2746 MAGE-A3 Co-Expression With IDH1-R132H And IL-1β In Colon Cancer

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Abstract

Colorectal cancer (CRC) remains a significant cause of cancer death worldwide with an estimated 52,000 deaths in 2023. Over the past few decades, the incidence rate of CRC in individuals younger than 50 has been steadily increasing. Currently, diagnostic biomarkers play an important role in the detection and treatment of CRC. MAGE-A3 has been shown to be expressed in many tumor types, including colon cancer by RNA expression. Due to their high sequence homology, members of the MAGEA family have been difficult to evaluate and screen for their protein expression. However, in a previous study, highly specific antibodies were identified using CytoSections for MAGE-A3 immunohistochemistry. IL-1β is a cytokine that induces inflammatory responses and when localized to the nucleus in colon cancer cells it can promote stemness. IL-1β expression has been shown to be increased in wildtype IDH1 positive glioblastoma. However, the relationship between IL-1β and IDH1 has not been studied in colon cancer. In this study, the difference in the expression of MAGE-A3, IL-1β, and wildtype IDH1 compared to mutant IDH1-R132H in colon cancer tissues was evaluated. The results show that IL-1β, wildtype IDH1, and MAGE-A3 were co-expressed in 27 of the 38 colon cancer tumors. However, IDH1-R132H expression was only seen in 20% of the tumors. This study suggests that MAGE-A3, IL-1β, and IDH1 are candidate biomarkers for immunotherapy due to their high expression in colon cancer tissues.

Introduction

Colon cancer, also known as colorectal cancer (CRC), can be caused by a combination of several different factors, including genetic predisposition and mutations, diet, environmental exposures, inflammation, and age. Melanoma Associated Antigen 3 (MAGE-A3) belongs to the Cancer Testis Antigen (CTA) family, and they are normally expressed in germ cells and not in adult somatic tissues. However, MAGE-A3 has been shown to be aberrantly expressed in various cancer types, including CRC, and plays an important role in oncogenesis. Additionally, because CTAs are not expressed in normal adult tissues, they are being studied for their potential as prognostic and diagnostic biomarkers for cancer detection. Inflammation, particularly chronic inflammation in the colon, has been shown to significantly increase the risk of developing CRC. Interleukin-1β (IL-1β) plays a significant role in inducing inflammation and in the pathogenesis of various cancers, such as CRC. Isocitrate dehydrogenase 1 (IDH1) is involved in cellular metabolism and serves as a key enzyme in the tricarboxylic acid (TCA) cycle. IDH1-R132H is a commonly occurring mutation in several tumor types that can alter cell differentiation and gene expression, which can lead to metabolic dysregulation and oncogenesis.

In this study, highly specific antibodies for MAGE-A3, IL-1β, IDH1, and IDH1-R132H were used on colon cancer tissues. Specific antibodies were verified using CytoSections (Fig 1). Their expression in the colon cancer tissues was reviewed and their co-expression patterns were determined.

Design & Methods

Immunocytochemistry

Manual IHC staining of paraffin-embedded CytoSections and colon cancer tissues with anti-MAGE-A3, anti-IL-1β, anti-IDH1, and anti-IDH1-R132H were performed. Heat-induced epitope retrieval (HIER) was used for all antibodies. OriGene's Citrate Buffer (B05C-100B) was used for Mage-A3 and IDH1 antibodies. TEE Buffer (B21-100) was used for IL-1β and IDH1-R132H antibodies. MAGE-A3 antibody was incubated overnight at 1:1000 in 4℃, IL-1β antibody was incubated for 1 hour at 1:200 in room temperature (RT), IDH1 antibody was incubated for 30 minutes at 1:600 in RT, and IDH1-R132H antibody was incubated overnight at 1:50 in 4℃. OriGene's Polink-1 HRP Mouse DAB Kit (D12-6) was used on MAGE-A3, IL-1β, and IDH1 antibodies. Polink-1 HRP Rat-NM DAB Kit (D35-6) was used on the IDH1-R132H antibody. The percentage of positive cells was scored and reviewed.

