A Panel of Rabbit Monoclonal Antibody Marker for Prostate Cancer

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Abstract

Tumor markers are useful in identification of tumor origin and in analysis of the biological characteristics of specific tumor. The objective of this study was to determine the immunohistochemical application of a panel of rabbit monoclonal antibodies against human prostate tumor specific antigens Alpha-methylacyl-CoA racemase (AMACR or P504s), androgen receptor (AR), ETS-related gene (ERG), prostatic acid phosphatase (PAP) and prostate specific antigen (PSA) on formalin-fixed and paraffin-embedded tissues. These antibodies were developed with a unique and proprietary RabMAb® technology, designated as EP clone product line (EP Clones™). All the antibodies showed excellent signal-to-noise ratio in immunohistochemical staining on formalin-fixed and paraffin-embedded tissues. Rabbit monoclonal antihuman AMACR, AR, ERG, PAP and PSA are valuable for immunohistochemical analysis of prostate cancer. (*The J Histotechnol* 33(4):179–181, 2010)

Key words: immunohistochemistry (IHC), prostate cancer, rabbit monoclonal antibodies

Introduction

Prostate cancer is one of the most common cancers in men worldwide and a leading cause of death among all cancer types (1). Immunohistochemical analysis of tumor markers has been useful in pathological diagnosis and understanding the biological characteristics of tumor. Alpha-methylacyl-CoA racemase (AMACR or P504s), androgen receptor (AR), ETS-related gene (ERG), prostatic acid phosphatase (PAP) and prostate-specific antigen (PSA) are specific markers for prostate cancer (2–4). Individual use of these markers has been helpful in prostate cancer analysis. Combined use of these markers provides improved management for prostate cancer.

At Epitomics Inc., (Burlingame, CA), RabMAb®, a unique and proprietary method for making monoclonal antibodies from rabbits rather than the conventional method of starting with mice, has been established. RabMAb® technology offers the combined benefits of superior antigen recognition with the specificity and consistency of a monoclonal antibody, yielding the greatest possible quality antibody. A panel of rabbit monoclonal antibodies directed against human AMACR, AR, ERG, PAP, and PSA was developed with the use of RabMAb® technology. These uniquely characterized rabbit monoclonal antibodies, designated as EP clone product line (EP Clones™), are ideal for immunohistochemical detection of target proteins in formalin-fixed and paraffin-embedded (FFPE) tissues.

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Materials and Methods

Antibodies

The rabbit antihuman monoclonal antibodies (Table 1) developed and affinity purified in Epitomics, Inc. have been used for immunohistochemistry.

Human Tissues

Human prostate tissue microarray constructed with FFPE tissue was used for immunohistochemical staining. The tissue microarray contains five normal prostate, 18 prostate hyperplasia, and seven prostate adenocarcinoma tissue samples.

Detection Systems and Ancillary Reagents

All detection systems and ancillary reagents were from Epitomics, Inc. EpiPrecision™ Rabbit HRP Kits, a Streptavidin-HRP Biotin Detection system (cat. no. DK-0001, DK-0003), and EpiVision™ Rabbit HRP Kits, a polymer detection system (cat. no. DK-0002, DK-0004), were used in this study.

Immunohistochemistry

Tissue sections (4 μM) were deparaffinized in xylene and rehydrated through a serials of graded ethanol. Epitope retrieval was performed using either Citrate buffer (Epitomics) or Tris EDTA buffer (Epitomics) with a pressure cooker (Biocare, Concord, CA) according to the manufacturer's instruction. Endogenous peroxidase activity was blocked by peroxidase solution (Epitomics) for 10 min at room temperature. After blocking with Blocking solution (Epitomics), sections were incubated with primary antibodies (Table 1) for 30 min at room temperature. All concentrated primary antibodies were diluted in primary antibody dilution buffer (Epitomics) at 1:100 dilution. Immunoreactivity was detected with EpiPrecision™ or EpiVision™ following the manufacturer's instruction. Staining was visualized by DAB (Epitomics). Photographs were taken with a Leica microscope and image system (Leica, Bannockburn, IL).

