Biochimie xxx (2018) 1–10

Contents lists available at ScienceDirect

Biochimie

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

Research paper

27-hydroxycholesterol decreases cell proliferation in colon cancer cell lines

Jessica Warns, Gurdeep Marwarha, Natalie Freking, Othman Ghribi*

Department of Biomedical Sciences, University of North Dakota School of Medicine and Health Sciences, Grand Forks, ND, 58202, USA

ARTICLE INFO

Article history:
Received 26 February 2018
Accepted 11 July 2018
Available online xxx

Keywords:
27-hydroxycholesterol
AKT
Cholesterol
Colorectal cancer
Oxysterols
Proliferation

ABSTRACT

Colorectal cancer (CRC) is the third most diagnosed cancer in the western world, affecting 1 out of approximately 22 people in their lifetime. Several epidemiological studies suggest a positive association between high plasma cholesterol levels and colorectal cancer. However, the molecular mechanisms by which cholesterol may alter the risk of colorectal cancer (CRC) are ill-defined as the cholesterol lowering drugs statins do not appear to decrease a patient's risk of developing colorectal cancer. Cholesterol is metabolized to active derivatives including *cholesterol oxidization products* (COP), known as oxysterols, which have been shown to alter cellular proliferation. These metabolites and not cholesterol *per se*, may therefore affect the risk of developing colorectal cancer. The cholesterol metabolite or the oxysterol 27-hydroxycholesterol (27-OHC) is the most abundant oxysterol in the plasma and has been shown to be involved in the pathogenesis of several cancers including breast and prostate cancer. However, the role of 27-OHC in colorectal cancer has not been investigated. We treated Caco2 and SW620, two well characterized colon cancer cells with low, physiological and high concentrations of 27-OHC, and found that 27-OHC reduces cellular proliferation in these cells. We also found that the effects of 27-OHC on cell proliferation are not due to cellular cytotoxicity or apoptotic cellular death. Additionally, 27-OHC-induced reduction in cell proliferation is independent of actions on its target nuclear receptors, liver-X-receptors (LXR) and estrogen receptors (ER) activation. Instead, our study demonstrates that 27-OHC significantly decreases AKT activation, a major protein kinase involved in the pathogenesis of cancer as it regulates cell cycle progression, protein synthesis, and cellular survival. Our data shows that treatment with 27-OHC substantially decreases the activation of AKT by reducing levels of its active form, p-AKT, in Caco2 cells but not SW620 cells. All-together, our results show for the first time that the cholesterol metabolite 27-OHC reduces cell proliferation in colorectal cancer cells.

1. Introduction

In the United States, Colorectal Cancer (CRC) is the third most diagnosed cancer, affecting 1 out of approximately 22 people in their lifetime. Globally, it is the third most common type of cancer, leading to an estimated 1.4 million patients being diagnosed in 2016 [1] with more than 600,000 deaths [1,2]. There are several risk factors for CRC including age [3], family history of polyps [4], alcohol consumption [5], consumption of westernized diet [6,7], and inflammatory bowel disease [8]. Cholesterol has also been considered a risk factor for cancer, but data about the involvement of cholesterol levels in CRC are still controversial. Several epidemiological studies suggested that higher cholesterol levels were associated with increased risk of developing cancer [9]. Several studies have shown that lowering cholesterol levels with statins, reduced the risk of some cancers [10]. Specifically in CRC, an observational study found a decreased risk of CRC with statin therapy [11,12]. However, other recent studies reported that lower cholesterol levels are associated with an increased risk of CRC [13].

Cholesterol is a precursor to several important molecules such as bile acids, steroid hormones, vitamin D, and oxysterols. Oxysterols are cholesterol oxidation products formed by the addition of a polar group such as hydroxyl, keto, or epoxy groups [14,15]. Oxysterols are not only metabolites that regulate cholesterol concentrations, but active molecules that regulate several functions

* Corresponding author. Department of Biomedical Sciences, University of North Dakota School of Medicine and Health Sciences, 1301 North Columbia Road, Grand Forks, ND, 58202, USA.
E-mail address: othman.ghribi@med.und.edu (O. Ghribi).

https://doi.org/10.1016/j.biochi.2018.07.006
0300-9084/

Abbreviations

27-OHC	27-hydroxycholesterol
ABCA1	ATP-binding cassette, sub-family A, member 1
ABCG1	ATP-binding cassette, sub-family G, member 1
AF	Afuresertib
BSA	Bovine Serum Albumin
COP	cholesterol oxidation products
CRC	Colorectal Cancer
DMSO	dimethyl sulfoxide
ECHS	5α-6α-epoxycholesterol-3-sulfate
E2	Estradiol
ER	Estrogen Receptors
ETOH	Ethanol
GW	GW3965
LDH	Lactate dehydrogenase
LXR	Liver X Receptor
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
SERM	Selective Estrogen Receptor Modulator
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labeling
Veh	Vehicle

