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Lack of AMACR Immunostaining is an Independent Predictor of Poor Prognosis in Colorectal Carcinoma

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Abstract

Background: AMACR (Alfa-Methylacyl-CoA Racemase) overexpression has become a useful biomarker of prostate cancer. In the present cohort we are aiming to analyse AMACR immunostaining in normal colonic mucosa, colorectal adenoma, and colorectal carcinoma (CRC) to explore the significance of immune-staining in relation to clinic-pathological features, prognosis, and survival.

Materials and Methods: The study included 38 normal colonic mucosae, 40 colorectal adenomas, 196 CRC, and 49 associated lymph node metastasis. Tissue microarrays were designed and constructed and immunostaining was done using anti-AMACR antibody.

Results: AMACR was absent in normal colonic mucosa while it showed positive immunostaining in 47.5% of adenomas, 53.6% colorectal carcinomas and 36.7% of nodal metastasis. There was no statistically significant difference between AMACR immunostaining in primary CRC in relation adenomas, and nodal metastasis. Low AMACR immunostaining showed significant association with the occurrence nodal metastasis (p=0.039) and distant metastasis (p=0.022). There was no significant association between AMACR immune-staining and other clinic pathological parameters. Regression analysis revealed that reduced AMACR immunostaining was an independent predictor of positive surgical resection margins, presence of Lymph vascular invasion, distant metastasis, and lymph node metastasis. AMACR immunostaining was not related to both diseases free survival and overall survival.

Conclusion: AMACR immune-staining correlated with nodal metastasis and distant metastasis. Loss of immunostaining of AMACR is an independent predictor of lymph vascular invasion, positive surgical margin, nodal and distant metastasis. AMACR may serve as biomarker of progression and prognosis of CRC.

Keywords: AMACR; Immunohistochemistry; Colorectal carcinoma; Prognosis

Abbreviations

AJCC: American Joint Committee on Cancer; AMACR: Alfa-Methylacyl-CoA Racemse; CRC: Colorectal carcinoma

Introduction

Colorectal carcinoma (CRC) is a major cause for morbidity and mortality globally [1]. CRC is related to genetic and environmental factors. Epidemiological studies suggested obesity, diabetes mellitus, smoking, alcohol intake, and dietary habits as environmental factors contributing to CRC [2]. Low fibre diet and increased consumption of fatty acids are proposed the most contributing factors. The main source of branching fatty acids in humans is red meat and dairy products [3-5] Prolonged meat and fatty meals consumption increases the risk of incidence of CRC to 20-30% and is also linked to an increased incidence of mortality in CRC patients [3]. Some reports suggested that fatty acid may promote human carcinogenesis through stimulation of colonic cell proliferation or through genetic mutations that may affects enzymes responsible for detoxification of harmful metabolites resulting from its consumption [4,5].

AMACR (Alfa-Methylacyl-CoA Racemse) was identified in rat liver as mitochondrial and peroxisomal enzyme. It catalyses a key step in the metabolism of fatty acids and fatty acid derivatives via conversion of (2R) fatty acids into their S-stereoisomers, which can be further metabolised and degraded in order to produce energy. An increase in utilisation of energy from fat is the hallmark of many cancer cells [6,7]. AMACR immunostaining was detected in normal hepatocytes, epithelial cells of renal tubules, bronchial epithelium, salivary glands and gall bladder, whereas, it was absent in normal epithelium of

prostate, lung, colon, skin, ovary and endometrium [8,9]. Severe reduction of AMACR activity is associated with neurological disorder due to accumulation of R-2-methyl fatty acids [10]. On the other hand, the consistent overexpression of AMACR in high grade intraepithelial neoplasia of prostate and primary and metastasising prostatic carcinoma pointed to its role in the biology of prostatic cancer [11-14]. Further studies revealed AMACR is overexpressed in other organ cancers as colon [15,16], liver [17], lung [18], bladder [19], lymphoma [20], renal cell carcinoma [21], and melanoma [22]. AMACR is rarely expressed in gastric [23], ovarian [24], and breast [25] carcinomas.

