International Immunopharmacology 52 (2017) 342–351

Contents lists available at ScienceDirect

International Immunopharmacology

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

Enhancing miR-132 expression by aryl hydrocarbon receptor attenuates tumorigenesis associated with chronic colitis

Abdullah M. Alzahrani[a,*,1], Hamza Hanieh[a,1], Hairul-islam Mohamed Ibrahim[a], Omar Mohafez[b,c], Tamer Shehata[b,d], Mohammad Bani Ismail[a], Manal Alfwuaires[a]

[a] Biological Sciences Department, College of Science, King Faisal University, 31982 Hofouf, Saudi Arabia
[b] Pharmaceutical Science Department, College of Clinical Pharmacy, King Faisal University, 31982 Hofouf, Saudi Arabia
[c] Biochemistry Department, College of Pharmacy, Al-Azhar University, Assiut, Egypt
[d] Department of Pharmaceutics, Faculty of Pharmacy, Zagazig University, Egypt

ARTICLE INFO

Keywords:
Ahr
miR-132
AChE
Cholinergic anti-inflammation
CAC

ABSTRACT

Background: Chronic inflammation in ulcerative colitis (UC) patients is the major risk factor for colitis-associated colon cancer (CAC). Recent evidences have shown that microRNAs (miRNAs) are implicated in CAC pathogenesis. However, the interaction of miRNAs with the transcription factors that alleviate CAC has not been reported.

Methods: 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) or 3,3′-diindolylmethane (DIM) were used to activate aryl hydrocarbon receptor (Ahr) in azoxymethane (AOM)/dextran sodium sulfate (DSS)-induced CAC in mice. Real-time PCR was used to quantify the mRNAs of miRNA and coding genes while western blot and ELISA were used to quantify protein levels. Silencing miRNA was carried out by means of electroporation and locked nucleic acid (LNA)-miRNA.

Results: Inducing CAC in mice upregulated miR-132 expression in the colon, spleen and lymph nodes at all stages of disease development. Activation of Ahr by TCDD or DIM boosted miR-132 expression and alleviated CAC severity by suppression of macrophage infiltration and pro-inflammatory cytokines. Interestingly, TCDD, but not DIM, augmented a cholinergic anti-inflammation by inducing acetylcholinesterase (AChE)-targeting miR-132. This anti-inflammation was manifested by suppressed production of TNF-α, IL-1β and IL-6. Silencing miR-132 in vivo in TCDD-treated mice abrogated the cholinergic anti-inflammation and exacerbated CAC. In addition, inhibition of miR-132 in vitro in $CD4^+$ cells and macrophages mitigated the inhibitory effect of TCDD on AChE catalytic activity.

Conclusion: Our findings identify miR-132 as a new molecule implicated in CAC pathogenesis, and reveal that miR-132 mediates the ameliorating effects of TCDD on CAC, suggesting miR-132 as a promising therapeutic candidate to control autoimmune inflammation and tumorigenesis in CAC patients.

1. Introduction

Colorectal cancer exemplifies the fundamental link between chronic inflammation and tumorigenesis. Patients with inflammatory bowel disease (IBD) such ulcerative colitis (UC) have much higher risk (~30 folds) to develop colon cancer [1]. Therefore, the IBD-associated dysplasia and colon cancer embodies a major complication in patients with IBD [2]. The colitis-associated colon cancer (CAC) is an inflammation-dysplasia-carcinoma that shows higher malignancy and decreases life expectancy compared with sporadic colon cancer [3]. Previous studies have elucidated transcriptional and posttranscriptional mechanisms for the pathophysiological process of CAC [4,5]. However, a clear strategy to control CAC development is yet to be established.

