Cancer Letters 378 (2016) 51–58

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Original Articles

Tissue-infiltrating plasma cells are an important source of carboxylesterase 2 contributing to the therapeutic efficacy of prodrugs

Anja A. Kühl [a], Ulrike Erben [a], Constanze Cieluch [a], Simone Spieckermann [a], Jörn Gröne [b], Philipp Lohneis [c], Ulrich Frank Pape [d], Ruza Arsenic [c], Nalân Utku [e,*]

[a] Medical Department, Division of Gastroenterology, Infectiology and Rheumatology/ Research Center ImmunoSciences, Campus Benjamin Franklin, Charité – Universitätsmedizin Berlin, Germany
[b] Department of General, Visceral and Vascular Surgery, Campus Benjamin Franklin, Charité – Universitätsmedizin Berlin, Germany
[c] Institute for Pathology, Campus Mitte, Charité – Universitätsmedizin Berlin, Germany
[d] Medizinische Klinik, Hepatology & Gastroenterology, Campus Mitte, Charité – Universitätsmedizin Berlin, Germany
[e] Institute for Medical Immunology, Campus Virchow-Klinikum, Charité – Universitätsmedizin Berlin, Germany

ARTICLE INFO

Article history:
Received 29 February 2016
Received in revised form 19 April 2016
Accepted 27 April 2016

Keywords:
Colorectal cancer
Tumors
Inflammatory bowel disease
Immunohistochemistry
Plasma cells

ABSTRACT

Carboxylesterase 2 (CES-2) is instrumental for conversion of ester-containing prodrugs in cancer treatment. CES-2 expression was analyzed by immunohistochemistry in colorectal cancer (CRC) compared to colonic inflammation as well as in liver and peripheral blood.

In CRC, tumor grades showed no correlation with levels of CES-2 expression, which was heterogeneous within these tumors. Cellular infiltrates in the immediate tumor vicinity expressed high levels of CES-2. Thus, tissue adjacent to the tumor was a substantial source of CES-2 with high expression in plasma cells. CES-2^{high} plasma cells were abundantly found in the colon of patients with inflammatory bowel disease. CES-2 expression is strong in hepatocytes of normal livers, while CES-2 expression in peripheral blood mononuclear cells of healthy donors was overall low at protein and mRNA levels.

In summary, the conversion of ester-containing prodrugs by CES-2 is mainly to occur in the periphery, during liver passage and in the colon after enterohepatic recirculation. We here demonstrated plasma cells as strong producers of CES-2. Further studies should elucidate the role of CES-2^{+} plasma cells in intestinal inflammation and cancer.

Introduction

Carboxylesterases (CES) are members of the serine esterase superfamily and located in the endoplasmic reticulum and the cytosol of cells in many tissues throughout the body, especially within the liver, the kidney, the small intestine and the colon [1]. These enzymes catalyze the hydrolysis of esters, amides, thioesters and carbamates, including environmental toxins and drugs [2], such as the cytostatic drugs used in tumor treatment [2,3]. The enzymatic activity of CES can be exploited to cleave inactive prodrugs, releasing the active (and often more toxic) component of a prodrug at the site of a tumor [4]. An example of this is irinotecan, which is converted to 7-ethyl-10-hydroxy-camptothecin by the CES isoform, CES-2 [5].

Immunohistochemical evaluation has shown moderate expression of CES-2 in normal human colon tissue and a broad range of expression levels in CRC [5,6]. CES-2 is down-regulated with disease progression in CRC as shown by Western-blot analysis from tumor tissues [7].

The FOLFIRI regimen (folic acid, 5-fluorouracil and irinotecan) is commonly used for chemotherapy of advanced colorectal cancer (CRC). Its cytotoxicity can vary, and this variability may be attributed to the tumor stage-dependent differential expression of CES-2 in the tumor tissue, or to the occurrence of splice variants that might interfere with the enzymatic activity of the CES-2 isoform [5,8].

Although a CES-mediated first pass effect within the liver might significantly contribute to the systemic availability of an esterase activity-dependent bioactive drug [9], the main tumor-specific effect should be increased when mediated by its local release leading to an accumulation of the respective drug in tumor tissue. However, high expression of CES-2 does not correlate to a superior outcome in the treatment of metastatic CRC with ester-prodrugs like irinotecan

Abbreviations: AP, alkaline phosphatase; CCC, cholangiocellular carcinoma; CD, Crohn's disease; CES, carboxylesterase; CRC, colorectal cancer; FFPE, formalin-fixed paraffin-embedded; IBD, inflammatory bowel disease; HCC, hepatocellular carcinoma; H&E, hematoxylin and eosin; NEC, neuroendocrine carcinoma; TMA, tissue microarray; UC, ulcerative colitis.

