

# a-Methylacyl coenzyme A racemase is highly expressed in the intestinal-type adenocarcinoma and high-grade dysplasia lesions of the stomach

Wenbin Huang, Jianhua Zhao, Li Li, Yue Huang, Xiaobin Yang, Jinsong Wang and Tonghai Zhang

Department of Pathology, Nanjing Medical College Affiliated Nanjing First Hospital, Nanjing Jiangsu, P. R. China

**Summary.** To study a-Methylacyl coenzyme A racemase (AMACR) expression in gastric intestinal-type adenocarcinoma and its precursors, we performed an immunohistochemical assay (using an avidin-biotin-peroxidase complex method) on 106 paraffin-embedded gastric mucosal biopsy samples and 25 gastrectomy samples (37 negative for dysplasia; 30 indefinite for dysplasia; 22 low-grade dysplasia; 25 high-grade dysplasia; and 34 invasive intestinal adenocarcinoma). The results showed that AMACR staining was uniformly negative in the groups negative for dysplasia and indefinite for dysplasia. Only 1 of 22 (4.5%) low-grade dysplasia showed weak staining for AMACR. In the groups of high-grade dysplasia and invasive intestinal-type adenocarcinoma, however, 19 of 25 (76%) and 18 of 34 (52.9%) were positive for AMACR respectively. Expression of AMACR was not correlated with location, *H. Pylori* infection or intestinal metaplasia. These results suggested that AMACR may play a role in the intermediate stage of gastric carcinogenesis. The high level expression of AMACR in high-grade dysplasia and carcinoma suggests that it may be a useful biomarker in distinguishing high-grade dysplasia and carcinoma from low-grade dysplasia.

**Key words:** AMACR, Gastric carcinoma, Dysplasia, Low-grade, High-grade

## Introduction

Gastric cancer remains a great challenge for clinicians and scientists. It is one of the most frequent cancers worldwide, and the second most common cause of cancer-related deaths (Fuchs and Mayer, 1995; Tahara et al., 1996; Delchier et al., 1998). In countries other than Japan, the vast majority of gastric cancers diagnosed are already in an advanced stage, with a five year survival rate lower than 20% (Pagnini and Rugge, 1985; Neugut et al., 1996). Gastric carcinogenesis has been shown to be a multistage process in which the occurrence of stomach cancer is preceded by a sequence of precancerous stages: chronic gastritis, atrophy, intestinal metaplasia and dysplasia (Correa, 1992). Histologically identified dysplasia in gastric epithelium, subdivided into low-grade and high-grade categories, is a precursor lesion of gastric intestinal-type adenocarcinoma. Because high-grade dysplasia is a powerful prognostic factor for the development of adenocarcinoma, the presence of this lesion in an endoscopic gastric mucosal biopsy sample is a strong indication for further surgical intervention (Rugge et al., 2003). Unfortunately, there is considerable disagreement in distinguishing low-grade dysplasia from high-grade dysplasia, even among gastrointestinal pathologists.

a-methylacyl-coenzyme A racemase (AMACR) is a cytoplasmic enzyme that plays an essential role in -oxidation of branched-chain fatty acids by catalyzing interconversion of several (2R)-methyl-branched-chain fatty acyl-coenzyme A esters to their (S)-stereoisomers. This protein was originally identified in mitochondria and peroxisomes of rat liver cells (Schmitz et al., 1994; Amery et al., 2000; Ferdinandusse et al., 2000), and was subsequently isolated by cDNA subtraction and microarray analysis of prostate adenocarcinoma (Xu et al., 2000). Apart from atrophic, foamy gland, and pseudohyperplastic variants, AMACR

immunostaining has been shown to be quite sensitive for the diagnosis of prostate carcinoma (Hameed and Humphrey, 2005).

