Clinical assessment of MUC1 protein expression in FFPE tissue: Development and validation of an immunohistochemistry assay as a predictive assay for response to MUC1 vaccines

Abstract B297

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Introduction

Mucin-1 (MUC1, epithelial membrane antigen, EMA) is a trans membrane glycoprotein that is expressed on the apical membrane of epithelial cells of many tissues including breast, prostate, lung, pancreas, stomach, ovaries, intestines, and kidneys. In tumor cells, MUC1 is often over-expressed and aberrantly glycosylated, revealing new epitopes that trigger a cytotoxic T-cell response (Ramlau *et al.*, 2008, Quiox *et al.*, 2011, Sinn *et al.*, 2013). Several approaches are currently being pursued for targeting MUC1 in cancer therapy, mainly focused on vaccines targeting MUC1 antigens. We have developed an immunohistochemistry (IHC) assay to measure MUC1 protein expression in FFPE tissues, and thereby enable evaluation of MUC1 expression as a potential biomarker for MUC1-targeted therapies.

Materials and Methods

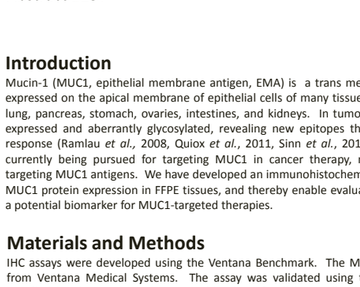
IHC assays were developed using the Ventana Benchmark. The MUC1 antibody was obtained from Ventana Medical Systems. The assay was validated using the recommended protocol supplied by the vendor using a combination of cell line pellets and formalin-fixed paraffin-embedded (FFPE) human tissue specimens purchased from ILSbio and BioServe. A breast cancer tissue array containing 16 matched adjacent normal and tumor tissue was purchased from BioChain Institute. Tumor positive specimens were scored based on expression of MUC1 in at least 25% of tumor cells (Quiox *et. al.*, 2011). In addition, distribution of staining (apical, membranous, cytoplasmic and combination staining patterns) was noted.

Figure 1: Specificity of MUC1 antibody staining

1A: Cell Line T47D (20X)
1C: Cell Line B16 MUC1 (20X)
1B: Cell Line HMEC (20X)
1D: Cell Line B16 (20X)

Figure 1 shows positive and negative control MUC1 staining in cell lines. Panel 1A is the breast cancer cell line T47D, and Panel 1B is control normal human mammary epithelial cell line HMEC. Panel 1C is the genetically engineered mouse B16 cell line over-expressing MUC1, and Panel 1D is the parental cell line. The specificity of the MUC1 IHC assay is apparent by the robust staining of the T47D and B16 MUC1 cells, but not the normal breast epithelial cell line or the B16 parental line.

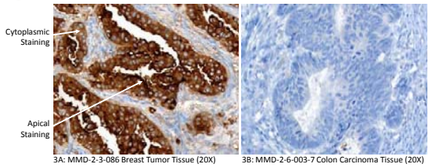
Figure 2: Expression of MUC1 in normal tissues



2A: MMD-1-4-012A Jejunum (10X)
2B: MMD-2-4-028A Lymph Node (20X)
2C: MMD-1-1-014 Skin (10X)
2D: MMD-2-3-090C Prostate (20X)

Several normal FFPE tissues were evaluated for MUC1 expression. As shown in **Figure 2**, we did not observe staining of MUC1 in lymph node, skin, prostate and jejunum except for the crypts in the jejunum.

Figure 3: MUC1 staining patterns in tumor tissues



3A: MMD-2-3-086 Breast Tumor Tissue (20X)
3B: MMD-2-6-003-7 Colon Carcinoma Tissue (20X)

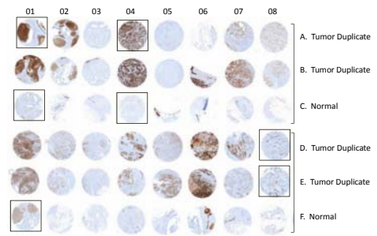
Figure 3 demonstrates positive and negative MUC1 staining in breast tumor (Panel 3A) and colon carcinoma tissue (Panel 3B), respectively. Note that we observed different types of staining patterns in MUC1 positive tissues which included apical, cytoplasmic, membranous or combinations; apical and cytoplasmic staining are both apparent in Panel 3A.