Despite the above promising data, there is still limited information about the relation of AMACR immune-staining to clinic-pathological variables and prognostic parameters of CRC. Exploration of characteristic AMACR immunostaining may add to understanding of mechanisms by which diet affects cancer colon development and may afford new insights into cancer prognosis, and therapy. The aim of the present study is to analyse AMACR immunostaining in normal, dysplastic and malignant colorectal tissues, and to explore significance of immunostaining in relation to clinic-pathological variables and free survival period.

Materials and Methods

Patients

The material of the present study represent paraffin blocks from 38 normal colonic mucosa, 40 colorectal adenoma, 196 colorectal carcinoma cases and 49 lymph nodes with metastatic CRC. Paraffin blocks were retrieved from database of the Department of Pathology, King Abdulaziz University from 1995 to 2010. Patients' clinical information, follow up data and treatment outcome data were collected from hospital patients records. The following Clinicopathological parameters for CR adenomas and CRC cases were used; age, sex, tumour site and size, histological type and grade, stage at time of diagnosis (following the AJCC staging system) [26], margins of excision, and the presence of distant metastasis. Details of clinic-pathological findings are listed (Table 1).

Parameter		Number (%)
Age	<60 years	100 (51%)
	≥ 60 years	96 (49%)
Sex	Male	109 (55.6%)
	Female	87 (44.4%)
Grade	Well-differentiated	40 (20.4%)
	Moderately-differentiated	129 (65.8%)
	Poorly-differentiated	27 (13.8%)
Tumour location	Right colon	57 (29.1%)
	Left colon	116 (59.2%)
	Rectum	23 (11.7%)
Tumour size	<5 cm	88 (44.9%)
	≥ 5 cm	108 (55.1%)
Primary tumour	T1	4 (2%)
	T2	28 (14.3%)
	T3	145 (74%)
	T4	19 (9.7%)
Lymphovascular invasion	Negative	169 (86.2%)
	Positive	27 (13.8%)
Margin status	Free	185 (94.4%)
	Involved	11 (5.6%)
Nodal metastasis	Negative	104 (53.1%)
	Positive	85 (43.3%)
	Not applicable	7 (3.6%)
Distant metastasis	Negative	139 (70.9%)
	Positive	57 (29.1%)
Survival	Alive	140 (71.4%)
	Died of disease	42 (21.4%)
	Data not available	14 (7.2%)
Relapse	Negative	119 (60.7%)
	Positive	77 (39.3%)

Table 1: Clinicopathological parameters of CRC cases.

T1: Tumour invades sub-mucosa; T2: Tumour invades muscularis propria; T3: Tumour invades through the muscularis propria into the sub-serosa or into non-peritonealised pericolic or perirectal tissues; T4: Tumour directly invades other organs or structures, and/or perforates visceral peritoneum

The study was approved by the Research Committee of the Biomedical Ethics Unit, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia. All patients included in this study gave an informed written consent for utilisation of their material in research and was accepted by Research Committee of the Biomedical Ethics Unit.

Tissue microarray and immunohistochemistry

Paraffin blocks were sliced and stained routinely with haematoxylin and eosin to review histopathological characteristics of cases as histological type and grade, presence of lymph-vascular and perineural invasion, status of surgical margins, as well lymph node status. Using an automated microarrayer (Master 3D Histech) [27], two replicate tissue cores (1.5 mm each) were sampled and inserted into tissue microarray blocks. Blocks were sliced into 4 micron meter sections, embedded in sialinated slides in order to be stained by immunohistochemical staining. Immunostaining of AMACR was performed using Avidin-Biotin procedure following manufacturer's kit instructions. Sections were stained immunohistochemically with primary antibody (mouse polyclonal antihuman AMACR antibody, 1:200 dilutions, Dako cytomation, Norden, Glostrup, Denmark) using automated immune-stainer (Ventana, Benchmark-XT). For each test, positive controls were selected from malignant prostatic tissue previously known to stain for AMACR, and as negative control we

replaced primary antibody by Tris buffered saline. Immunohistochemical stain was evaluated semi-quantitatively by two separate expert pathologists (EE and WG) by counting the extent (%) of staining of AMACR in cells. The extent Immunostaining was scored as follows; negative when positivity is found in less than 10% of cells, low immunostaining (10-15% positivity), and high immunostaining when positivity is detected in more than 50% of cells.