In gastrointestinal tract tumors, expression of AMACR has been shown in 25% of gastric adenocarcinomas, 75% of colonic adenomas and in most moderately and well-differentiated colonic carcinomas, but not in normal gastric, small intestinal, and colonic epithelium (Jiang et al., 2003a,b). AMACR is expressed in dysplastic epithelium in both Barrett's esophagus and inflammatory bowel diseases, with a reasonably high sensitivity and complete specificity, and is highly specific in distinguishing high-grade dysplasia from reactive atypia in Barrett's esophagus (Dorer and Odze, 2006; Lisovsky et al., 2006). These studies suggest that AMACR can be a biomarker for precancerous lesions and epithelial malignancies of the gastrointestinal tract. The aim of this study was to evaluate the expression of AMACR in the intestinal-type adenocarcinoma and its precursor lesions, and to determine whether the expression of AMACR can be used to help identify high-grade dysplastic epithelium in gastric mucosa.

## Materials and methods

### Case selection

Formalin-fixed, paraffin-embedded gastric tissue samples were selected from the archives of the Department of Pathology, Nanjing Medical College Affiliated Nanjing First Hospital between the years 2005 and 2006 including 106 gastric mucosal biopsy samples and 25 gastrectomy samples (92 males, 39 females). The mean age of patients was 45.2 years (range 23-81). Histologically, 37 samples were negative for dysplasia, 30 samples were indefinite for dysplasia, 22 samples were low-grade dysplasia, 25 samples were high-grade dysplasia, and 34 samples were invasive intestinal-type adenocarcinomas. Of 25 high-grade dysplasia, 17 were taken from paracancerous mucosa. Among the 25 high-grade dysplasia, there were 17 cases with *H. Pylori* infection and 8 without, according to clinical information. Intestinal metaplasia (IM) typing was determined by PAS-AB (pH2.5) and HID-AB (pH2.5) staining (Liu et al., 2007). Among the 75 samples with IM, 51 had complete IM and 24 had incomplete IM, in which 9 complete IMs and 11 incomplete IMs were in high-grade dysplasia. The morphologic diagnostic criteria of negative for dysplasia, indefinite for dysplasia, low- and high-grade dysplasia in stomach were made following the revised Vienna Classification (Dixon, 2002). For diagnosis of low-grade dysplasia, the glands are slightly crowded but show a rather similar size and shape, and there is a regular arrangement of basally oriented, spindle-shaped and only mildly to moderately hyperchromatic nuclei. High-grade dysplasia is defined as "The glands are crowded and have a variable shape and size, but there is no invasion into the lamina propria mucosa, the nuclei have a short spindle

shape, are moderately hyperchromatic and show moderate stratification". The hematoxylin and eosin-stained sections were reevaluated by two pathologists (XH L and KW F), and only cases where consensus on the diagnosis was achieved were included in the study. Interobserver disagreement, which occurred in 13% of the cases, was resolved by reviewing the slides at a multiheaded microscope by the two pathologists. In the instances of disagreement, the most severe grade of dysplasia was used as the final diagnosis.

### Immunohistochemistry

Immunohistochemical studies were performed on formalin-fixed, paraffin-embedded sections using an avidin-biotin-peroxidase complex method. Sections were cut at 4  $\mu\text{m}$ , placed on positively charged slides, deparaffinized in organic solvents, then treated with 3% hydrogen peroxide to quench endogenous peroxidase activity, and rehydrated. Following rehydration, heat-induced epitope retrieval was performed by placing sections in 10 mM citrate buffer (pH 6.0) and heating for 5 minutes in a pressure cooker. Immunostaining was performed on a Lab Vision Autostainer (Lab Vision, Carpinteria, CA). The slides were incubated with a monoclonal anti-AMACR antibody p504S (1:80 dilution; Zeta Corp, Sierra Madra, CA) for 1 h. Then, sections were sequentially incubated with secondary biotinylated antibodies and with avidin-peroxidase complex, and reacted with diaminobenzidine and hydrogen peroxide using DakoCytomation EnVision+ System-HPR (DakoCytomation). All slides were counterstained with hematoxylin. A tissue section of prostate cancer was used as a positive control for AMACR expression in each staining. Appropriate negative controls for the immunostaining were prepared using nonimmune mouse IgG.