Table 1: MUC1 IHC data from 36 FFPE tissues

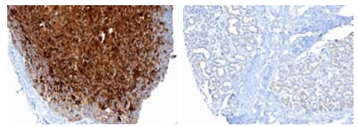
	Sample ID	Site	Pathology	Combined Result	Localization of Signal
1	MMD-2-5-001	Thyroid	Tumor	Negative	None
2	MMD-2-2-068	Lung	Papillary adenocarcinoma	Positive	Apical, Cytoplasmic, Membranous
3	MMD-2-6-008	Tumor of unknown origin	Squamous Cell Carcinoma	Positive	Cytoplasmic, Membranous (Weak)
4	MMD-2-4-038	Tumor of unknown origin	Spindle Cell Neoplasm	Negative	None
5	MMD-2-2-049	Lung	Adenocarcinoma, mixed type - acinar-papillary and solid adenocarcinoma structures, moderately differentiated	Positive	Cytoplasmic, Membranous
6	MMD-2-2-050	Lung	Adenocarcinoma, mixed type - acinar-papillary, moderately differentiated	Positive	Cytoplasmic, Membranous
7	MMD-2-2-051	Lung	Adenocarcinoma, mixed type - acinar-papillary (including micropapillary)	Positive	Cytoplasmic, Membranous
8	MMD-2-2-052	Lung	Adenocarcinoma, mixed type - acinar-papillary, moderately differentiated	Positive	Cytoplasmic, Membranous
9	MMD-2-2-053	Lung	Huge metastasis from primary adenocarcinoma in the lymph nodes	Positive	Cytoplasmic, Membranous
10	MMD-2-2-054	Lung	Adenocarcinoma, mixed type - acinar-papillary (including macro and micropapillary)	Positive	Apical, Cytoplasmic, Membranous
11	MMD-2-2-055	Lung	Adenocarcinoma, mixed type - acinar-papillary (including micropapillary)	Positive	Cytoplasmic, Membranous
12	MMD-2-2-056	Lung	Massive infiltration from lung cancer -adenocarcinoma with papillary structures	Positive	Cytoplasmic, Membranous
13	MMD-2-2-057	Lung	Adenocarcinoma, mixed type - acinar-papillary with infiltration of the bronchial wall	Positive	Cytoplasmic, Membranous
14	MMD-2-2-048	Lung	adenocarcinoma, acinar-papillary type	Positive	Cytoplasmic, Membranous
15	MMD-2-3-056	Uterus	Endometrioid adenocarcinoma	Positive	Cytoplasmic, Membranous
16	MMD-2-3-066	Ovary	Ovarian Cancer	Positive	Apical, Cytoplasmic, Membranous
17	MMD-2-3-076	Breast	Tumor	Positive	Cytoplasmic, Membranous
18	MMD-2-3-077D	Breast	Tumor	Positive	Cytoplasmic, Membranous
19	MMD-2-3-075	Ovary	Metastatic adenocarcinoma from breast primary	Positive	Cytoplasmic, Membranous
20	MMD-2-3-078	Breast	Tumor	Positive	Apical, Cytoplasmic, Membranous
21	MMD-2-3-086	Breast	Infiltrating Ductal Carcinoma	Positive	Apical, Cytoplasmic, Membranous
22	MMD-2-6-003-1	Breast	Carcinoma	Positive	Cytoplasmic
23	MMD-2-6-003-2	Breast	Carcinoma	Positive	Cytoplasmic
24	MMD-2-6-003-3	Breast	Carcinoma	Positive	Cytoplasmic and Membranous
25	MMD-2-6-003-4	Kidney	Normal	Positive	Cytoplasmic
26	MMD-2-6-003-5	Liver	Carcinoma	Negative	None
27	MMD-2-6-003-6	Ovary	Carcinoma	Positive	Cytoplasmic
28	MMD-2-6-003-7	Colon	Carcinoma	Negative	None
29	MMD 2 6 003 8	Colon	Carcinoma	Positive	Cytoplasmic
30	MMD-2-6-003-9	Colon	GIST	N/A-Tissue exhausted	None
31	MMD-2-6-003-10	Lung	Carcinoma	Positive	Cytoplasmic
32	MMD-2-6-003-11	Lung	Carcinoma	Positive	Cytoplasmic
33	MMD-2-6-003-12	Prostate	Carcinoma	Negative	None
34	MMD-2-6-003-13	Tumor of unknown origin	Squamous Cell Carcinoma	Positive	Cytoplasmic
35	MMD-2-6-003-14	Thyroid	Carcinoma	Negative	None
36	MMD-2-6-003-15	Prostate	Normal	Negative	None