* Corresponding author.
E-mail address: nalan.utku@charite.de (N. Utku).

http://dx.doi.org/10.1016/j.canlet.2016.04.041
0304-3835/

Table 1
Patient characteristics of CRC tumor tissue samples.

Number of patients	86
Gender	42 female/44 male
Age	Median 67 years (range: 22–85 years)
Tumor localization	Cecum (n = 8), colon (n = 56), rectum (n = 22)
Tumor grade*	G1 (n = 4), G2 (n = 47), G3 (n = 35)

* Definition according to the WHO Classification of Tumours of the Digestive System [12].

[10]. By specifically addressing the spatial distribution of cells that express CES-2, the major intestinal CES [11], our study will help to understand how the tumor and non-tumor tissues can contribute to efficacy of ester prodrugs used for the therapy of advanced CRC.

Materials and methods

Human tissue and blood samples

The following archived formalin-fixed paraffin-embedded (FFPE) samples were retrieved from the tissue bank of the Charité – Universitätsmedizin, Zentrale Biomaterialbank (ZeBanC; http://biobank.charite.de/en/service/) (Berlin, Germany): intestinal tissue from patients with CRC including tissue microarray (TMA; Table 1), with neuroendocrine carcinomas (NEC) of the large intestine (cecum, n = 2; colon, n = 3; rectum, n = 2), Crohn's disease (CD, n = 4) or ulcerative colitis (UC, n = 5); liver tissue from patients with hepatocellular (HCC, n = 5) or cholangiocellular carcinoma (CCC, n = 6); normal colon tissue from patients with CRC. Normal tissues were taken from resection margins with normal intestinal morphology assessed after hematoxylin/eosin (H&E) staining. Whole blood samples from healthy donors were obtained from filters from leukapheresis. The study was approved by the ethics committee of the Charité – Universitätsmedizin Berlin (registration number EA1-157-13).

Histopathology

Thin sections of archived patient samples (1–2 μm) were either stained with H&E or subjected to heat-induced epitope retrieval prior to incubation with antibodies specific for CES-1 (clone EP1376Y; Biozol Diagnostica, Eching, Germany) or CES-2 (#ab64867, polyclonal rabbit; Abcam; Cambridge, United Kingdom). These were visualized using the EnVision+ HRP System (#K4011; Dako, Glostrup, Denmark), with diaminobenzidine (DAB; Dako) as chromogen. The nuclei were counterstained with hematoxylin and slides cover-slipped with glycerol gelatin (both Merck, Darmstadt, Germany). The AxioImager Z1 microscope (Carl Zeiss MicroImaging, Jena, Germany) was used for image acquisition. All evaluations were performed in a blinded manner.

To evaluate CES expression in tissue samples, the expression levels and the percentages of CES-expressing tumor cells were added to create an overall score from 2 to 8 as follows: Expression level – 1, low expression; 2, medium expression; 3, strong expression. Percentage – 1, <10%; 2, 10–30%; 3, 31–60%; 4, 61–90%; 5, >90% (Fig. 1).

For detection of CES-2$^+$ leukocytes, sections were subjected to a heat-induced epitope retrieval step prior to blocking of endogenous alkaline phosphatase (AP), using the Dual Endogenous Enzyme-Blocking Reagent (Dako). After rinsing, sections were incubated with anti-CES-2 antibodies, followed by biotinylated goat anti-rabbit antibodies (Dianova, Hamburg, Germany) and AP-labeled streptavidin (Dako). AP was visualized with the VECTOR Blue substrate kit (#SK-5300; Vector Laboratories, Burlingame, USA). Proteins were then inactivated by pressure cooking and the sections were incubated with antibodies specific for CD3 (clone M-20; Santa Cruz, San Diego, USA), CD11b (clone EP1345Y; Abcam), CD20 (clone L26; Dako), CD68 (clone PG-M1; Dako), CD138 (clone MI15; Dako), CD163 (clone 10D6; Leica Biosystems, Nussloch, Germany) or MPO (#A0389, polyclonal rabbit; Dako) followed by biotinylated secondary antibodies (anti-goat, anti-mouse or anti-rabbit; Life Technologies, Carlsbad, USA) and AP-labeled streptavidin (Dako). AP was visualized with the chromogen FastRed (Dako). The nuclei were counterstained, the slides cover-slipped as described above, and the images acquired using the AxioImager Z1 microscope. As FastRed and Vector Blue are both fluorescent, coexpression was also detected using fluorescence microscopy (emission peaks: Red/560 nm, Vector Blue/680 nm). For immunohistochemical detection of CES-2 expressing plasma cells, sections were subjected to heat-induced epitope retrieval step prior to blocking of endogenous AP employing Dual Endogenous Enzyme-Blocking Reagent (Dako). This was followed by incubation with anti-MUM1 (clone MUM1p, Dako) followed by the LSAB™+, Dako REAL™ Detection System (#K5005, Dako). After color development, the proteins were inactivated by pressure cooking and endogenous peroxidase was blocked by Peroxidase-blocking solution (Dako). Sections were incubated with anti-CES-2 followed by the EnVision+ HRP System and DAB. Nuclei were counterstained with hematoxylin and slides cover-slipped with glycerol gelatin. Additionally, immunohistochemistry and fluorescence were combined for costaining of CES-2 and CD138. Sections were subjected to a heat-induced epitope retrieval step prior to blocking of endogenous AP employing Dual Endogenous Enzyme-Blocking Reagent (Dako). After rinsing, sections were incubated with anti-CES-2 followed by biotinylated goat anti-rabbit antibodies (Dianova) and AP-labeled streptavidin (Dako). AP was visualized with LSAB™+, Dako REAL™ Detection System. Proteins were then inactivated by pressure cooking and the sections were incubated with anti-CD138 (clone MI15; Dako) followed by biotinylated anti-mouse secondary antibody and Alexa488-labeled streptavidin (Invitrogen). Nuclei were counterstained with DAPI (Sigma-Aldrich, St. Louis, USA) and sections cover-slipped with Fluoromount G (BIOZOL, Eching, Germany). Negative controls were carried out as above, omitting the primary antibodies.