Results
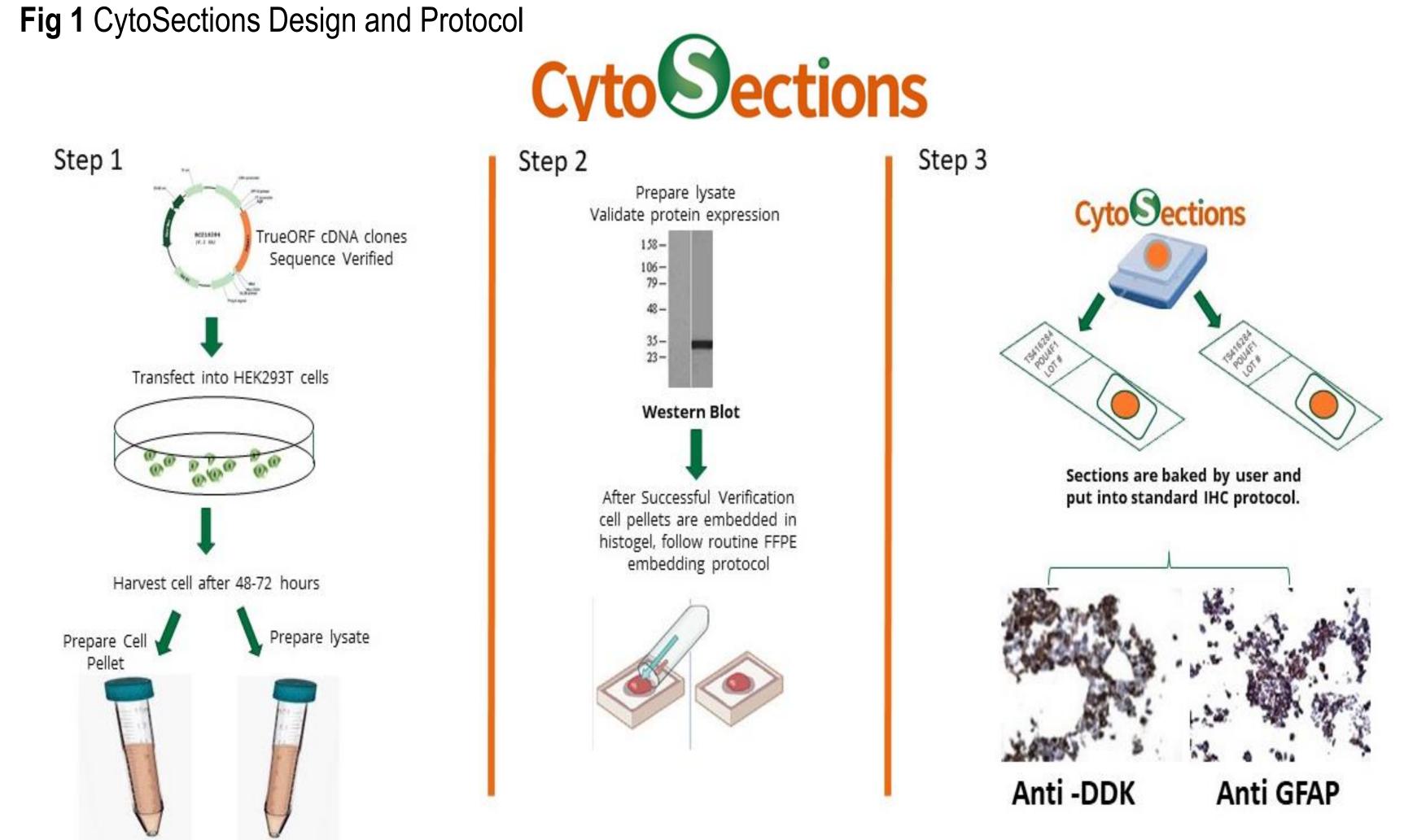

Fig 1 CytoSections Design and Protocol

Fig 2 DDK and MAGEA3 Antibodies on MAGEA1-12 CytoSections
Mouse anti-DDK clone OTI11C3 SKU# TA180144
Mouse anti-MAGEA3 clone OTI1H1 SKU# TA800826