Table 1 summarizes the MUC1 IHC data from 36 FFPE tissues (both normal and tumor) from lung, ovary, breast, colon, kidney, liver, thyroid and prostate. 27/36 specimens were scored positive for MUC1 staining. Negative staining was reported in thyroid, liver, colon and prostate tumor tissues.

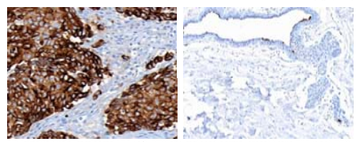
Figure 4: Evaluation of MUC1 staining in a set of matched breast cancer and adjacent normal tissues



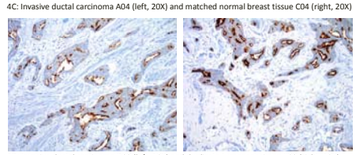
4A: FFPE tissue array of breast cancer duplicates and adjacent paired normal specimens; detail views of boxed specimens included in panels below



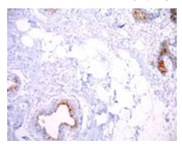
4B: Invasive ductal carcinoma A01 (left, 10X) and matched normal breast tissue C01 (right, 10X)



4C: Invasive ductal carcinoma A04 (left, 20X) and matched normal breast tissue C04 (right, 20X)



4D: Invasive ductal carcinoma D08 (left, 10X) and duplicate tumor specimen E08 (right, 10X)



4E: Normal breast tissue F01 (10X)

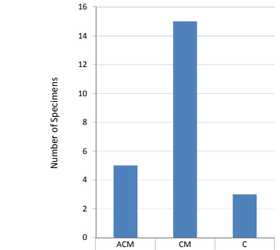
An FFPE tissue array of 16 matched tumor and adjacent normal tissue specimens were evaluated for MUC1 expression. **Figure 4** shows stained array (Panel 4A) as well as various detail views of the expression pattern of MUC1 (Panels 4B-4E). A summary of all the data from the array is presented in **Table 2**. MUC1 positive staining was observed in 13/16 tumor specimens with majority having no staining in the matched normal tissue. Interestingly, one of the tumor tissues exhibited distinct apical staining pattern that was unique in this set of 16 breast cancer tissues (Panels 4D). In a few instances, we also observed MUC1 staining in normal tissues (Panel 4E).