Statistical analysis

Statistical analysis was performed using SPSS® Release 16.0. Statistical significance was determined at p value ≤ 0.05 and was 2-sided. The following tests were used. Mann Whitney test and Kruskal Wallis test to compare two and three groups of variables respectively. One sample non-parametric chi-square tested variance along one variable. Binary logistic regression analysis was used to prediction of lymph noel metastasis, distant metastasis, surgical resection margins involvement, lymph-vascular invasion, and local disease recurrence in relation AMACR immunostaining. The Kaplan-Meier procedure was used for disease-free and overall survival probabilities.

Results

AMACR immunostaining profile

AMACR immunostaining was shown exclusively in the cytoplasm of atypical and malignant cells. All normal colonic mucosa were negative to AMACR staining. 47.9% of colonic adenomas showed low positivity to AMACR for CRC cases, 42.9% showed low immunostaining profile while 10.7% of cases showed strong immunostaining (Table 2).

Tissue	(n)	AMACR immunostaining			p value*
		Negative immuno staining	Low immuno staining	High immuno staining	
Normal colonic mucosa	38	38 (0%)	0 (0%)	0 (0%)	<0.001
Colorectal adenoma	40	21 (52.5%)	19 (47.5%)	0 (0%)	0.752
Colorectal carcinoma	196	91 (46.4%)	84 (42.9%)	21 (10.7%)	<0.001
Lymph node metastasis	49	31 (63.3%)	16 (32.7%)	2 (4.1%)	<0.001

Table 2: Distribution of scoring categories of AMACR immunostaining in different tissues examined.

n = number of cases falling in each category; *One sample non-parametric chi-square test

AMACR was downregulated in primary tumours (p<0.001) and in nodal metastasis (p<0.001). However, in adenoma there was no difference between low and high immunostaining (Figure 1).

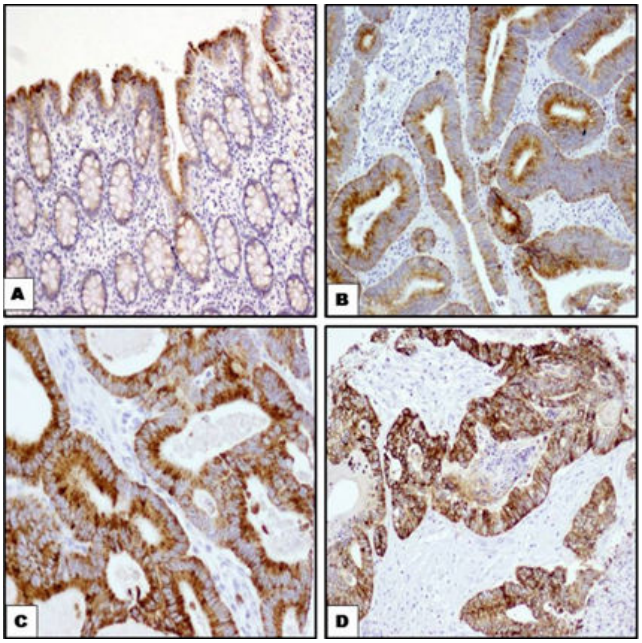

Figure 1: Immunohistochemical immunostaining of AMACR.

A: Normal colonic mucosa showing focal staining in cytoplasm of surface epithelium only (100 x); B: Colorectal adenoma showing weak cytoplasmic expression (100x); C: Primary CRC carcinoma showing diffuse strong cytoplasmic immunoreactivity (100x); D: CRC metastasising to lymph nodes showing strong cytoplasmic staining (100x). Immunohistochemical labelling of AMACR was using anti-AMACR antibody. Diaminobenzidine was used as a chromogen and haematoxylin as a counterstain.

