Biochemical and Biophysical Research Communications 391 (2010) 1122–1126

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Carbon monoxide enhance colonic epithelial restitution via FGF15 derived from colonic myofibroblasts

Kazuhiko Uchiyama, Yuji Naito *, Tomohisa Takagi, Katsura Mizushima, Natsuko Hayashi, Akihito Harusato, Ikuhiro Hirata, Tatsushi Omatsu, Osamu Handa, Takeshi Ishikawa, Nobuaki Yagi, Satoshi Kokura, Toshikazu Yoshikawa

Molecular Gastroenterology and Hepatology, Kyoto Prefectural University of Medicine, 465 Kajiicho Hirokoji Kawaramachi Kamigyo-ku, Kyoto 602-8566, Japan

ARTICLE INFO

Article history:
Received 5 December 2009
Available online 11 December 2009

Keywords:
Carbon monoxide
Myofibroblast
Restitution
FGF15

ABSTRACT

Carbon monoxide (CO) has been reported to ameliorate colonic inflammation and improve experimental colitis. It is well known that mucosal restitution is important to improve colitis as well as reduction of mucosal inflammation. However, it has not been clear whether CO effects to colonic mucosal restitution or not. In general, colonic myofibroblast (MF) has been reported to play an important role of colonic epithelial cell restitution via constitutive secretion of TGF-β. In this study, we showed CO (supplied by CO-releasing molecule; CORM) treated MF conditioned medium enhanced colonic epithelial cell (YAMC) restitution and we determined gene expression in colonic MF treated with CO using microRNA. The microRNA array suggested that miR-710 was significantly reduced in MF by CO treatment and the target gene of miR-710 is determined to fibroblast growth factor (FGF)15. The CO treated MF conditioned medium which FGF15 expression was silenced extinguished the enhancement effect of epithelial cell restitution. Our findings demonstrate that CO treatment to MF increased FGF15 expression via inhibition of miR-710 and FGF15 enhanced colonic epithelial cell restitution.

Introduction

Endogenous CO shows a variety of beneficial actions including potent anti-inflammatory and anti-apoptotic effects [1–3], suppression of atherosclerotic lesions following aortic transplantation [4], prevention of reprefution-induced ventricular fibrillation in the myocardium [5], and protection against ischemic lung injury [6].

Haem-oxygenase-1 (HO-1) is the rate limiting enzyme in the conversion of haem into biliverdin/bilirubin, iron and carbon monoxide (CO). It has been reported that induction of HO-1 shows protective effect against colonic mucosal damage in experimental colitis model [7–11]. This protective effect has been suggested by the anti-inflammatory effect by CO, as local production of CO can be markedly increased upon activation of HO-1 [12,13]. It has been reported that the disease based on colonic mucosal damage such as inflammatory bowel disease, mucosal healing is important to control the disease as well as anti-inflammatory therapy [14,15]. The protective effect of CO against experimental colitis model might be, in part, included in anti-inflammatory effect by CO, however, it has not been clear if CO enhance colonic epithelial mucosal restitution.

Gastrointestinal epithelial tissues are often superficially injured, and if the basement membrane underlying the sloughed epithelium is intact, residual epithelial cells at the edges of the wound become motile and move along the basement membrane until they meet advancing epithelial cells from the other side of the wound and form new tight junctions. This process is called restitution [16,17]. Myofibroblast-secreted growth factors such as TGF-β, TGF-α, EGF, aFGF, and bFGF and inflammatory cytokines such as IL-1β and IFN-γ also promote restitution [16,18–21].

In this study, we have investigated the beneficial effect of CO to enhance colonic epithelial restitution via the factor derived from colonic myofibroblasts treated with CO.

Material and methods

Materials. Tricarbonyldichlororuthenium (II) dimmer (CORM-2) was obtained from Sigma–Aldrich (St. Louis, MO, USA) and solubilized in DMSO to obtain a 10 mmol/l stock. Same amount of ruthenium was also used in some experiments as negative control.

Cell culture. The YAMC (Young Adult Mouse Colon) cell line was used as mouse colonic epithelial cell. This cell line is derived from the immortimouse that express a temperature-sensitive SV40 large T antigen transgene under control of the IFN-γ-sensitive segment of the class MHC II promoter (Whitehead, 1993 #2). The cells were generous gift of Dr. R. Whitehead (Vanderbilt University, Nashville, TN).

* Corresponding author. Fax: +81 75 251 0710.
E-mail address: ynaito@koto.kpu-m.ac.jp (Y. Naito).

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doi:10.1016/j.bbrc.2009.12.035

YAMC cells were grown at the permissive temperature of 33 °C in RPMI 1640 medium with 5% (vol/vol) FBS, 5 U/ml murine IFN-γ, 2 mM glutamine, 50 mg/ml streptomycin, and 50 U/ml penicillin (FBS, penicillin/streptomycin, and glutamine were all from Invitrogen, Grand Island, NY) and also supplemented with 6.25 mg/l insulin, 6.25 mg/l linolenic acid (ITS + Premix; Collaborative Biomedical Products, Bedford, MA). Stock cultures were maintained at 33 °C and were split at a ratio of 1:6 every 4 days. VUPF (Vanderbilt University Pericryptal Fibroblast) myofibroblasts were grown at the permissive temperature of 37 °C in RPMI 1640 medium with 5% (vol/vol) FBS, 2 mM glutamine, 50 mg/ml streptomycin, and 50 U/ml penicillin, supplemented with ITS + Premix. For studies using myofibroblast conditioned medium, myofibroblasts were grown to confluence in tissue culture flasks in complete medium. After they were washed, the cells were cultured for 24 h in 0.5% FBS–RPMI medium. The myofibroblast conditioned medium was filtered and stored at −80 °C until used for studies on YAMC cells.