Table 2: MUC1 IHC data from matched breast cancer and adjacent normal tissues

Position	Pathology	Result	Localization of Signal
A01	Invasive Ductal Carcinoma	Positive	Cytoplasmic and Membranous
B01	Invasive Ductal Carcinoma	Positive	Cytoplasmic and Membranous
C01	Uninvolved breast tissue of A01,B01	Negative	None
A02	Invasive Ductal Carcinoma	Positive	Cytoplasmic
B02	Invasive Ductal Carcinoma	Positive	Cytoplasmic
C02	Uninvolved breast tissue of A02,B02	Negative	None
A03	Invasive Ductal Carcinoma	Negative	None
B03	Invasive Ductal Carcinoma	Negative	None
C03	Uninvolved breast tissue of A03,B03	Negative	None
A04	Invasive Ductal Carcinoma	Positive	Cytoplasmic and Membranous
B04	Invasive Ductal Carcinoma	Positive	Cytoplasmic and Membranous
C04	Uninvolved breast tissue of A04,B04	Negative	None
A05	Invasive Ductal Carcinoma	Negative	None
B05	Invasive Ductal Carcinoma	Positive	Cytoplasmic
C05	Uninvolved breast tissue of A05,B05	Positive	Apical and small amount Cytoplasmic
A06	Invasive Ductal Carcinoma	Negative	None
B06	Invasive Ductal Carcinoma	Positive	Cytoplasmic and Membranous
C06	Uninvolved breast tissue of A06,B06	Negative	None
A07	Invasive Ductal Carcinoma	Positive	Cytoplasmic
B07	Invasive Ductal Carcinoma	Positive	Cytoplasmic
C07	Uninvolved breast tissue of A07,B07	Negative	None
A08	Invasive Ductal Carcinoma	Positive	Cytoplasmic
B08	Invasive Ductal Carcinoma	Positive	Cytoplasmic
C08	Uninvolved breast tissue of A08,B08	Negative	None
D01	Invasive Ductal Carcinoma	Positive	Cytoplasmic
E01	Invasive Ductal Carcinoma	Positive	Cytoplasmic and small amount Membranous
F01	Uninvolved breast tissue of D01,E01	Positive	Membranous and Cytoplasmic
D02	Invasive Ductal Carcinoma	Positive	Cytoplasmic
E02	Invasive Ductal Carcinoma	Positive	Cytoplasmic
F02	Uninvolved breast tissue of D02,E02	Negative	None
D03	Invasive Ductal Carcinoma	Positive	Cytoplasmic
E03	Invasive Ductal Carcinoma	Positive	Cytoplasmic
F03	Uninvolved breast tissue of D03,E03	Negative	None
D04	Invasive Ductal Carcinoma	Positive	Apical and Membranous
E04	Invasive Ductal Carcinoma	Positive	Cytoplasmic and small amount Membranous
F04	Uninvolved breast tissue of D04,E04	Negative	None
D05	Invasive Ductal Carcinoma	Positive	Cytoplasmic and Membranous
E05	Invasive Ductal Carcinoma	Positive	Cytoplasmic and Membranous
F05	Uninvolved breast tissue of D05,E05	Negative	None
D06	Invasive Ductal Carcinoma	Positive	Cytoplasmic and Membranous
E06	Invasive Lobular Carcinoma	Positive	Cytoplasmic
F06	Uninvolved breast tissue of D06,E06	Positive	Cytoplasmic
D07	Invasive Ductal Carcinoma	Positive	Apical and Cytoplasmic
E07	Invasive Ductal Carcinoma	Positive	Cytoplasmic
F07	Uninvolved breast tissue of D07,E07	Negative	None
D08	Invasive Ductal Carcinoma, tubular type	Positive	Apical
E08	Invasive Ductal Carcinoma, tubular type	Positive	Apical
F08	Uninvolved breast tissue of D08,E08	Negative	None

Figure 5: Graphical representation of MUC1 staining patterns

5A: Frequency of staining patterns in 36 FFPE tissues evaluated

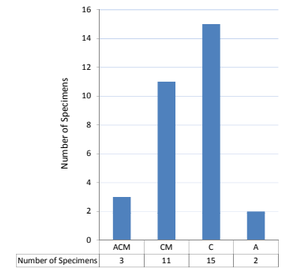


	ACM	CM	C
Number of Specimens	5	15	3

ACM Apical, cytoplasmic & membranous
CM – Cytoplasmic & membranous
C – Cytoplasmic
A – Apical

Localization of MUC1 Staining Pattern

5B: Frequency of staining patterns in 16 duplicate matched breast cancer and adjacent normal FFPE tissues evaluated



	ACM	CM	C	A
Number of Specimens	3	11	15	2

ACM Apical, cytoplasmic & membranous
CM – Cytoplasmic & membranous
C – Cytoplasmic
A – Apical

Localization of MUC1 Staining Pattern

A summary of the MUC1 staining patterns observed for the FFPE tissues is presented in **Figure 5**.

Conclusions

We have developed a robust assay for MUC1 that clearly distinguishes MUC1 expression in normal versus tumor tissue, as well as demonstrates distinct MUC1 expression patterns in a variety of tumor tissues. We are currently using this assay to explore the utility of MUC1 protein expression as a biomarker for ONT-10, a novel liposomal cancer vaccine that is currently in clinical trials.

For further information please contact info@molecularmd.com or visit www.molecularmd.com.

References

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Pkg Ins, anti-MUC1 (H23) Mouse Monoclonal Primary Antibody, English. Distributed by Ventana Medical Systems, Inc. Tucson, Arizona.

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