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Original article

Low acyl-CoA synthetase 5 expression in colorectal carcinomas is prognostic for early tumour recurrence

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ABSTRACT

It has been shown that the metabolism of long chain fatty acids is involved in colorectal carcinogenesis. Acyl-CoA synthetases (ACSL) activate free fatty acids by synthesis of acyl-CoA thioesters. ACSL isoform 5 (ACSL5) is involved in enterocytic differentiation and maturation by regulating both pro-apoptotic and anti-proliferative effects. Whilst impaired expression of ACSL5 has been associated with sporadic colorectal carcinogenesis, little is known about ACSL5 as a prognostic factor. Aim of this retrospective study was to characterize the prognostic impact of ACSL5 expression levels in sporadic colorectal adenocarcinomas. A total of 72 patients with a median follow-up of 54 months was included. Using a standardized immunohistochemical approach, colorectal adenocarcinomas with low (n = 41; group 1) or high (n = 31; group 2) ACSL5 levels were identified. In a one-year follow-up, tumour recurrence was significantly increased in group 1 ($p = 0.0279$). The finding was independent of the TNM- and UICC-stage in the surgical resections. Frequency of lymph node metastasis and mortality was not different between the groups. In a long-time follow-up no differences were found between the ACSL5 groups. The data indicate that ACSL5 could be an independent prognostic factor for early recurrence of sporadic colorectal adenocarcinoma.

1. Introduction

Colorectal adenocarcinoma (CRC) is one of the most frequent malignant tumours in industrialized countries. In current pathophysiological concepts of colorectal carcinogenesis, long-chain fatty acid metabolism is suggested as an important player of tumourigenesis in addition to aberrant Wnt-signaling [1,2]. Evidence is given that long-chain fatty acids and their derivatives modify several tumour-related pathways by protein lipidation resulting in disturbed molecular signaling [3]. In the last few years, enzymes probably involved in the carcinogenesis-related lipid metabolism have reached more and more the focus of intestinal carcinogenesis research [4,5].

Acyl-CoA synthetase long-chain isoform 5 (ACSL5) is one of the lipid metabolizing enzyms that is expressed and synthesized by intestinal epithelial cells [6]. The enzyme catalyzes activation of palmitic acid to acyl-CoA thioesters. In the intestine, the nuclear-coded mitochondrial ACSL5 protein is found abundantly in epithelial cells of small intestinal villi, but its expression is rare in epithelia lining small or large intestinal crypts. Throughout the intestine, an ACSL5 expression gradient exists from intestinal crypt bottom either to the small intestinal villus tip (crypt-villus axis; CVA) or the mucosal plateau in the large intestine (crypt-plateau axis; CPA). The characteristic gradient reflects different ACSL5 functions in the metabolism of long-chain fatty acids including fatty acid activation after lipid absorption, mitochondrial lipid channeling, and energy balancing.

Recently, it has been shown that ACSL5 is a modifier of cell vitality [7–9]. In different epithelial cell types including enterocytes and hepatocytes, ACSL5 expression mediates pro-apoptotic activities. These ACSL5-related functions are associated with disturbed

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expression of TRAIL-receptors and accumulation of pro-apoptotic complex lipid species.

In intestinal mucosa, the pro-apoptotic ACSL5 function is aggravated by an ACSL5-related silencing of Wnt-activity. Mitochondrial palmitic acid, activated by ACSL5 to palmitoyl-CoA, modifies Wnt2B protein by palmitoylation. Due to this lipidation Wnt2B is arrested in mitochondria and not able to translocate into the nucleus for activation of Wnt signaling [3].

In a previous study [5], disturbed expression and synthesis of ACSL5 in sporadic CRCs was shown with characterization of two ACSL5-related tumour groups (CRC^{ACSL5+}: CRCs with high ACSL5 expression; CRC^{ACSL5-}: CRCs with low ACSL5 expression). In the present study we hypothesized that the expression level of ACSL5 in CRCs could be a prognostic variable for patient's outcome.

2. Material and methods

2.1. Patients

In a clinical database search, all CRC patients treated at the Surgical Department of the Luisenhospital Aachen and the University Hospital Aachen between 2002 through 2007 were identified using the items 'carcinoma' and 'colon' or 'rectum'. Non-R0 resected patients (RX, R1, R2), patients with CRC recurrence, and non-sporadic CRCs were excluded from the study. Based on these criteria 72 patients (47 men; 25 women) were included in the study. All CRCs were diagnosed according to conventional histological criteria (WHO) using hematoxylin and eosin-stained sections of formalin-fixed and paraffin-embedded tissues.

The clinical follow-up for all patients was performed at the Surgical Department and routinely included colonoscopy, imaging with CT-scan/sonography, and measurement of serum CEA as important tumour marker. Tumour recurrence was defined as local tumour recurrence, distant metastasis or cancer-associated death. Study design and procedure were approved by the local ethics committee at the University Hospital at RWTH Aachen University (EK 179/12).