Fig 3 DDK, IDH1 Wild-Type, and IDH1-Mutant Antibodies on IDH1 and IDH1-R132H CytoSections
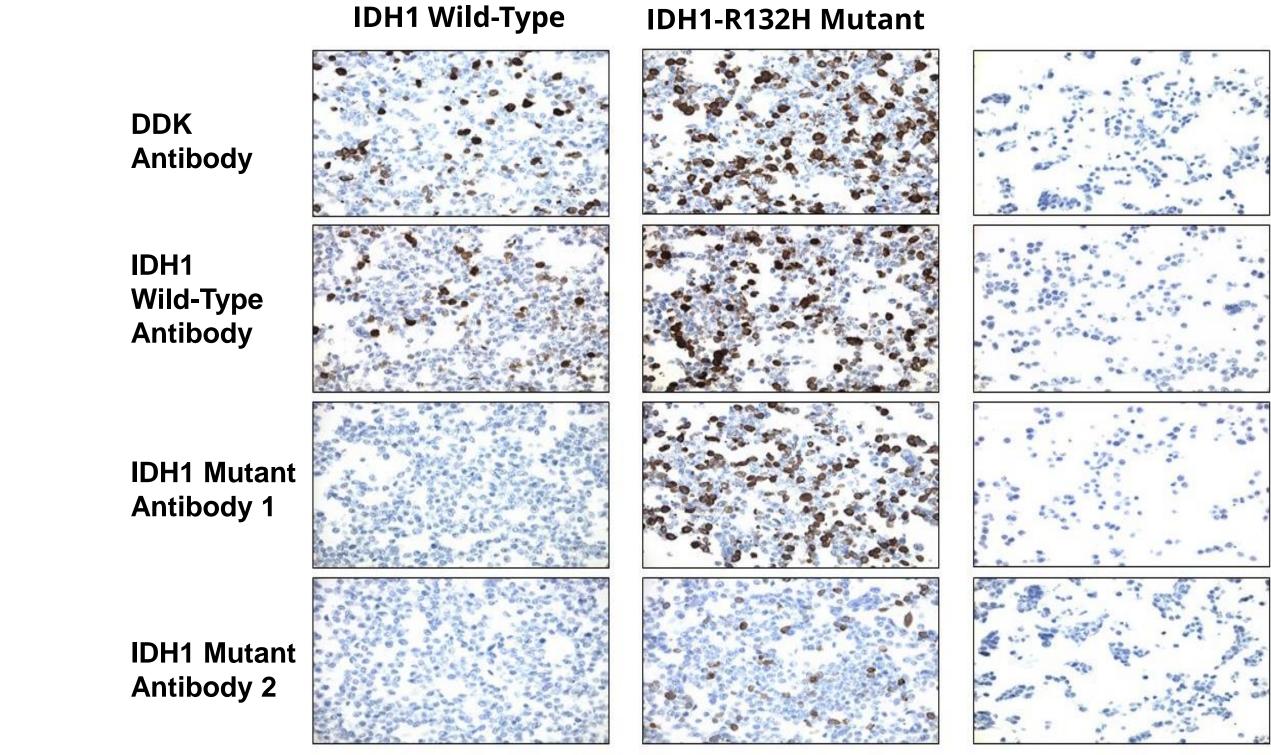

