 1

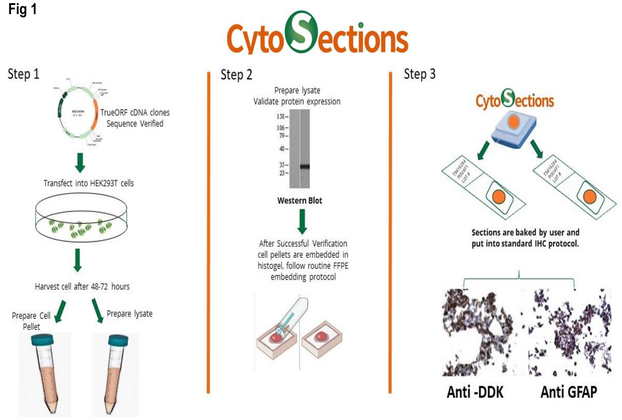
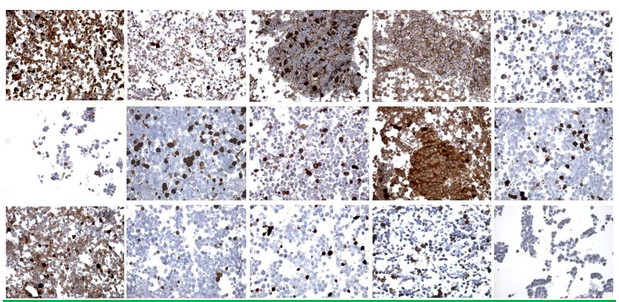


Table 1 MAGEA Family Member 1-12 CytoSections Images Map

MAGEA1-12 CytoSection Map				
MAGEA1 TS402134	MAGEA2 TS423561	MAGEA3 TS403288	MAGEA4v1 TS418952	MAGEA4v2 TS423938
MAGEA4v3 TS404482	MAGEA4v4 TS423561	MAGEA5 TS418575	MAGEA6 TS423578	MAGEA8 TS429878
MAGEA9 TS401760	MAGEA10 TS402501	MAGEA11 TS402471	MAGEA12 TS429868	HEK293T CONTROL

Fig 2 DDK and MAGEA3 antibodies on MAGEA1-12 CytoSections

Ms anti-DDK clone OTI1C3 SKU# TA180144



Ms anti-MAGEA3 clone OTI1H1 SKU# TA800826

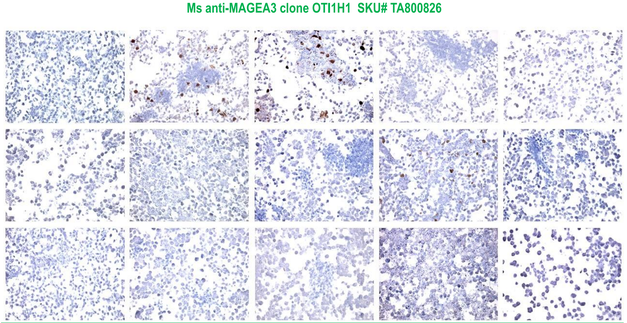


Table 2 MAGEA3 and 4 Antibody Detection Pattern on MAGEA1-12 CytoSections

Antibody sku #	OTI11C3	OTI1H1	OTI1G9	OTI2F10	OTI1A9
AntibodyTarget	DDK -	MAGE-A3	MAGE-A3	MAGE-A3	MAGE-A3
CytoSection	Dilution = 1:600	Dilution = 1:10000	Dilution = 1:2000	Dilution = 1:2000	Dilution = 1:2000
MAGE-A1	95	0	0	5	0
MAGE-A2	95	5	0	95	5
MAGE-A3	100	10	100	95	95
MAGE-A4 v1	100	0	0	95	0
MAGE-A4 v2	10	0	0	95	0
MAGE-A4 v3	10	0.01	0	na	0
MAGE-A4 v4	10	0	0	10	0
MAGE-A5	10	0	0	95	0
MAGE-A6	100	10	10	95	10
MAGE-A8	10	0	0	0	0
MAGE-A9	10	0	0.1	0	0
MAGE-A10	10	0	0	0	0
MAGE-A11	10	0.01	0	0.1	0
MAGE-A12	10	0	10	3	0
NEG CONTROL	0	0	0	0	0

Fig 3 DS203A Kit Provides a Reliable Way to Stain with Two Mouse Primary Antibodies

