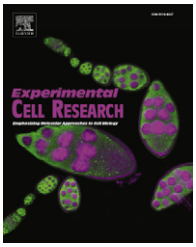
EXPERIMENTAL CELL RESEARCH 318 (2012) 768–778

Available online at www.sciencedirect.com

SciVerse ScienceDirect

www.elsevier.com/locate/yexcr



Research Article

Up-regulation of 12(S)-lipoxygenase induces a migratory phenotype in colorectal cancer cells

T. Klampfl[a,1], E. Bogner[a,1], W. Bednar[a,1], L. Mager[a], D. Massudom[a], I. Kalny[a], C. Heinzle[a], W. Berger[a], S. Stättner[b], J. Karner[b], M. Klimpfinger[b], G. Fürstenberger[c], P. Krieg[c], B. Marian[a,*]

[a]Department of Internal Medicine 1, Institute of Cancer Research, Medical University Vienna, Borschkegasse 8a, 1090 Vienna Austria
[b]Social Medical Centre South, Vienna, Austria
[c]German Cancer Research Centre, Heidelberg, Germany

ARTICLE INFORMATION

Article Chronology:
Received 11 November 2011
Revised version received
15 December 2011
Accepted 21 December 2011
Available online 29 December 2011

Keywords:
Lipoxygenase
12(S)-HETE
Cell migration
Malignant phenotype
E-cadherin
Integrin

ABSTRACT

12(S)-Lipoxygenase (LOX) and its product 12(S)-hydroxyeicosatetraenic (HETE) acid have been implicated in angiogenesis and tumour invasion in several tumour types while their role in colorectal cancer progression has not yet been studied. We have analysed 12(S)-LOX expression in colorectal tumours and found gene expression up-regulated in colorectal cancer specimens for which the pathology report described involvement of inflammation.

Using cell line models exposed to 12(S)-HETE or over-expressing 12(S)-LOX malignant cell growth as well as tumour cell migration was found to be stimulated. Specifically, Caco2 and SW480 cells over-expressing 12(S)-LOX formed fewer colonies from sparse cultures, but migrated better in filter-migration assays. SW480 LOX cells also had higher anchorage-independent growth capacity and a higher tendency to metastasise in vivo. Knock-down or inhibition of 12(S)-LOX inhibited cell migration and anchorage-independent growth in both 12(S)-LOX transfectants and SW620 cells that express high endogenous levels of 12(S)-LOX. On the cell surface E-cadherin and integrin-β1 expression were down-regulated in a 12(S)-LOX-dependent manner disturbing cell–cell interactions. The results demonstrate that 12(S)-LOX expression in inflammatory areas of colorectal tumours has the capacity to induce an invasive phenotype in colorectal cancer cells and could be targeted for therapy.

Introduction

Lipoxygenases (LOX) and their eicosanoid products are involved in many physiological and pathophysiological processes like growth, differentiation, vascularisation, inflammation and arteriosclerosis [1,2]. Specifically, 12- and 15-LOX enzymes have been described as regulators of inflammation and immune-response [3]. Leukocyte, reticulocyte and epidermal forms of the enzyme are well characterised as to their product pattern and physiological function [3–5]. By contrast, the platelet-derived 12(S)-LOX (ALOX12) is still insufficiently understood. It produces almost exclusively 12(S)-hydroxyeicosatetraenic

* *Corresponding author at:* Institute of Cancer Research, Department of Medicine 1, Medical University Vienna, Borschkegasse 8a, 1090 Vienna, Austria. Fax: +43 1 4277 9651.
E-mail address: brigitte.marian@meduniwien.ac.at (B. Marian).
Abbreviations: AA, arachidonic acid; HETE, hydroxyeicosatetraenic acid; LOX, lipoxygenase.
[1] T.K, E.B and WB. contributed to this manuscript equally.

0014-4827/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.yexcr.2011.12.017

acid (HETE) [6,7] that has been shown to act as a growth and/or survival factor in gastric [8], pancreatic [9], melanoma and prostate tumour cells [10,11] and to induce a metastatic phenotype in prostate cancer [12]. In addition 12(S)-HETE may also affect other tissue components to enhance neovascularisation and tumour progression [10,12–15]. For this reason 12(S)-LOX is regarded as a protumorigenic gene [16]. Protumorigenic roles of LOX-enzymes are also suggested by the observation that inhibitors of LOX isoenzymes like quercetin, nordihydroguaretic acid or baicalein can protect from carcinogenesis in both the skin and the colon [17–19]. Such inhibitors are not specific for individual LOX enzymes, however, so that a more specific approach is needed to demonstrate the actual role of 12(S)-LOX in tumour development.