Fig 4 DDK and IL-1β antibodies on IL-1β CytoSections

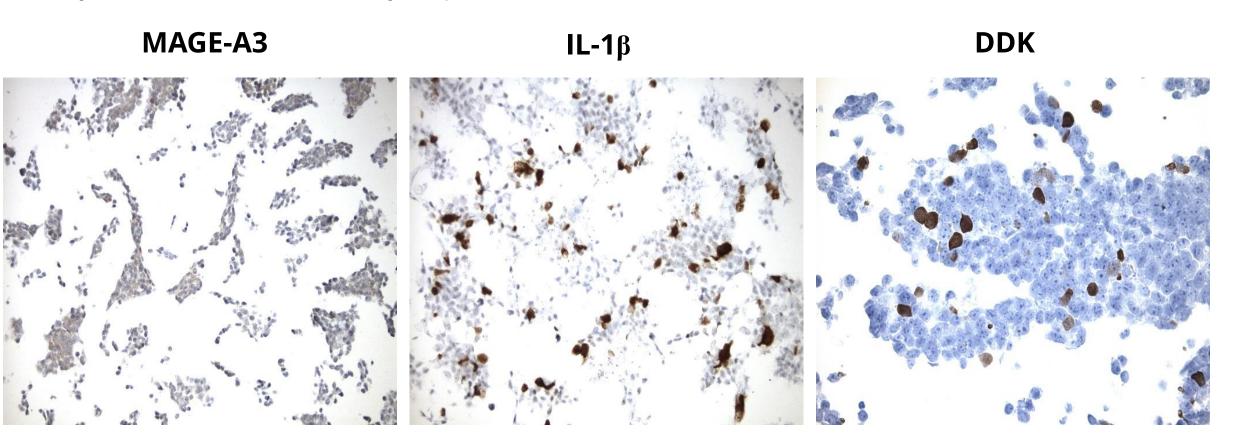