There was no statistically significant difference between AMACR immunostaining in primary CRC in relation to normal colonic mucosa, adenoma, and nodal metastasis (Table 3).

	p value*
Malignant *vs.* Normal mucosa	1.000
Malignant *vs.* Adenoma	0.283
Malignant *vs.* Nodal metastasis	0.646
Nodal metastasis *vs.* Normal mucosa	1.000
Nodal metastasis *vs.* Adenoma	0.974
Adenoma *vs.* Normal mucosa	1.000

Table 3: Differences in AMACR immunostaining in different tissues examined; *Mann-Whitney Test.

The relationship between AMACR Immuno-staining and clinic-pathological features of CRCs

Low AMACR immune-staining in CRC is associated with the occurrence of lymph node metastasis and distant metastasis. However, there was no significant association between AMACR immunostaining and age, sex, degree of differentiation, depth of tumour invasion, stage,

lymph-vascular invasion, and status of surgical resection margins (Table 4).

	p value
Age	0.430✪
Sex	0.882✪
Grade	0.835*
Tumour location	0.482 *
Tumour size	0.834✪
Depth of invasion (pT)	0.452*
Lymphovascular invasion	0.689✪
Margin status	0.238✪
Nodal metastasis	0. 039✪
Distant Metastasis	0.022✪
Relapse	0.195✪

Table 4: AMACR immunostaining in relation to clinicopathological parameters, * Kruskal-Wallis Test, ✪ Mann-Whitney test.

Regression analysis revealed that AMACR downregulation was an independent predictor of surgical resection margins, lymphovascular invasion, nodal metastasis, and distant metastasis (Table 5).

Variable	Exp B	95% CI for Exp B	p value
Surgical resection margins	0.067	0.036-0.123	<0.001
Lymph-vascular invasion	0.182	0.121–0.275	<0.001
Nodal Metastasis	2.9109	1.019–8.303	0.046
Distant metastasis	0.354	0.141–0.885	0.026
Relapse	0.550	0.222–1.367	0.198

Table 5: Binary logistic regression analysis for AMACR immunostaining.

AMACR immunostaining was not related to both diseases free survival Log Rank (Mantel-Cox)=0.676, p=0.414, and overall survival Log Rank (Mantel-Cox)=0.330, p=0.566 (Figures 2 and 3).

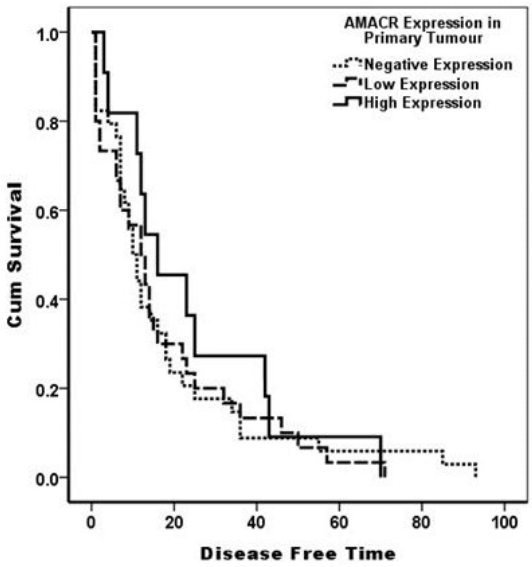

Figure 2: Disease-free survival curve according to AMACR immunostaining showing the survival probabilities of negative, low and high immunostaining. There is no statistically significant difference in survival probabilities (log-rank=0.676, p=0.713).

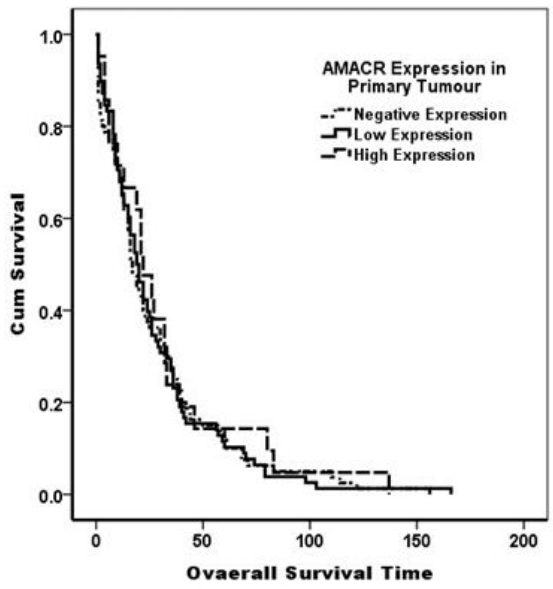

Figure 3: Overall survival curve according to AMACR immunostaining showing the survival probabilities of negative, low and high immunostaining. There is no statistically significant difference in survival probabilities (log-rank=0.333, p=0.848).