2.2. Immunohistochemistry

Using routine procedures, sections of paraffin-embedded tissues from each primary CRC and associated normal large intestinal mucosa (n = 72) were performed [6,8]. Briefly, tissue sections about 3 μm thick were dewaxed, pre-treated with citratbuffer pH 6.0, incubated in blocking solution, and afterwards incubated with the first antibody directed against ACSL5 (Abnova, Walnut, CA; H00051703-M01, dilution 1:50). The isotype-matched immunoglobulin was used as control. Immunobinding was detected with the Envision-System (DAKO, Glostrup, Denmark).

2.3. Evaluation of ACSL5 expression in CRCs

In a previous study, strong correlation of ACSL5 immunostaining with ACSL5 expression (qRT-PCR) as well as ACSL5 synthesis (Western blot) in CRCs and intestinal tissues was found [5]. The finding prompted us to evaluated ACSL5 expression in CRCs by relative values when compared with the adjacent normal mucosa. For this purpose a grading system was established using the strongest anti-ACSL5 immunostaining in normal mucosa as base line/reference value. In a scaled system with 0.5 steps (−4 through +4) ACSL5 expression in CRCs was evaluated. Zero was indicating for identical immunostaining of mucosa and tumour. Values below zero (−4 through 0) were used to characterize diminished ACSL5 expression in CRCs, whereas ACSL5 overexpression in CRCs was judged by values from 0 through +4. In order to estimate heterogeneity of ACSL5 immunostaining/expression in CRCs, three different tumour levels, each evaluated in two separate randomly chosen areas (40× objective, 18 mm field of view ocular), were used: apical (surface), middle (center), basal (invasion front). Using the algorithm, ACSL5 expression in CRCs was characterized by the median of the six values. Evaluation of anti-ACSL5 immunstainings was independently performed by two pathologists (FH and NG).

2.4. Statistical analysis

Categorical data are shown as frequencies and percentages. Continuous variables are presented by the median and the range. The calculation of the median follow-up was based on the patients without any event. The recurrence-free survival was defined as the number of months between primary CRC operation and a local tumour recurrence, metastasis or tumour-associated death. Data of patients without any tumour-related event at the date of their last follow-up were used as censored observation. Survival probabilities were estimated and plotted according to the Kaplan-Meier method, separately for the $ACSL5^{high}$ and $ACSL5^{low}$ CRC patients. Differences between survival curves were evaluated using the log-rank test. In a sensitivity analysis, a cox proportional hazard regression was used to evaluate the survival curves for the $ACSL5^{high}$ and $ACSL5^{low}$ CRC patients taking into account the prognostic variables T- and N-stage.

In order to evaluate ACSL5 as prognostic marker of early tumour recurrence, recurrence-free survival within the first year after CRC surgery was separately analyzed. For this purpose, patients with a tumour manifestation within the first year after initial CRC surgery were counted and all other patient data were used as censored observations after one year follow-up without any tumour-related event. Statistical analyses were conducted using SAS version 9.2 (SAS Institute, Cary, NC, USA) and R Version 3.3.0/RStudio Version 0.99.902 (RStudio Inc., Boston, MA, USA).

3. Results

3.1. Classification of CRCs

In the present study, 72 CRC patients (47 men; 25 women) were included with a median follow-up time (i.e. period between primary surgical CRC resection and last clinical follow-up) of 54 months. The median age at first tumour surgery was 65 years (39–85 years). CRCs were most often found in the rectum (41.67%) followed by sigmoid colon (30.56%), ascending colon (12.50%), cecum (6.95%), left colic flexure (4.17%), right colic flexur (2.78%), and the rectosigmoidal junction (1.39%).

Tumour grading was performed following suggestions of the WHO. Grading G2 was given in 86.11% (n = 62), G3 in 13.89% (n = 10). The T-stage was variable with two T1 tumours (2.78%), 19 T2 (26.39%), 42 T3 (58.33%), and 9 T4 (12.5%). Lymph node metastasis with N1-category was given in 31.94% (n = 23), N2-category in 16.67% (n = 12). At primary CRC surgery, hematogenic metastasis was found in 8.33% (n = 6).

The UICC stages were different among the patients and include stage I in 23,61% (n = 17), stage II in 26,39% (n = 19), stage III in 41,67% (n = 30) and stage IV in 8,33% (n = 6). From 72 CRC patients, tumour recurrence (recidive, metastasis, or tumour-associated death) was found in 43 patients (59.72%). The median time between surgical tumour resection and recurrence was 18 months (3–78 month).

3.2. Classification of CRCs in $ACSL5^{high}$ and $ACSL5^{low}$ tumours

From each surgical resection, tissue sections of formalin-fixed and paraffin-embedded adenocarcinoma and normal intestinal mucosa were anti-ACSL5 immunostained. Taking strongest ACSL5