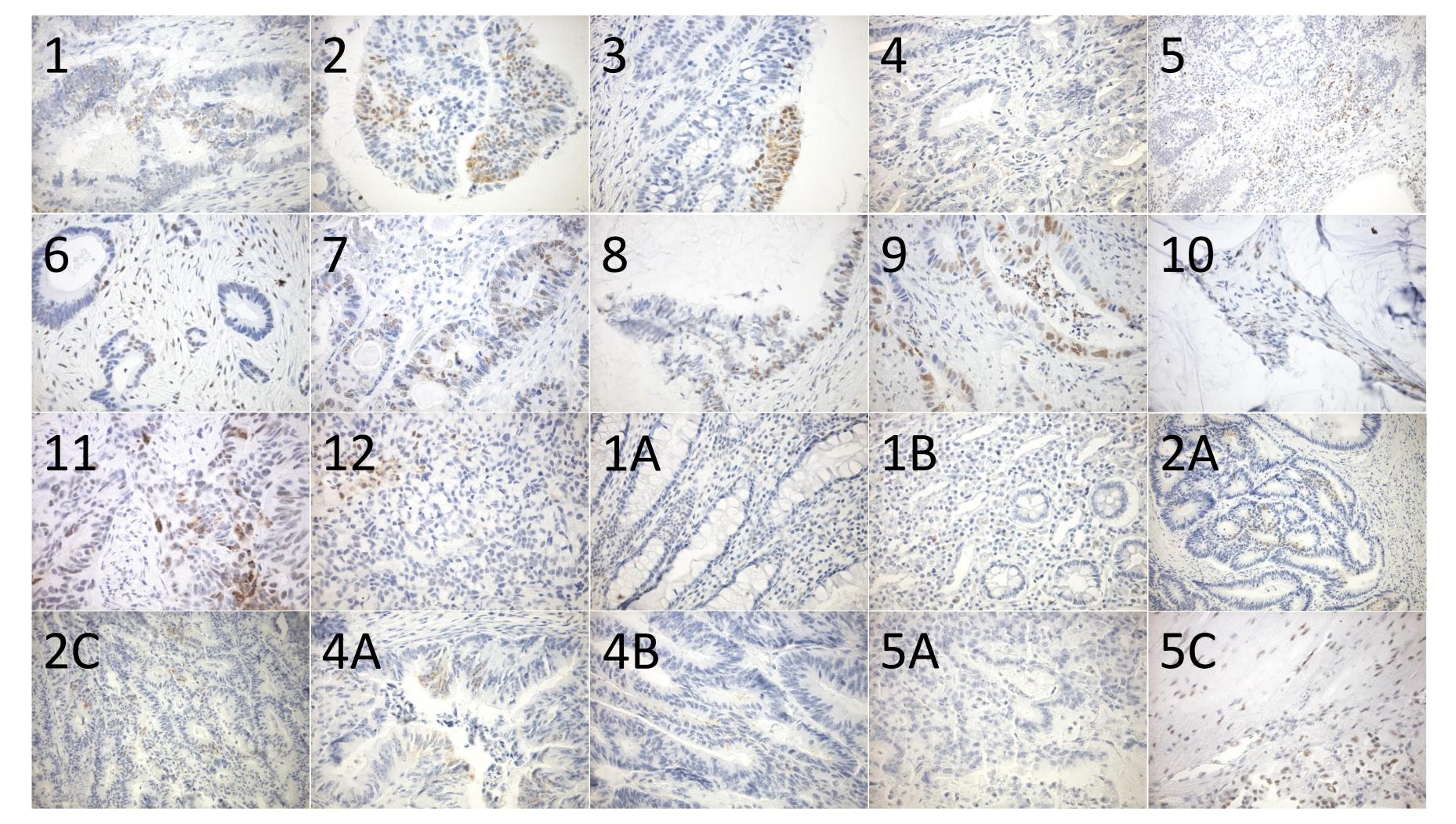Fig 5 MAGE-A3 Antibody on Colon Cancer Tissues
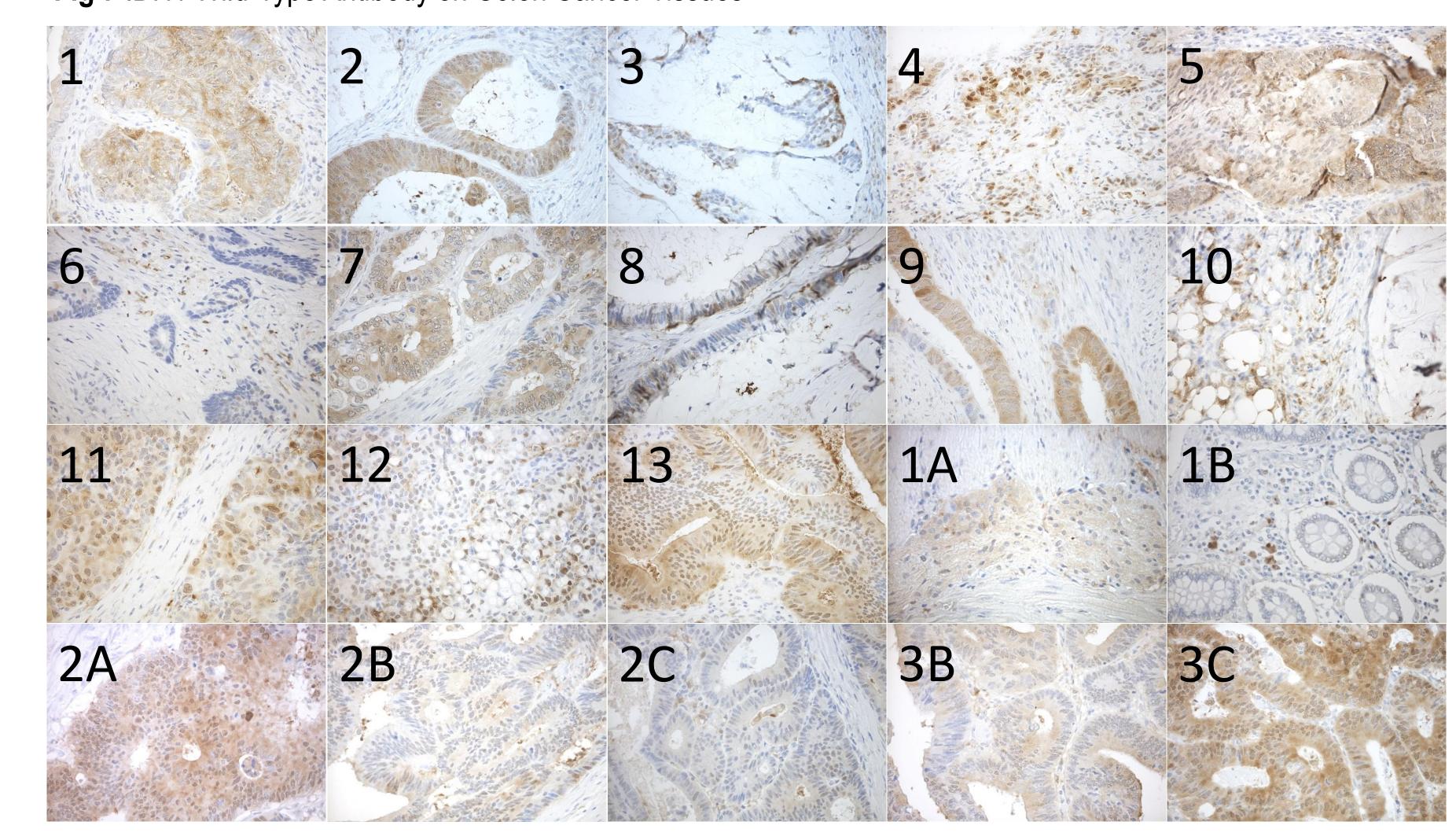

