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A Novel Rabbit Monoclonal Antibody against Arginase-1 is a more Sensitive Marker than Rabbit Polyclonal Arginase-1 Antibody in Hepatocellular Carcinomas

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Background

Hepatocellular carcinoma (HCC) is the fifth most common cancer in men and seventh most common in women. The discrimination of primary HCC from other malignancies often presents a diagnostic challenge when distinguishing benign proliferative lesions from well-differentiated, primary and metastatic lesions of HCCs. Definitive criteria are still essentially required for differential diagnosis in certain problematic cases of HCCs. Identification of a more sensitive and specific diagnostic marker delineating primary HCC from tumors metastatic to the liver is of immense clinical significance. HepPar-1 (also called Hepatocyte Specific Antigen) and Glypican-3 are commonly used as markers of hepatic differentiation, but suffer from lower sensitivity in high grade HCC.

Arginase-1 (ARG-1), a urea cycle metalloenzyme is predominantly expressed in the liver, where it plays a role in detoxification of ammonia. ARG-1 is now considered as a key target for the differential diagnosis of primary HCC from tumors metastatic to the liver. Only a few limited studies have been published on the use of ARG-1. Most publications utilizing immunohistochemistry (IHC) have used a rabbit polyclonal antibody from Sigma (reference antibody). However, several other ARG-1 monoclonal and polyclonal antibodies are also commercially available for IHC, but have not been well characterized and reported in the literature.

The purpose of the present study was to identify a sensitive and specific ARG-1 antibody from the available monoclonal and polyclonal antibodies to Arginase-1, and to ascertain its usefulness in differential diagnosis of primary HCCs and tumor metastasis to the liver. All antibodies were compared with the reference antibody and HepPar-1.

Design

Tissue Microarrays (TMAs) were used consisting of various normal (n=33) and neoplastic tissues (n=675); and HCCs (Grade I, II, III, n=209). Five commercially available mouse and rabbit antibodies to ARG-1 were evaluated for their sensitivity and specificity. These included rabbit monoclonal antibody (RMAb) ARG-1 [EPR 6672B] (Biocare Medical), the reference rabbit polyclonal antibody to ARG-1 (Sigma), mouse monoclonal ARG-1 [Clone 19] (BD Biosciences), and two rabbit polyclonal antibodies to ARG-1 (Thermo and Acris).

All TMA sections were retrieved in a modified citrate buffer (DIVA, Biocare Medical) in a pressure device (Decloaking Chamber, Biocare Medical) at 125°C for 30 seconds. Antibodies were optimized and applied to the tissues, followed by an anti-mouse or anti-rabbit micro-polymer HRP detection system.

Scoring Method for Interpretation

Scoring and interpretation methods were developed based on those previously reported by Yan *et. al.* (2010)[1] and Timek et. al. (2012)[2]. For each antibody, cases were considered positive, if 1% or more of tumor cells were stained. Cases with <1% staining and no focal areas of positive staining were scored as negative. Cases that were mostly negative, but contained tumor clusters in which almost all tumor cells exhibited positivity were classified as focally positive. McNemar's test was employed to evaluate differences in sensitivity observed between the RMAb ARG-1 antibody and the HepPar-1 antibody.

Results

ARG-1 RMAb and the reference polyclonal ARG-1 antibody from Sigma were superior to all other antibodies tested in this study. The remaining three antibodies demonstrated poor specificity, and thus were eliminated for further comparative evaluation. In normal liver, ARG-1 RMAb stained cytoplasmic and nuclear components of normal hepatocyte cells (Fig. 1) and also marked infiltrating inflammatory cells in HCC (Fig. 2).

ARG-1 RMAb demonstrated superior sensitivity in staining Grades I, II, and III HCC as compared to the reference polyclonal antibody to ARG-1 and the HepPar-1 antibody (Table 1). However, ARG-1 RMAb did not stain pancreatic tumors as reported in previous studies (Table 2). ARG-1 RMAb also stained more tumor cells when compared to the reference antibody (Fig. 3); and staining sharpness was also improved (Fig. 4). ARG-1 RMAb showed limited staining in normal tissues except liver, pancreas, and kidney (Table 3). ARG-1 RMAb also stained 50% of cholangiocarcinomas (n=14).

Modern Pathology 2013; 26, Supplement 2: 411A.