The microRNAs (miRNAs) are short nucleotides that regulate the transcriptome at the posttranscriptional level by complementary binding to the 3′ untranslated region (3′UTR) of the target mRNA. A recently formed paradigm has clearly linked numerous miRNAs with tumorigenesis of several tumors such as gastric, lung and breast cancer [6,7,8]. The miRNAs such as miR-543 and miR-517 enhance tumorigenesis of colon cancer by targeting phosphatase and tensin homolog

Abbreviations: UC, Ulcerative colitis; CAC, Colitis-associated colon cancer; Ahr, Aryl hydrocarbon receptor; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; DIM, 3,3′-diindolylmethane; miRNA, MicroRNA; AChE, Acetylcholinesterase; Ach, Acetylcholine; AOM, Azoxymethane; DSS, Dextran sodium sulfate; IBD, Inflammatory bowel disease

* Corresponding author.
E-mail address: aalzahra@kfu.edu.sa (A.M. Alzahrani).
[1] Contributed equally.

http://dx.doi.org/10.1016/j.intimp.2017.09.015
Received 20 August 2017; Accepted 18 September 2017
Available online 07 October 2017

(PTEN) and forkhead box J3 (FOXJ3), respectively [9,10]. In contrast, miR-320 suppresses colon cancer by targeting SRY-related HMG-box4 (SOX4), FOXM1 and FOXQ1 [11]. In animal model of CAC, deletion of miR-155 exacerbates disease conditions by greater activation of transforming growth factor-β (TGF-β)/SMAD pathway [12]. Furthermore, inhibition of miR-214 in CAC mice reduces inflammation and tumor by increasing PTEN, PDZ and LIM domain 2 (PDLIM2) [13].

MiR-132 is a highly conserved miRNA belonging to a cluster named miR-212/132. Several studies have shown that miR-132 suppresses breast [14], ovarian [15], and prostate [16] cancers. In colon cancer, miR-132 inhibits metastasis and cell invasion of different colon cancer cell lines by targeting zinc finger *E*-box-binding homeobox 2 (ZEB2) [17]. Furthermore, inhibition of miR-132 is associated with poor prognosis and enhanced cell invasion in colorectal cancer [18,19]. Several studies have demonstrated an anti-inflammatory role of miR-132 in different inflammatory immune responses [20,21,22]. This anti-inflammatory role is attributed mainly to targeting acetylcholinesterase (AChE) that hydrolyzes acetylcholine (ACh), a neurotransmitter that suppresses pro-inflammatory cytokines by interrupting nuclear factor κB (NFκB) nuclear translocation [23]. In addition, AChE enhances anchorage independent growth of colon cancer [24]. Although miR-132 possesses anti-inflammatory and anti-tumor properties in colon cancer, its implication in CAC has not previously been explored.

Recently, we showed that the expression of miR-212/132 cluster is regulated by aryl hydrocarbon receptor (Ahr) [25,26,27] which is an environmentally-responsive transcription factor. This heterodimeric transcription factor acts as a tumor suppressor in inflammation-associated intestinal neoplasia [28]. Importantly, activation of Ahr by exogenous ligands such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and 3,3′-diindolylmethane (DIM) attenuates the inflammatory symptoms of UC [29,30], and its activation by indole-3-carbinol (I3C) suppresses CAC in mice [31]. Notably, the TCDD-activated Ahr induces miR-132/AChE module and augments cholinergic anti-inflammatory mechanism or scheme characterized by suppression of pro-inflammatory cytokines [21]. Therefore, we hypothesized that ligand-activated Ahr could induce the miR-132/AChE axis to suppress development of CAC.

To examine this hypothesis, the effect of TCDD and DIM on CAC pathogenesis and miR-132 expression was investigated in mice. Our data identified miR-132 as a new molecule implicated in CAC development, and revealed that TCDD alleviates CAC by augmenting a cholinergic anti-inflammation via enhancing the expression of AChE-targeting miR-132.

2. Materials and methods

2.1. Animals

Female 8–10 weeks old C57BL/6 mice were purchased originally from Charles River and maintained in specific-pathogen-free (SPF) conditions. The experiments were performed at the animal housing unit and the Physiology Laboratory in the College of Science, King Faisal University, Saudi Arabia, in accordance with the protocol approved by the Standing Research Ethics Committee.