Human cell lines

Human cell lines were from the American Type Culture Collection (ATCC; Bethesda, USA) or the German Collection of Microorganisms and Cell Cultures (DSMZ;

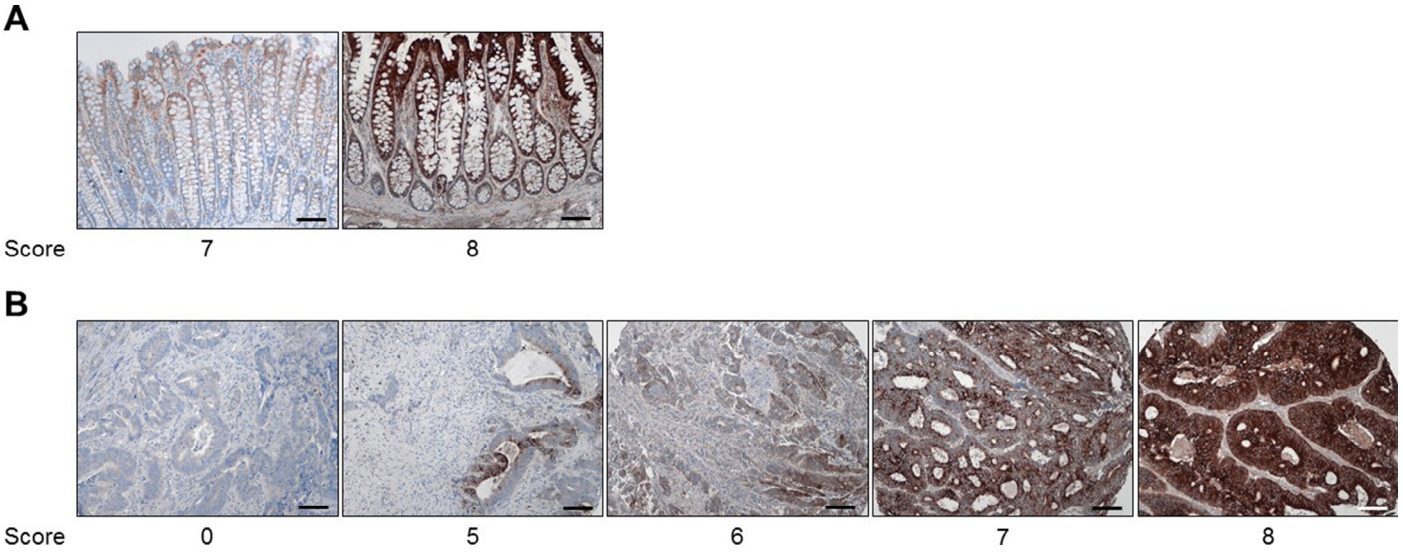

Fig. 1. Scoring of CES expression. (A) Sections of normal tissue or (B) TMA spots from graded CRC were stained for CES-2 by immunohistochemistry (brown); nuclei with hematoxylin (blue). (A) Characteristic CES-2 distribution in normal colon tissue with strong expression in epithelial cells along the crypts. (B) Score 0: all tumor cells devoid of CES-2 expression; score 5: strong CES-2 expression in about 20% of the tumor cells; score 6: medium CES-2 expression in up to 90% of tumor cells; score 7: strong CES-2 expression in up to 90% of tumor cells; score 8: strong CES-2 expression in over 90% of tumor cells. Representative images; original magnification ×100. Bars represent 100 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)