Table 1. Primary antibodies for antigen detection

Antibody	*Clone No.*	*Cat. No. (concentrated)*	*Cat. No. (prediluted)*
AMACR	EP37	AC-0036	AP-0036
AR	EP120	AC-0070	AP-0070
ERG	EP111	AC-0071	AP-0071
PAP	EP53	AC-0051	AP-0051
PSA	EP109	AC-0070	AP-0070

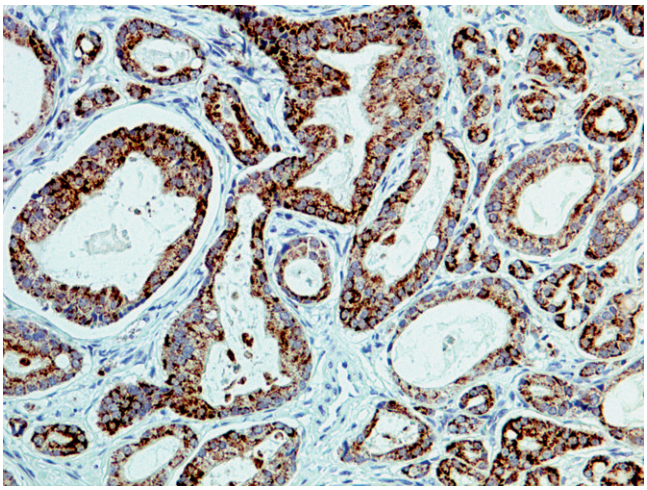

Figure 1. Staining of AMACR in human prostate cancer. Pretreatment: citrate; detection: EpiPrecision™.

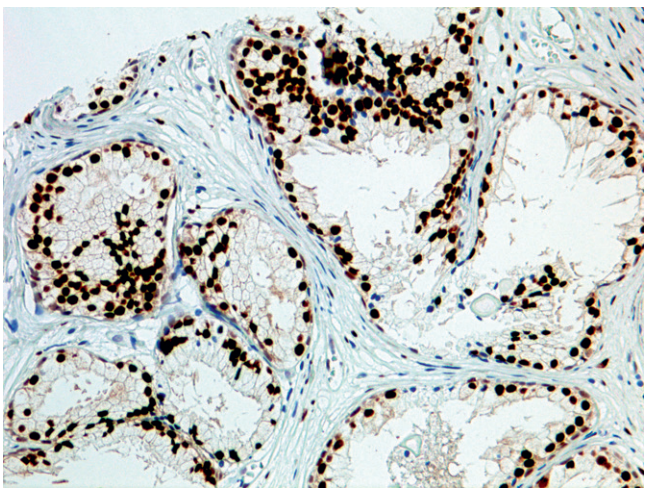

Figure 2. Staining of AR in human prostate hyperplasia. Pretreatment: citrate; detection: EpiPrecision™.

Results

The staining for all five antibodies in respective tissues showed clear signal; no background staining was observed. Anti-AMACR (Figure 1) and anti-ERG (Figure 3) showed immunoreactivity in cancer cells but not in normal prostate epithelial cells. Anti-AMACR stained cytoplasm of cancer cells in a granular pattern, whereas ERG was localized in nuclear of cancer cells. Anti-AR labeled nuclear of both normal prostate epithelial cells and tumor cells, with strong staining seen in cancer cells (Figure 2). Antibody to PAP (Figure 4) or PSA (Figure 5) strongly