Table 1: Comparison of ARG-1 RMAb, ARG-1 Rb Polyclonal, and HepPar-1 antibodies in HCC specimens of various grades (n=56)

Antibody	Grade I	Grade II	Grade III	All Grades
ARG-1 RMAb	100% (15/15)	96.6% (28/29)	75% (9/12)	92.9% (52/56)*
Reference Rb Polyclonal ARG-1	100% (15/15)	86.2% (25/29)	66.7% (8/12)	85.7% (48/56)
HepPar-1	87% (13/15)	72.4% (21/29)	50% (6/12)	71.4% (40/56)*

*Increased sensitivity of ARG-1RMAb relative to HepPar-1 was determined statistically significant by McNemar's test ($p<0.0015$).

Table 2: ARG-1 RMAb Staining in Various Neoplastic Tissues

TMAs: Various Neoplastic Tissues	Positive Cases
Breast (infiltrating ductal carcinoma)	0/40
Melanoma	0/12
Kidney (renal cell carcinoma)	0/71
Pancreas (adenocarcinoma)	0/89
Prostate adenocarcinoma	3/64
Seminoma	0/12
Ovarian cancer	0/80
Lung (squamous cell carcinoma and adenocarcinoma)	0/77
Endocrine Tumors	0/46
Colon cancer	0/184
Total	3/675 (0.4%)

Table 3: Specificity of ARG-1 RMAb in Normal Tissues

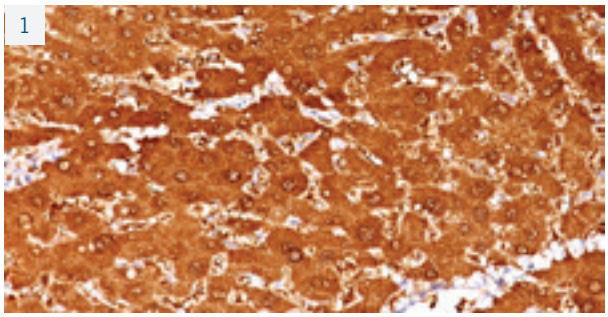
Tissue	Positive Cases	Tissue	Positive Cases
Adrenal gland	0/3	Ovary	0/3
Bladder, urinary	0/3	Pancreas	2/3
Bone marrow	1/1	Parathyroid	0/3
Eye	0/2	Pituitary gland	0/2
Breast	0/3	Placenta	0/3
Brain, cerebellum	0/3	Prostate	0/3
Brain, cerebral cortex	0/3	Skin	2/2
Fallopian tube	0/3	Spinal Cord	0/2
Esophagus	0/3	Spleen	2/2
Stomach	0/3	Skeletal Muscle	0/3
Intestine, small	0/3	Testis	0/3
Intestine, colon	0/3	Thymus	0/3
Intestine, rectum	0/3	Thyroid	0/3
Heart	0/3	Tonsil	0/3
Kidney	5/5	Ureter	0/3
Liver	3/5	Uterus cervix	0/3
Lung	0/3	Uterus endometrium	0/3

Table 4: IHC staining of ARG-1 RMAb in Grades I, II, II of HCC (n=209)

Tumor Grade	ARG-1 RMAb
Grade I	94.7% (18/19)
Grade II	88% (95/108)
Grade III	69.5% (57/82)

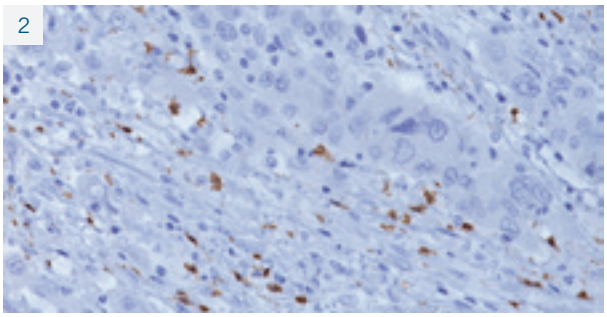
Figures

1



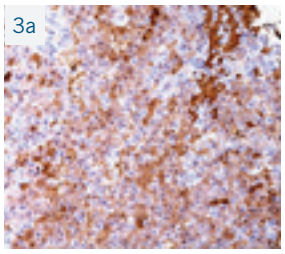
Arginase-1 RMAb in Normal Liver (20x)

2



Arginase-1 RMAb Staining Inflammatory cells in HCC (20x)

3a



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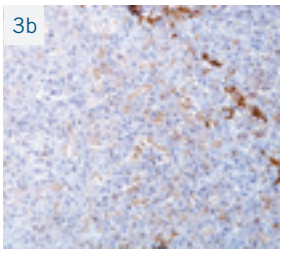

