The Development of a Highly Specific IHC Monoclonal Antibody against Ki-67

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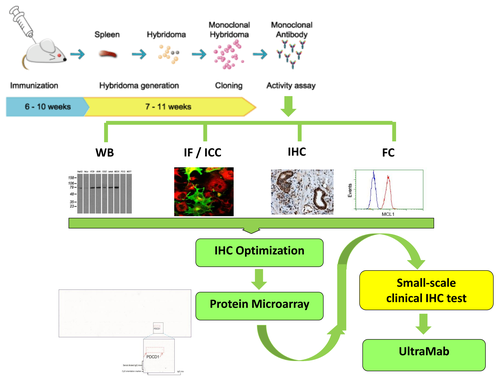
Introduction

Human Ki-67 protein is strictly associated with cell proliferation. It is expressed during all active phases of the cell cycle (G1, S, G2, and mitosis) except resting cells (G0). The Ki-67 positive tumor cells (the Ki-67 labeling index) is often correlated with the clinical course of the disease. New monoclonal antibodies that react with the Ki-67 equivalent protein from rodents now extend the use of Ki-67 protein as a proliferation marker to laboratory animals in basic research. The most popular anti-Ki-67 clone is MIB1. However, MIB1 does not cross-react with mouse Ki-67. We generated a new ultra-specific mouse anti-Ki-67 monoclonal antibody (UMAB107) validated by 10K protein microarray chip. UMAB107 can be used to detect the expression of human and mouse Ki-67 by Western Blot (WB), Immunofluorescence (IF) and Immunohistochemistry (IHC). From the IHC study on 627 human cancer tissues, UMAB107 shows high concordance of the staining patterns with MIB1.

Design & Methods

Figure 1. UltraMAB Development Procedure



1. **Antibody generation**: Antibodies to Ki-67 were generated by immunizing Balb/c mice with a purified recombinant protein fragment corresponding to amino acids 1160-1493 of human MKI67 (NP_002408) produced in E.coli. Enriched B cells from immunized animals were fused to myeloma SP2/0 myeloma cells (ATCC) to generate hybridomas using standard techniques. Single clones were obtained by limiting dilution assays through 3-round screening (by ELISA and IHC) and subcloning, and then screened with different kinds of FFPE human tissues by IHC.
2. **IHC:** IHC staining was performed on 4-µm thick paraffin tissue sections. Briefly, de-paraffinized slides were treated in 1 mM EDTA (pH 8.0 or pH9.0) antigen retrieval solution at 120 degree for 2.5 min in a high pressure cooker. Slides were incubated in primary antibodies for 90 min at room temperature (RT) without serum blocking. ZSBIO's PV9000 detection system was used in IHC assays manually.
3. **Protein array and assays:** Protein chips were printed and protein assays were executed as described (1).

Results ---- Validation Assays

Screening Ki-67 specific antibodies by Immunohistochemical assay

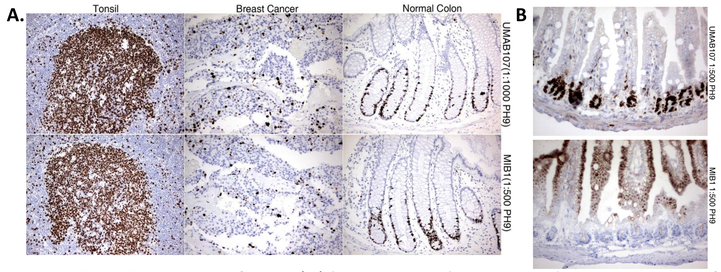


Figure 2. IHC staining of FFPE (A) human tonsil tissues, adenocarcinoma of human breast tissue, human colon tissue, and (B) mouse colon tissue by using UMAB107 and MIB1, respectively.

Specificity analysis of UMAB107 by 10K protein microarray chip

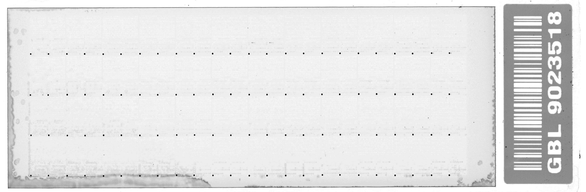


Figure 3. No cross-reactive protein was found by 10k protein microarray chips.