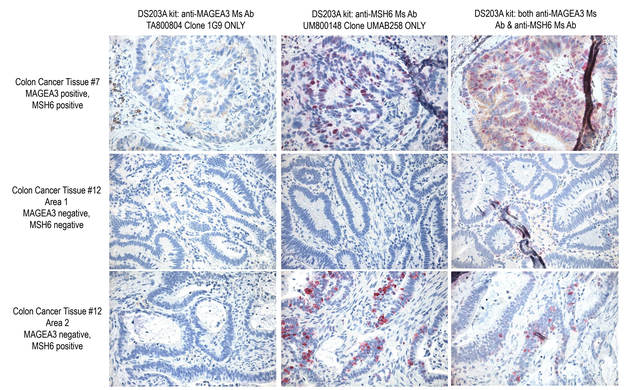


Table 3 Double Staining with TA800804 and UM800148 Scoring

Colon Cancer Tissue	MSH6 (AP) Scoring	MAGEA3 (DAB) Scoring
1	3 (95% of the tissue)	2- (95% of the tissue)
2	2+ (80% of the tissue)	1- (40% of the tissue) 2 (20% of the tissue)
3	2+ (100% of the tissue)	1+ (50% of the tissue)
4	3 (90% of the tissue)	2+ (70% of the tissue)
5	3 (95% of the tissue)	2+ (70% of the tissue)
6	3 (10% of the tissue)	3+ (90% of the tissue)
7	2+ (75% of the tissue)	1-- (10% of the tissue)
8	3+ (40% of the tissue)	2 (98% of the tissue)
9	3 (80% of the tissue) 2 (10% of the tissue)	1- (10% of the tissue)
10	3 (90% of the tissue)	2 (70% of the tissue)
11	1- (80% of the tissue)	negative
12	3 (3% of the tissue) 2- (10% of the tissue)	1 (5% of the tissue)
13	3 (25% of the tissue) 2 (25% of the tissue)	2- (30% of the tissue)
14	3+ (95% of the tissue)	2- (100% of the tissue)
15	3 (50% of the tissue)	2- (50% of the tissue)
16	2+ (70% of the tissue)	3- (70% of the tissue)
17	3 (10%of the tissue) 2 (40% of the tissue)	1+ (50% of the tissue)
18	3 (50% of the tissue)	2- (70% of the tissue)
19	3 (70% of the tissue)	3- (80% of the tissue)
20	2+ (40% of the tissue)	2+ (60% of the tissue)
21	2+ (20% of the tissue)	2 (60% of the tissue)
22	3 (10% of the tissue) 2 (25% of the tissue)	2 (80% of the tissue)
23	3 (90% of the tissue)	2+ (100% of the tissue)

Fig 4 Colon Cancer Double Staining with DS203A Kit Using Two Mouse Primary Antibodies

OriGene Double Stain Kit
DS203A
(2 Ms Primary Abs)
TA800804 Clone 1G9
UM800148
Clone UMAB258

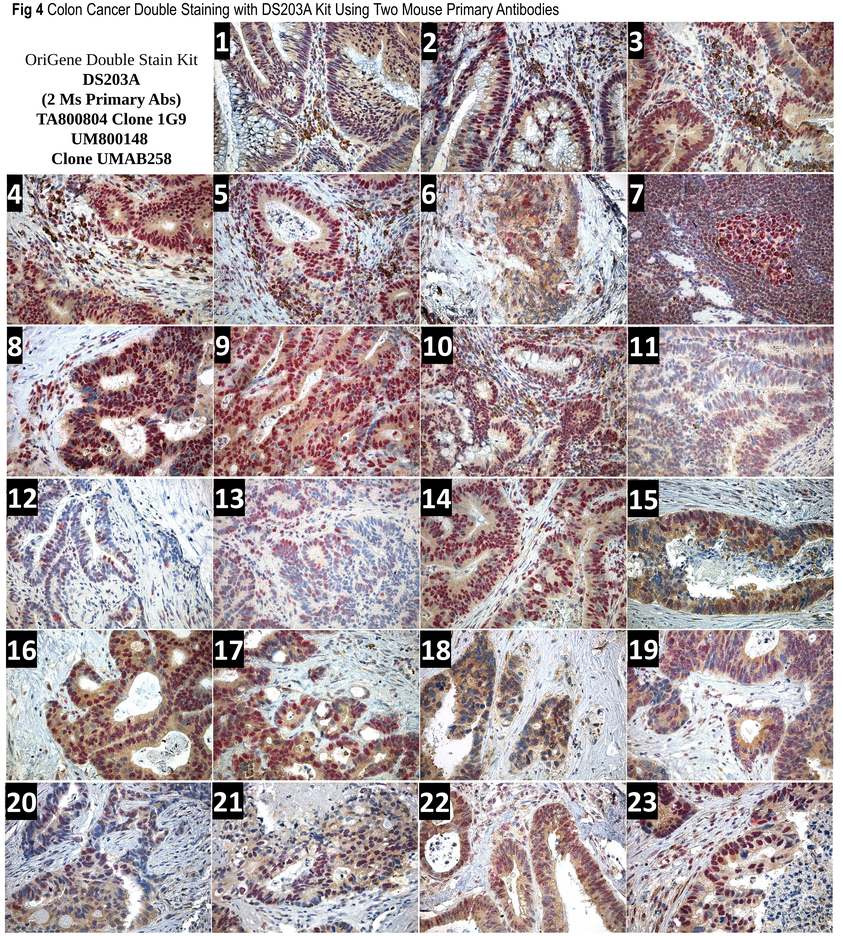
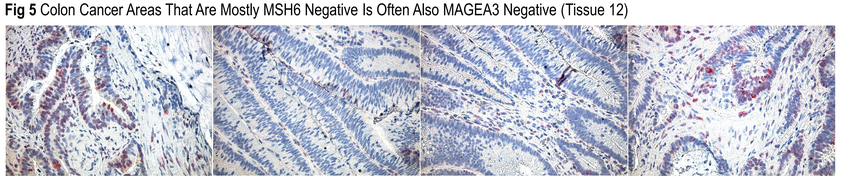


Fig 5 Colon Cancer Areas That Are Mostly MSH6 Negative Is Often Also MAGEA3 Negative (Tissue 12)



Conclusion

- Specific antibody for MAGEA3 was identified by using CytoSections for screening tissue
- DS203A provides a reliable way to stain human tissue with two different mouse antibodies
- Areas of the tumor that widely express MSH6, tumor epithelial cells were positive for MAGEA3;MAGEA3 and MSH6 is co-expressed in the tumor epithelial cells, with MAGEA3 being expressed in the cytoplasm of the cell that is positive for MSH6 in the nucleus
- Some of the infiltrating immune cells near the tumor cells express MSH6 and/or MAGEA3
- Areas of the tumor that widely do not express MSH6 were also MAGEA3 negative