### Immunohistochemical assessment and statistical analysis

All immunohistochemical staining results were evaluated semiquantitatively. AMACR expression was scored as negative, weak (faint diffuse cytoplasmic or granular apical staining), moderate (mild granular cytoplasmic staining), and strong (intense granular cytoplasmic stain). Cases with less than 5% of lesional cells staining positive were scored as 0; 5% to 14%, 1+; 15% to 50%, 2+; more than 50%, 3+. Cases with more than 5% of lesional cell with moderate and strong staining were considered positive. The Fisher's exact probability test was used for statistical analysis of the results. P-values less than 0.05 were considered significant.

## Results

The immunohistochemical findings are summarized in Table 1. In all cases of negative for dysplasia (n=37)

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**Table 1.** Expression of AMACR in gastric adenocarcinoma and precursor lesions.

Degree of Dysplasia	N	AMACR Expression [No. (%)]				Total Positive [No. (%)]
		0	1+	2+	3+	
Negative for dysplasia	37	37 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Indefinite for dysplasia	30	30 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Low-grade dysplasia	22	21 (95.5)	1 (4.5)	0 (0)	0 (0)	1 (4.5)
High-grade dysplasia	24	6 (24)	3 (12)	13 (52)	3 (12)	19 (76)
Adenocarcinoma	34	16 (47.1)	5 (14.7)	10 (29.4)	3 (8.8)	18 (52.9)

and cases indefinite for dysplasia (n=30), the epithelium were uniformly negative for AMACR (Fig. 1a,b). Only 1 of 22 (4.5%) specimens of low-grade dysplasia was weakly positive for AMACR (Fig. 1c). In the group with high-grade dysplasia, 19 of 25 (76%) specimens were positive for AMACR (Fig. 1d). The differences between high- and low-grade dysplasia are very significant (P=0.001). Of 34 invasive intestinal-type adenocarcinomas, 18 (52.9%) were positive for AMACR (Fig. 1e). AMACR expression was higher in high-grade dysplasia than in carcinoma, but the difference between them was statistically insignificant (P=0.070). In 17 cases with high-grade dysplasia in their paracancerous mucosas, the incidences of AMACR expression in high-grade dysplasia and in carcinomas are 76.5% (13/17) and 58.8% (10/17) respectively. But the difference between them is not significant (P=0.271). Although the incidence of AMACR expression in high-grade dysplasia with *H. Pylori* infection (14/17, 82.4%) was higher than those without *H. Pylori* infection (5/8, 62.5%), no significant difference between them was found (P=0.344).

### Discussion

Dysplastic epithelium replacing the normal stomach glands is thought to be the precursor lesion for most tubular adenocarcinomas, and also for the so-called intestinal-type gastric carcinomas (Bajtai and Hidvegi, 1998). Gastric dysplasia is a non-invasive neoplasia or intraepithelial neoplasia, which is divided into low-grade and high-grade. The risk for development to carcinoma is believed to increase with the histological severity of dysplasia. In the revised Vienna Classification, morphological description is adopted for classification and diagnosis of these lesions (Dixon, 2002). However, the description is simple and often poses difficulties for pathologists to differentiate reactive hyperplasia from dysplasia, or low-grade from high-grade dysplasia. There is significant interobserver variability in diagnosis of dysplasia, with poor agreement in separation of negative, indefinite, low-grade dysplasia, and high-grade dysplasia. Furthermore, not all patients with dysplasia progress to adenocarcinoma. Thus, there is an ongoing need to find new biomarkers that can help pathologists accurately categorize dysplastic lesions and provide clinically useful prognostic information.

The value of AMACR in distinguishing high-grade dysplasia from reactive atypia in Barrett's esophagus and inflammatory bowel disease has been reported. Dorer et al. (2005) reported that AMACR was not expressed in any case negative for dysplasia, but was significantly increased in foci of low-grade dysplasia (38%), high-grade dysplasia (81%) and adenocarcinoma (72%) in patients with Barrett's esophagus. Similarly, in patients with inflammatory bowel disease, AMACR was not expressed in any foci of cases negative for dysplasia, but its expression was significantly increased in the foci of low-grade dysplasia (96%), high-grade dysplasia (80%) and adenocarcinoma (71%) (Dorer and Odze, 2006). Lisovsky et al. (2006) found that AMACR staining was uniformly negative in the groups negative for dysplasia and with reactive atypia, only 2 (11%) of 19 specimens with low-grade dysplasia showed positive immunostaining, compared with 14 (64%) of 22 in high-grade dysplasia group in Barrett's esophagus. Of 16 specimens, 12 (75%) showed positive staining for AMACR in the adenocarcinoma group. These findings suggest that AMACR immunoreactivity is fairly sensitive in identification of high-grade dysplasia and is highly specific in distinguishing high-grade dysplasia from reactive atypia in Barrett's esophagus.