2.2. CAC model and treatments

The CAC was induced by an adopted method described by Thaker et al. [32]. Briefly, the mice were injected *i.p.* with azoxymethane (AOM; Sigma Aldrich, MO, USA) at 7.5 mg/kg on day -7, and received 2.5 % dextran sodium sulfate (DSS; MP Biomedicals, CA, USA) in drinking water on day 0–5 followed by 16 days of tap water. The cycle of DSS and tap water was repeated 3 times. The mice were injected *i.p.* with TCDD (1 μg; AccuStandard, CT, USA), DIM (25 μg; Sigma Aldrich) or corn oil (Sigma Aldrich) 2 days before DSS treatment. Disease activity index (DAI) score that includes weight loss, stool consistency and anal bleeding was calculated as described previously [33]. On day 63, the mice were euthanized for samples collection. The number and size of tumor nodules in the excised colons examined using a dissection microscope and Vernier caliper [32].

2.3. Histological studies

The sections from distal colon stained by the conventional hematoxylin & eosin (H & E) staining procedure. A pathologist who is blind of the experimental treatments calculated the histopathological score including loss of epithelium and cellular architecture arrangements as described by Scaldaferri et al. [34]. The immunohistochemistry study was performed to study macrophage infiltration using rat monoclonal anti-F4/80 antibodies (A3–1) and ImmunoCruz™ rat ABC Staining System (Santa Cruz Biotechnology, TX, USA), following manufacturer's instructions. Briefly, paraffin-embedded colonic tissues were cut at 5-μm sections and incubated with blocking serum, followed by anti-F4/80 antibodies and biotinylated secondary antibodies. The sections were then counter stained with hematoxylin. The imageJ software (https://imagej.nih.gov/ij/) [35] was used to quantify color segmentation of two-color staining, and macrophage infiltration.

2.4. Colon culture and cytokines quantification

Equal size of colon tissues were cut and cultured in 400 μL RPMI-1640 medium containing penicillin/streptomycin for 24 h. The cytokines levels in tissue culture supernatants and serum were quantified using ELISA kits (GenWay, CA, USA), following manufacturer's instructions.

2.5. Quantitative real-time PCR

Total RNA was isolated using RNeasy kit® (Qiagen, Hilden, Germany), and reverse transcribed in a thermal cycler using ReadyScript® cDNA Synthesis Mix (Sigma Aldrich). Amplification of cDNA was carried out in ViiA7 real-time PCR system with the following thermal profile (50 °C for 2 min and 95 °C for 10 min, then followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min). The TaqMan® Gene Expression of *Cyp1a1* (Mm00487218_m1), *AChE* (Mm00477274_g1), *Gapdh* (Mm99999915_g1), and TaqMan® microRNA assay of has-miR-132 (ID:00457), has-miR-212 (ID:00515) and *RNU6B* (ID: 1093) were utilized in this study. System, kits and reagents for real-time PCR were purchased from Applied Biosystems (CA, USA). The relative gene expression was calculated using the comparative ΔΔCt method.

2.6. AChE activity and western blot

The lysates were prepared using RIPA Lysis Buffer System® (Santa Cruz Biotechnology), and protein concentration was quantified using BCA Proetin Assay® kit (Thermo Scientific, MA, USA). The lysates were fractionated using SDS-PAGE system (Bio-Rad, CA, USA). The AChE and β-actin proteins were detected using anti-rabbit polyclonal antibodies (Santa Cruz Biotechnology). The AChE catalytic activity was quantified using modified Ellman's method [21]. Briefly, the hydrolysis of ACh (Sigma Aldrich) was measured in the presence of specific butyrylcholinesterase inhibitor (tetraisopropylpyrophosphoramide, Sigma Aldrich). Light absorbance was measured spectrophotometrically at 405 nm at 10 min intervals.

2.7. Transfection

The $CD4^+$ cells were isolated using MACS isolation kit (Miltenyi Biotec, Bergisch Gladash, Germany), following manufacturer's instructions, and macrophages were collected from peritoneal cavity after injecting 3% (*w/v*) Brewer thioglycollate medium (BD Biosciences, CA, USA). The antisense (as)-miR-132 and negative control were purchased