including plasma membrane permeability and signal transduction [16], apoptosis [17,18], and immune system [19–21]. The most abundant oxysterol in the plasma is 27-hydroxycholesterol (27-OHC) [22]. 27-OHC is a macrophage synthesized cholesterol metabolite that can enhance inflammation in macrophages which are detected in abundance in atheromatous lesions [23]. Multiple lines of study have demonstrated that cholesterol-enriched diets increase plasma 27-OHC levels [24–29]. This increase in plasma 27-OHC upon high cholesterol diet feeding was observed despite significant heterogeneity in these studies with regards to the relative fraction and percentage of cholesterol content present in the respective diets used in these studies that varied from 0.25% to 2.0% w/w. Furthermore, this phenomenon of high cholesterol diet-induced increase in 27-OHC was recapitulated in different animal species that included rabbits [24], rats [29], mice [26–28], as well non-human primates [25]. 27-OHC is a ligand for liver-X-receptor (LXR), a nuclear receptor that has a variety of functions [30]. For the last decade, 27-OHC has also been shown to act as a selective estrogen receptor modulator (SERM) [31–33]. This discovery implicated 27-OHC in ER-positive carcinogenesis in breast cancer [27]. We and others have also demonstrated that 27-OHC can be deleterious in prostate cancer cells [34,35]. However, because cholesterol metabolism is suspected to influence a variety of cancer progression, little is known about 27-OHC involvement in CRC. We determined in the present study the effects of 27-OHC on cell proliferation in two major cell line models for CRC, Caco2 and SW620 cells. We also determined the extent to which 27-OHC effects involve LXR, ER and the AKT pathway.

2. Material and methods

2.1. Materials

Caco2 cells (cat # HTB-37™), MTT Cell Proliferation Assay (cat # 30-1010 K), and SW620 cells (cat # CCL-227™) (ATCC, Manassas, VA). DMEM media, Leibovitz's L-15 media, 100U/ml penicillin, 100 μg/ml streptomycin, and 0.25μg/ml amphotericin (Life Technologies, Carlsbad, CA). FBS (Atlanta Biologicals, Flowery Branch, GA). GW3965 (cat # 2474), 27-hydroxycholesterol (cat # 3907), and estradiol (cat # 2824) (R&D systems from Minneapolis, MN). 6α-epoxycholesterol-3-sulfate (cat #C4136-000) (Steraloids, Newport, RI). Afuresertib (cat # 17988) (Cayman Chemicals from Ann Arbor, MI). CytoTox 96® non-radioactive cytotoxicity assay kit (cat #G1782) and DeadEnd Fluorometric TUNEL assay kit (cat #G3250) (Promega, Valencia, CA). Hard Set mounting medium with DAPI (cat # H-1500) (Vector Laboratories, Burlingame, CA). DNase (cat #AM2222), Alexa Fluor 594 nm (cat #A11037), Halt™ proteases and phosphatase inhibitor cocktail (cat #78446) (Fisher Scientific, Hampton, NH). Immun-Blot® PVDF membrane for protein blotting (cat # 1620177), Clarity™ Western ECL substrate (BioRAD, Hercules, CA). The antibodies used in this study are included in Table 1 below.

2.2. Cell culture

Caco2 non-metastatic cells were grown in DMEM media containing 10% FBS. SW620 metastatic cells were grown in Leibovitz's L-15 containing 10% FBS. 100U/ml penicillin, 100 μg/ml streptomycin, and 0.25μg/ml amphotericin were added. Cells were maintained at 5% CO_2 at 37 °C. Cells were treated with (0, 0.5, 1, 10, 50, or 100 μM) 27-hydroxycholesterol (27-OHC), 2 nM estradiol (E2), 100 nM ICI 182 780, 10 μM GW3965, and/or 10 μM of 5α-6α-epoxycholesterol-3-sulfate (ECHS). Circulating 27-OHC levels are 0.15–0.73 μM, and these concentrations can be in the millimolar range in some pathological situations such as atherosclerosis [14]. Our study was approved by the Institutional Biosafety Committee of the School of Medicine at the University of North Dakota.

2.3. MTT assay

14,000 cells were seeded in a 96-well plate and grown overnight. Next morning, different concentrations of 27-OHC (0-300 μM) was added and incubated for 24hrs. After 24hrs, MTT assay was performed following manufacture's protocol. Briefly, 10 μl of MTT reagent was added and cells were incubated at 37 °C until purple precipitate formed (2-4hrs). Media was removed and 100 μl of detergent reagent was added and was incubated at room temperature in dark for 2 h. Plate was read using a microplate reader at 570 nm. Ethanol (ETOH) was used as a vehicle control with treatment was made to 1 and the other samples were normalized to the ETOH treatment and the fold change was determined.

2.4. LDH assay

14,000 cells were seeded in a 96-well plate and grown overnight. Next morning, different concentrations of 27-OHC (0-300 μM) was added and incubated for 24hrs. After 24hrs, LDH assay was performed using CytoTox 96® non-radioactive cytotoxicity

Table 1
Antibodies and their sources and applications used in the present study.

Name	Catalog#	Species	Application
ABCA1	Novus biologicals: NB400-105	Rb	Western Blotting
ABCG1	Thermo Fisher: PA5-13462	Rb	Western Blotting
Actin	Santa Cruz: sc-47778	Rb	Western Blotting
AKT	Cell Signaling 9272S	Rb	Western Blotting
p-AKT	Cell signaling 4060S	Rb	Western Blotting
ERα	Santa Cruz: sc-787	Ms	Western Blotting
ERα	Thermo Fisher: PA5-16440	Rb	Immunofluorescence
ERβ	Thermo Fisher: PA1310B	Rb	Western Blotting
LXRα	Thermo Fisher: PA1330	Rb	Western Blotting
LXRβ	Thermo Fisher: PA1333	Rb	Western Blotting

Please cite this article in press as: J. Warns, et al., 27-hydroxycholesterol decreases cell proliferation in colon cancer cell lines, Biochimie (2018), https://doi.org/10.1016/j.biochi.2018.07.006