In the present study, we firstly evaluated the role of AMACR in detecting dysplasia and distinguishing high-grade dysplasia from low-grade dysplasia and indefinite for dysplasia. Our results showed that AMACR was not expressed in the gastric mucosal specimens with negative and indefinite for dysplasia, but it was observed in 40.8% gastric biopsy specimens with dysplasia, which suggested that AMACR may be a useful immunohistochemical marker for detecting dysplasia. However, further analysis found that AMACR expression was detected in only one biopsy with low-grade dysplasia, suggesting that AMACR had a limited usefulness in discriminating negative for dysplasia and indefinite for dysplasia from low-grade dysplasia. Interestingly, however, AMACR expression was detected in a majority of gastric specimens with high-grade dysplasia (76%) and intestinal-type adenocarcinoma (52.9%). Thus, our findings suggest that AMACR immunohistochemical staining may be useful for distinguishing high-grade dysplasia from low-grade dysplasia and indefinite for dysplasia. In addition, AMACR expression in adenocarcinoma was lower than that in matched high-

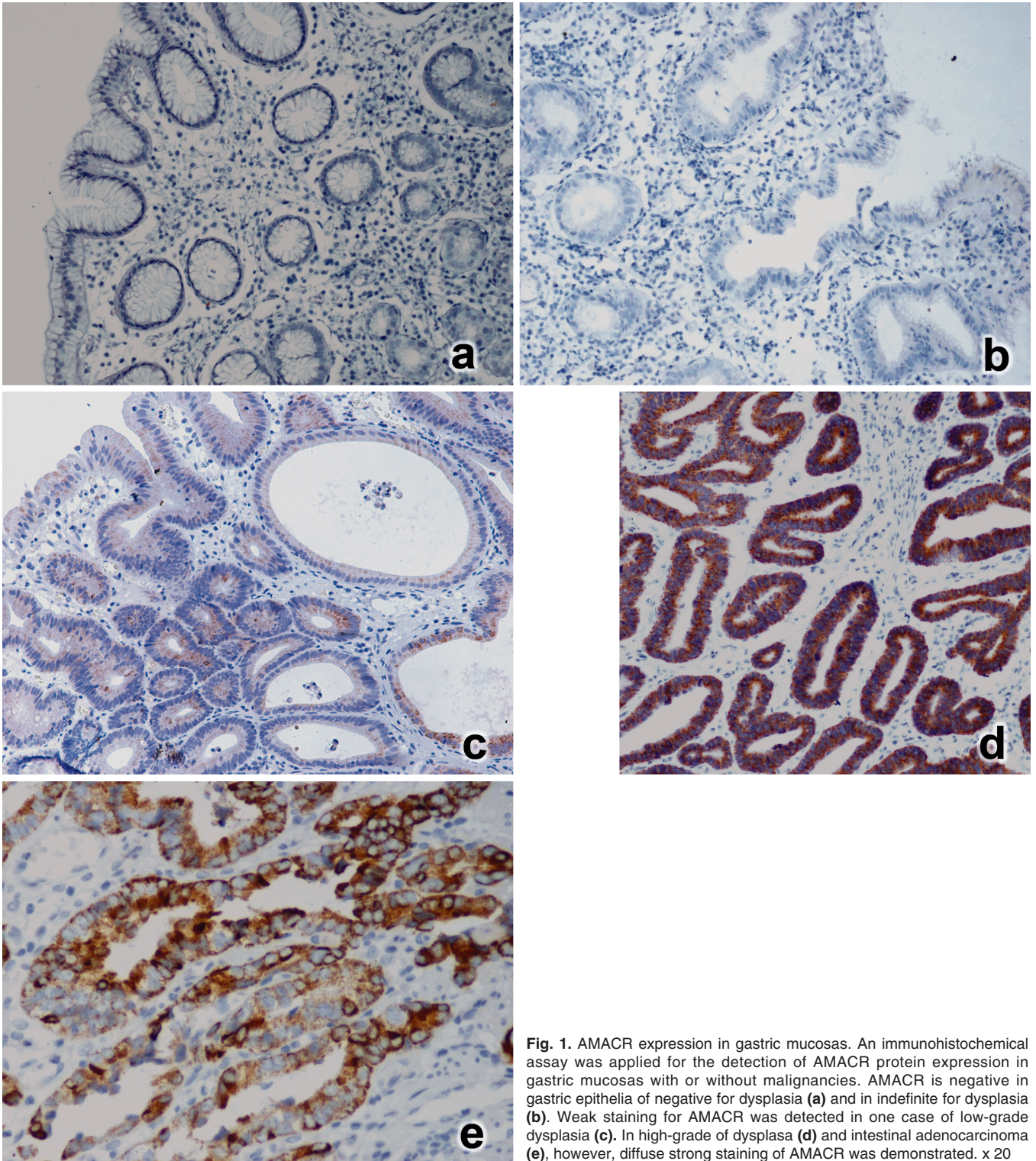


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grade dysplasia, but the mechanism for it was unclear.

Since *H. Pylori* infection was first described in 1983, it has been proven that *H. Pylori* is a definite carcinogen

in gastric cancer (Warren and Marshall, 1983). There were a few studies reporting an association between *H. Pylori* infection and the alteration of oncogene products.



**Fig. 1.** AMACR expression in gastric mucosae. An immunohistochemical assay was applied for the detection of AMACR protein expression in gastric mucosae with or without malignancies. AMACR is negative in gastric epithelium of negative for dysplasia (a) and in indefinite for dysplasia (b). Weak staining for AMACR was detected in one case of low-grade dysplasia (c). In high-grade of dysplasia (d) and intestinal adenocarcinoma (e), however, diffuse strong staining of AMACR was demonstrated. x 20

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However, no studies have explored the association between *H. Pylori* infection and AMACR expression to date. In the present study, we found no AMACR expression in any gastric specimens with negative for dysplasia and indefinite