Fig 6 IL-1β Antibody on Colon Cancer Tissues

Fig 7 IDH1 Wild-Type Antibody on Colon Cancer Tissues

Fig 8 IDH1-R132H Mutant Antibody on Colon Cancer Tissues

Table 1 MAGEA Family Member 1-12 CytoSections Image Map for Fig 1

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

Table 2 MAGE-A3, IL-1β, IDH1 Wild-Type and IDH1-R132H Expression Score on Colon Cancer Tissues

Colon Cancer	MAGE-A3	IL-1β	IDH1	IDH1-R132H
1	POS	POS	POS	NEG
2	POS	POS	POS	NEG
3	POS	NEG	POS	NEG
4	POS	NEG	POS	NEG
5	POS (WEAK)	NEG	POS	NEG
6	POS	POS	POS	NEG
7	POS	POS	POS	NEG
8	POS	POS	POS	NEG
9	POS	POS	POS	NEG
10	POS	POS (RARE)	POS	NEG
11	POS	POS	POS	NEG
12	POS (WEAK)	NEG	POS	NEG
13	POS (WEAK)	NEG	POS	NEG

Table 3 MAGE-A3, IL-1β, IDH1 Wild-Type and IDH1-R132H Expression Score on Colon Cancer Tissue Array

Colon Cancer	MAGE-A3	IL-1β	IDH1	IDH1-R132H
1A	N/A	POS	N/A	NEG
2A	POS	POS	POS	NEG
3A	N/A	N/A	NEG (NO TUMOR)	NEG
4A	POS	POS	N/A	NEG
5A	POS	POS	N/A	NEG
1B	N/A	NEG (NO TUMOR)	N/A	NEG
2B	POS	N/A	POS (WEAK)	NEG
3B	POS	POS (RARE)	POS (WEAK)	NEG
4B	POS	POS	POS	NEG
5B	N/A	N/A	N/A	NEG
1C	N/A	NEG	N/A	NEG
2C	POS	POS	POS	NEG
3C	N/A	NEG (NO TUMOR)	POS	NEG
4C	POS	NEG	POS (WEAK)	NEG
5C	POS	POS	POS	NEG

Conclusion

- CytoSections showed high specificity for MAGE-A3, IL-1β, IDH1, and IDH1-R132H and serve as excellent controls for specific antibody detection.
- There was a high co-expression of MAGE-A3, IL-1β, and IDH1 in the colon cancer tissues.
- IDH1-R132H expression was rare and was independent of MAGE-A3, IL-1β, and IDH1 expression.
- MAGE-A3, IL-1β, and IDH1 have the potential to serve as promising diagnostic biomarkers for colon cancer.
- The relationship between MAGE-A3, IL-1β, and IDH1 in the pathogenesis of CRC remains unclear. However, the results suggest that they play a role in the progression of CRC.