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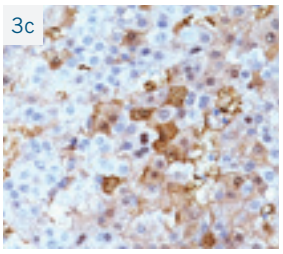

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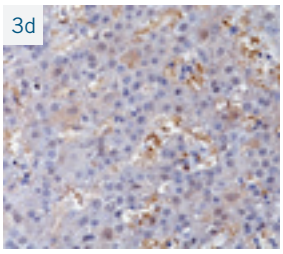
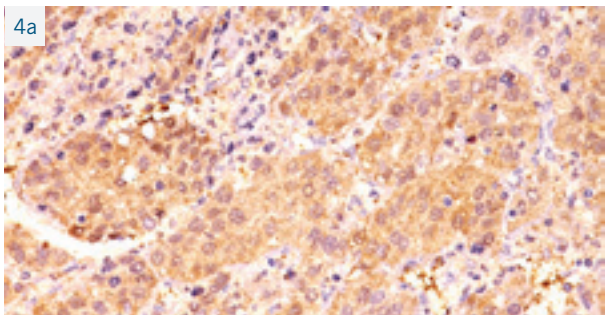


IHC Staining Comparison of Arginase-1 RMAb (a, c) vs. Arginase-1 Rb Poly Ab in HCC (b, d).

4a



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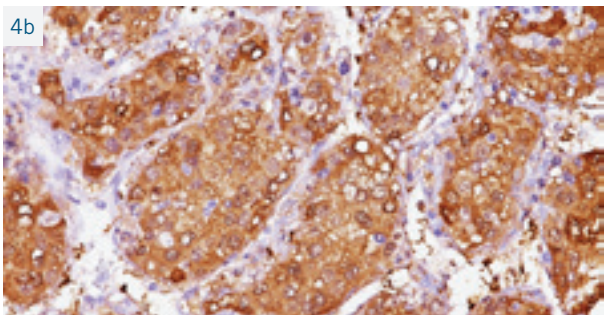

IHC Staining Comparison ARG-1 Rb Polyclonal (a) vs. ARG-1 RMAb (b) (Staining is much shaper with ARG-1 RMAb).

Discussion

The TMA data on a large series of HCC specimens and various neoplastic and normal tissues has clearly established the diagnostic sensitivity and specificity of ARG-1 RMAb. Remarkably, the sensitivity of ARG-1 RMab was also proportionately higher in HCC Grade II and III (96.6% and 75%, respectively) than that of the reference polyclonal antibody to ARG-1 (86.2% and 66.7% respectively). A meta-analysis on publications reporting the reference polyclonal antibody to ARG-1, showed virtually identical staining in non-hepatocellular carcinoma cases vs. ARG-1 RMAb; however, reports cited positive staining of the reference antibody in pancreatic tumors. In our study, none of the cases of pancreatic tumors stained with ARG-1 RMAb (0/89). In normal pancreas, the ARG-1 RMAb stained normal acinar cells, but not in islet of Langerhans.

The HepPar-1 marker suffers from a lower sensitivity and specificity compared to the ARG-1 RMAb. IHC staining of HepPar-1 is not considered entirely specific for hepatic differentiation as earlier reports have shown the expression of HepPar-1 in non-hepatic cancers such as lung adenocarcinomas, esophageal carcinomas and gastric cancers. However, the co-expression of both antibodies may contribute to higher specificity. In our study, ARG-1 was positive in all cases that were positive for HepPar-1.

Conclusion

Arginase-1 RMAb [Clone EPR 6672B] is superior in sensitivity and specificity, and provides sharper staining in comparison with the reference rabbit polyclonal ARG-1 and HepPar-1 antibodies; thus representing a potential marker for differential diagnosis of primary HCC vs. tumor of unknown origin.

References

1. Yan BC, *et al.* Arginase-1: A new Immunohistochemical marker of hepatocytes and hepatocellular neoplasms. Am J Surg Pathol. 2010; 34(8):1147-1154.

2. Timek DT, *et al.* Arginase-1, HepPar-1, and Glypican-3 are the most effective panel of markers in distinguishing hepatocellular carcinoma from metastatic tumor on fine-needle aspiration specimens. Am J Clin Pathol. 2012; 138: 203-210.