In colorectal cancer protumorigenic impact has been unequivocally demonstrated for cyclooxygenase 2 (COX-2) and its prostaglandin products [20] as well as for 5-LOX and its product leukotrien B4 [21,22]. Concerning 12(S)-LOX a genetic polymorphism that produces a more active enzyme is related to a higher cancer risk [23] suggesting that there is a protumorigenic impact of 12(S)-LOX on CRC. The pathophysiological impact of the enzyme on CRC has not yet been analysed in any detail, however. This study therefore, undertakes to determine the expression of 12(S)-LOX in colorectal carcinomas as compared to normal mucosa as well as the cell biological consequences of 12(S)-HETE and 12(S)-LOX expression in colorectal tumour cell lines.

Materials and methods

Tissue specimen

Tissue specimens were obtained from colorectal carcinomas and normal tumour-free mucosa at the resection margin from patients suffering from colorectal cancer. The study has been approved by the local ethics committee and the patients had given their informed consent. The tissue specimens were collected immediately after surgery and frozen in liquid N_2 until extraction.

Cell lines and transfection

SW480, SW620, and Caco2 colon carcinoma cells were obtained from the American Type Culture Collection. The cell lines were kept under standard tissue culture conditions using Minimal Essential Medium (MEM) containing 10% fetal calf serum (FCS).

12(S)-LOX over-expressing SW480 and Caco2 cells were produced by electroporation as described before [24]. Populations stably over-expressing 12(S)-LOX were cultivated in the presence of 1.6 and 0.2 mg/ml geneticin (G418) respectively.

Eicosanoid mediators, inhibitors and cell treatment

12(S)-HETE (0.1 mg/ml in ethanol) was purchased from Cayman chemicals (Ann Arbor, MI). Arachidonic acid (AA) and baicalein were obtained from Sigma (St. Louis, MO). Cells were plated at a density of 5×10^4 in 24-well plates and left to attach for 24 h before exposure to the compounds diluted into HEPES-buffered MEM containing 1 mg/ml bovine serum albumin (BSA). Experiments on 12(S)-LOX transfectants and their respective controls were done in the presence of 10 μM AA to provide sufficient substrate for eicosanoid synthesis.

Growth parameters

Viable cell number was determined by neutral red uptake as reported previously [24].

For clonogenicity assays cells were plated at a density of 100 cells/well (SW480) or 200 cells/well (Caco2, SW620) into six-well plates in growth medium. Unattached cells were removed 24 h later and the cultures then left to grow for 7 days. The number of colonies was assessed after staining with crystal violet.

Anchorage-independent growth was determined from 5000 cells/well in 0.25% agar prepared in RPMI medium containing 20% FCS and incubated for 2–3 weeks before counting the number of colonies microscopically.

Cell migration assay

Cell migration was analysed by filter migration assay as described before [25]. Specifically, 0.5×10^5 cells/cm² were seeded into 8-μm-pore-size filters (Becton Dickinson-Falcon, Franklin Lakes, NJ) and migration periods of 24 h (SW480, SW620) or 48 h (Caco2) were permitted.

Tumour growth in vivo

SW480-LOX and SW480-co cells were harvested, washed with PBS, and suspended in Ringer's solution. 1×10^6 cells in 50 μl Ringer's solution were subcutaneously injected into the rear flanks of immunodeficient SCID/Balb/c recipient mice (female, aged 4 weeks, Harlan Winkelmann, Borchen, Germany). Tumour formation was monitored periodically by palpation, and the tumour size was determined using a Vernier caliper. Tumour volume was calculated using the formula (smaller diameter$^2\times$larger diameter)/2. All experiments were performed in triplicate and carried out according to the Austrian and FELASA guidelines for animal care and protection. Tissue sections of experimental tumours were analysed by immunohistochemistry using antibodies directed against cytokeratin 20.

Mouse lungs were prepared for immunohistochemistry and stained with a monoclonal antibody recognising Ki67 (Novacastra, Leica Microsystems, Wetzlar, Germany). Serial sections were scored for Ki67-positive cells/field of vision and the following scores were assigned: <1 cell/field $=0$, 1–5 cells/field $=1$; 5–10 cells/field $=2$; 10–20 cells/field $=3$; >20 cells/field $=4$.

Knock-down of 12(S)-LOX expression

To knock down 12(S)-LOX expression 400 pmol (Caco2 transfectants), 10 nmol (SW480 transfectants) or 30 nmol (SW620 cells) of an antisense phosphothioate oligonucleotide directed against 12(S)-LOX with the sequence 5′-CTCAGGAGGGTGTAAACA-3′ [26] was introduced by lipofection. Lipofectamin (Invitrogen Life Technologies, Carlsbad, CA) was used for Caco2 cells and siLentFect (BioRad, Hercules, CA) for SW480 and SW620 cells. Controls were transfected with a scrambled oligonucleotide, the sequence of which was 5′-AAGATT GCGCGACGATGA-3′