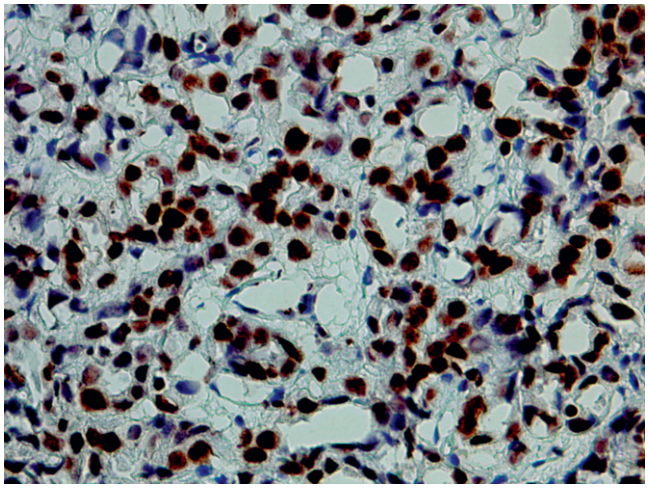

Figure 3. Staining of ERG in human prostate cancer. Pretreatment: Tris-ethylene diamine tetraacetic acid; detection: Epivision™.

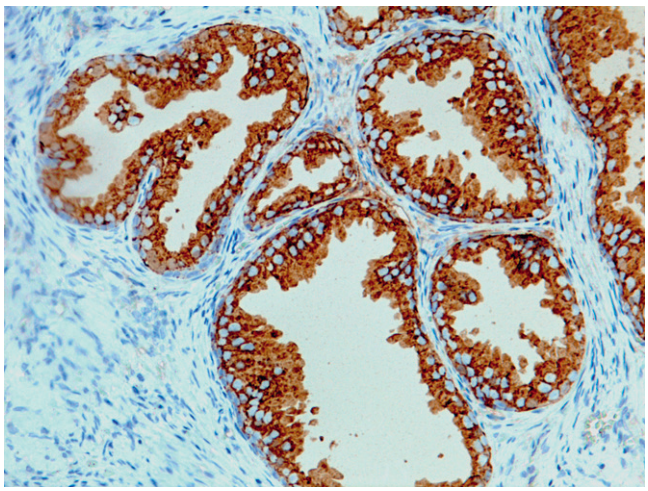

Figure 4. Staining of PAP in human prostate hyperplasia. Pretreatment: citrate; detection: EpiPrecision™.

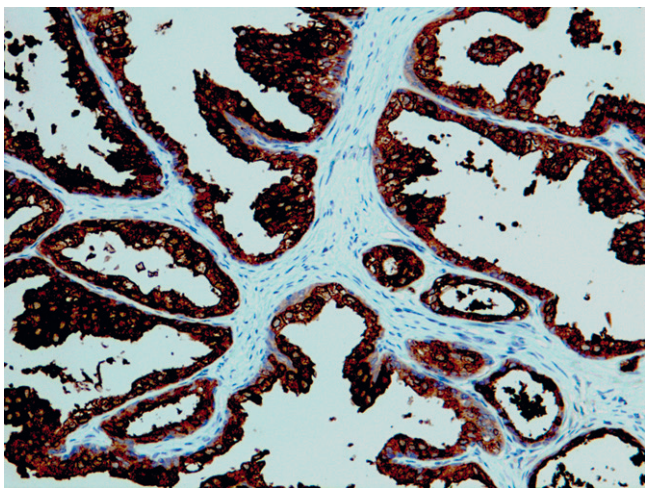

Figure 5. Staining of PSA in human prostate hyperplasia. Pretreatment: citrate; detection: EpiPrecision™.

stained cytoplasm of both normal prostate and tumor cells.

Conclusions

All five antibodies provided respective specific staining for their target proteins in benign and/or malignant prostate tissues. Antibody against AR or ERG gave nuclear staining whereas anti-AMACR, PAP and PSA gave cytoplasmic staining with the use of detection systems tailored to each antibody. These antibodies and detection systems give the excellent signal-to-noise ratio for all the tissue sections stained in this study. In conclusion, rabbit monoclonal anti-human AR, AMACR, ERG, PAP, and PSA are excellent antibodies for immunohistochemical analysis of prostate cancer in FFPE tissue.

References

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