for dysplasia, irrespective of *H. Pylori* infection. In high-grade dysplasia, the expression of AMACR in specimens with *H. Pylori* infection was higher than in those without *H. Pylori* infection, but the difference between them was not statistically significant. These findings suggested that AMACR expression in gastric mucosa may not be associated with infection with *H. Pylori*, but needs to be verified by large size samples.

Intestinal metaplasia (IM) is considered to be a precancerous condition according to the Correa's proposed cascade of gastric carcinogenesis (Correa, 1992). Individuals with IM are estimated to have a 10-fold increase in the risk of developing gastric malignancy (Uemura et al., 2001). However, the molecular pathways underlying progression of IM into cancer remains elusive. Past studies have identified a number of molecular alterations in IM. These alterations include p53 mutation, overexpression of transforming growth factor alpha and epidermal growth factor receptor, microsatellite instability and overexpression of cyclooxygenase-2 (To et al., 2002). In this study, we found no AMACR expression in any gastric specimens with negative for dysplasia and indefinite for dysplasia, irrespective of typing of IM. Similarly, there were no differences in AMACR expression between complete IM and incomplete IM in high-grade dysplasia. These findings suggested that AMACR expression in gastric mucosa has no relation with intestinal metaplasia.

Recently, Cho et al. (2007) reported an overexpression of AMACR in 7.7% of IM, 79.3% of adenoma and 62.9% of adenocarcinoma of the stomach, while there was no expression in the normal gastric epithelia. However, they did not explain the degree of dysplasia in their 8 gastric adenomas. In any case, combining our results with Cho's, AMACR may be involved in gastric carcinogenesis, specifically in an intermediate stage from low-grade to high-grade dysplasia transaction.

In summary, our study suggests that AMACR may play a role in the intermediate stage of gastric carcinogenesis. We have also shown that AMACR is expressed in high-grade gastric dysplasia with a high sensitivity and specificity. Based on our results, we deduce that AMACR may be helpful in distinguishing high-grade dysplasia from low-grade dysplasia in gastric biopsy.

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