Other applications of UMAB107

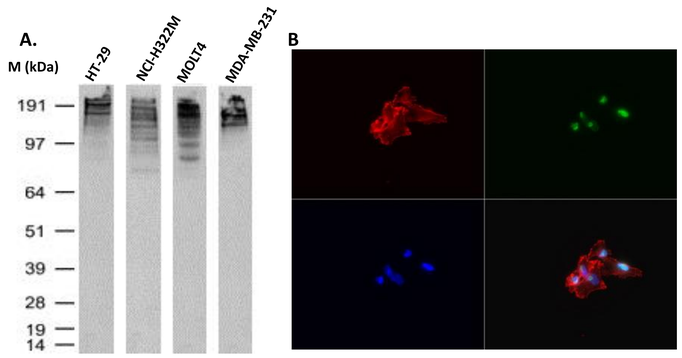


Figure 4. Western Blot and Immunofluorescence analyses of Ki-67 expression in cell lines by using UMAB107. (A) Western Blot analyses of HT-29, NCI-H322M, MOLT4 and MDA-MB-231 cell lysates (35µg).
(B) Immunofluorescent staining of MDA-MB-231 cells using UMAB107 (green, 1:100). Actin filaments were labeled with TRITC-phalloidin (red), and nuclear with DAPI (blue).

Results ---- IHC comparison between UMAB107 and MIB1

Table1. Performance comparison between UMAB107 and MIB1 on FFPE human tissue samples

Notes	Tissues	Positive/total Samples	
		UMAB107	MIB1
Human normal tissues	Breast	2/3	2/3
	Colon	3/3	3/3
	Kidney	0/2	0/2
	Liver	0/2	0/2
	Lung	1/3	1/3
	Ovary	0/3	0/3
	Pancreas	1/3	1/3
	Thyroid	1/3	1/3
	Endometrium	0/3	0/3
	Prostate	0/3	0/3
	Bladder	0/2	0/2
	Lymph Node	3/3	3/3
	Cerebrum	0/3	0/3
	Cerebellum	0/3	0/3
	Adrenal gland	0/3	0/3
	Heart	0/3	0/3
	salivary glands	0/3	0/3
	Skin	1/3	1/3
	Placenta	1/3	1/3
	Esophagus	0/3	0/3
	Stomach	0/3	0/3
	Intestine	0/3	0/3
	Mesothelium	0/3	0/3
	Tonsil	8/8	8/8
Human Cancer tissues	Breast CA	42/43	42/43
	Colon CA	50/50	50/50
	Kidney CA	2/3	2/3
	Liver CA	10/10	10/10
	Lung CA	57/57	57/57
	Ovary CA	9/9	9/9
	Pancreas CA	10/10	10/10
	Thyroid CA	69/69	69/69
	Endometrium CA	2/3	2/3
	Prostate_CA	2/3	2/3
	Bladder CA	1/2	1/2
	Lymphoma	57/58	57/58
	Sacoma	9/10	9/10
	Melanoma	1/1	1/1

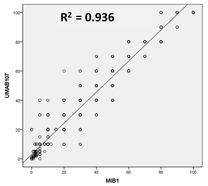


Figure 5. UMAB107 showed high concordance of the staining patterns with MIB1.

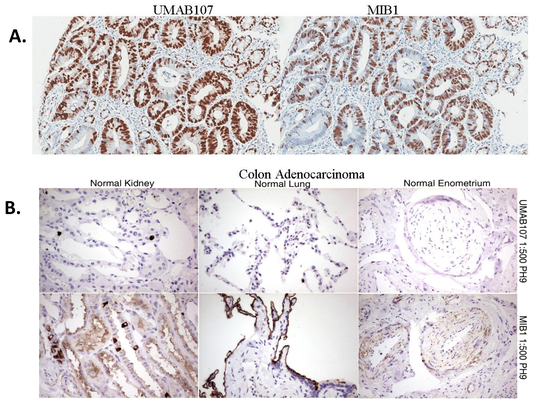


Figure 6. (A) UMAB107 showed a slightly stronger intensity than MIB1;
(B) UMAB107 showed no background staining in lung, kidney, and endometrium tissues.

Conclusions

1. **UMAB107 can be used in multiple applications such as WB, IF, and IHC.**
2. **UMAB107 can detect mouse Ki-67 with good performance and no obvious background staining. However, MIB1 does not recognize mouse Ki-67 and has abnormal staining patterns.**
3. **UMAB107 showed high concordance of the staining patterns with MIB1.**

References

1. **Ma D, et al. Using protein microarray technology to screen anti-ERCC1 monoclonal antibodies for specificity and applications in pathology. *BMC Biotechnology, 2012, 12: 88.***