Discussion

The biological role of AMACR overexpression is proved in carcinogenesis of many organ systems most probably due to its relation to beta-oxidation of branching fatty acids [7]. The greatest expression

of AMACR was reported in prostatic and colonic carcinoma accounting for 92% and 83% respectively [11,15]. Nowadays, AMACR overexpression is considered a promising biomarker to distinguish normal prostate from malignant tissue particularly in ambiguous needle prostatic biopsies and even in blood samples [11,28]. The differential expression of AMACR in some malignancies rather than others, make it useful diagnostic tool in contrasting carcinoma originating from various primaries as colon, ovary and breast [29], and endometrial carcinomas [30]. Recently, attention was paid to AMACR as biomarker in preneoplastic conditions in many organs. Many studies reported significant correlation between AMACR expression and degree of dysplasia in preneoplastic conditions of gastrointestinal tract as Barrett's esophagus and inflammatory bowel disease and suggested AMACR as additional risk factor in colorectal preneoplastic conditions and considered it as candidate adjunct marker for further evaluation regarding risk stratification of patients undergoing colonoscopy surveillance [31-34].

Many research efforts were performed to explore the relation between AMACR expression and clinic-pathological features in variable malignancies. Rubin et al. [32] reported correlation between AMACR expression and prostatic carcinoma recurrence and cancer specific death. Xu and Huang [17,35] suggested AMACR as prognostic biomarker for early recurrence and metastasis of hepatocellular carcinoma. Similar results were obtained in gastrointestinal stromal tumours [36], nasopharyngeal carcinoma [37].

Despite these promising data about value of AMACR as diagnostic and prognostic adjunct in surgical pathology, there are limited reports about the relation of AMACR immune-staining to clinic-pathological variables and prognostic parameters of colonic malignancies. In the present study, we analysed immunohistochemical expression of AMACR in normal colonic mucosa, colorectal adenomas, CRC and metastatic CRC to lymph nodes. None of normal colonic mucosae showed positive immunoreactions to AMACR except for occasional positivity in cytoplasm of some surface epithelial cells which were considered as negative staining. AMACR immune-positivity was noted in colorectal adenomas, but no significant correlation was found between its immune-staining and any clinic-pathological variables or degree of dysplasia. Our results are comparable to those of Went et al [37] who did not find any significant correlation between AMACR expression and clinic-pathological features of colorectal adenomas. On the other hand, Lakis [16] and Starter [34] reported that increased immunostaining of AMACR in colorectal adenomas was associated with villous pattern, adenomas larger than 1 cm in diameter, high grade dysplasia and /or carcinoma in situ, whereas adenomas with tubular features, small size and low grade dysplasia showed weak positive staining to AMACR. AMACR was expressed in 53.6% of CRC cases and in 36.8% of metastatic CRC to Lymph nodes. Our result is comparable to other investigators who reported AMACR positivity in 47 to 81% of CRC cases [38-40]. We found significant correlation between AMACR down regulation with the occurrence of lymph node metastasis and distant metastasis, but no association between AMACR immune-staining and clinic-pathological features as age, sex, degree of differentiation, depth of tumour invasion, tumour stage, lymph-vascular invasion, and status of surgical resection margins. Our results are contradictory to those of Marx, et al. [39] who reported positive relation between reduced AMACR immunostaining and high tumour grade, stage and occurrence on left colon but did not find any relation to lymph node status or distant metastasis.

Conclusion

AMACR immunostaining is correlated with nodal metastasis and distant metastasis. AMACR is an independent predictor of lymph-vascular invasion, positive surgical margin, nodal and distant metastasis. AMACR may serve as biomarker of progression and prognosis of CRC.

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