Isolation of total RNA. Total RNA was isolated from intestinal mucosal tissue by the acid guanidinium phenol chloroform method with an Isogen kit (Nippon Gene Co.) and the concentration of RNA was determined by absorbance at 260 nm in relation to that at 280 nm. The isolated RNA was stored at −70 °C until use in either the microRNA array analysis or real-time PCR.

Real-time PCR. The mRNA expression of VUPF cell TGF-β and FGF were determined by real-time PCR at 3 h after CORM treatment. Total RNA was isolated from VUPF cells by the acid guanidinium phenol chloroform method with an ISOGEN kit (Nippon Gene, Tokyo, Japan) and the concentration of RNA was determined by the absorbance at 260 nm in relation to that at 280 nm. The isolated RNA was stored at −70 °C until it was used for the real-time PCR. One microgram of extracted RNA was reverse transcribed into first-strand cDNA at 42 °C for 40 min, using 100 U/ml of reverse transcriptase (Takara Biomedicals, Shiga, Japan) and 0.1 μM of oligo(dT)-adapter primer (Takara Biomedicals) in a 50-μl reaction mixture. Real-time PCR for TGF-β and FGF was carried out with a 7300 real-time PCR system (Applied Biosystems, Foster City, CA) using the DNA-binding dye SYBR green I for the detection of PCR products. The reaction mixture (RT-PCR kit, Code RRO43A, Takara Biochemicals) contained 12.5 μl Premix Ex Taq, 2.5 μl SYBR green I, custom synthesized primers, ROX reference dye, and cDNA (equivalent to 20 ng total RNA) to give a final reaction volume of 25 μl. The PCR settings were as follows: the initial denaturation for 15 s at 95 °C was followed by 40 cycles of amplification for 3 s at 95 °C and 31 s at 60 °C, with subsequent melting curve analysis increasing the temperature from 60 to 95 °C. The primers had the following sequences: for FGF15, sense 5′-CCGCA CTTGGCCTCTCTTTA-3′; and antisense; 5′-CAGCC CGTGCTTTA-GAACTTG-3′ and for β-actin, sense 5′-TATCCA CCTTCCAGCA-GATGT-3′; and antisense; 5′-TATCCACCTTCCAGCAGATGT-3′. Relative quantification of gene expression with real-time PCR data was calculated relative to β-actin.

Western blot analysis. VUPF cells were treated with 1 and 10 mM of CORM. For Western blot analysis, cells were harvested by scraping in ice-cold PBS and centrifuged. Total proteins were mixed with SDS sample buffer. The samples were subjected to a 12% SDS–polyacrylamide gel electrophoresis (PAGE) and blotted onto a polyvinylidene difluoride (PVDF) membrane (Atto Corporation, Tokyo, Japan). The membrane was blocked with 2% bovine serum albumin in TBS–T (TBS and 0.1% Tween 20) at room temperature for 30 min. Western blotting was carried out using a specific anti-FGF15 antibody (SPA 801; Stressgen, Victoria, BC, Canada) at room temperature for 1 h. After three washes with TBS–T, the membrane was incubated with anti-rabbit IgG-HRP (GE Healthcare UK, Ltd., Chalfont St. Giles, UK) at room temperature for 45 min. The signals were visualized using an enhanced chemiluminescence (ECL) kit (GE Healthcare UK, Ltd.) according to the manufacture's instructions.

Evaluation of cell migration using wound healing assay. Wound healing assay was performed as previously described with minor modification (Petit, 2000 #1). Briefly the cells were seeded in P60 culture dishes and cultured until they reached confluence. The cells were then incubated with serum-free culture medium for 24 h, and scraped with a 10 μl extra long micro-pipette tip, denuding a strip of the monolayer approximately 500 μl in diameter. Variation in the wound diameter within experiments was approximately 5%. Cultures were washed twice with PBS to remove cell debris and incubated with serum-free culture medium for an additional 16 h with CO bubbled medium or normal medium. When specific inhibitors were used, an appropriate concentration of each inhibitor was added to the culture medium 30 min before PMA treatment. After incubation, the cells were photographed with a digital camera, and the migrated area was measured using NIH Image (v1.63) software. To ensure that the same wounds were compared, we used a permanent marker to make positioning marks at the bottom of the culture dishes. The migration area in the wound was calculated according to the following formula: cell free area at 0 h-cell free area at 12 h. At least eight fields were analyzed and the migrated area was expressed as a percentage of that in untreated control cells.

micro RNA array analysis. In this study, 5 μg of total RNA was labeled with the NCode™ rapid miRNA labeling system (Invitrogen Japan K.K., Tokyo, Japan) and hybridized to replicate Ncode human miRNA microarrays V3 (Invitorogen Japan K.K.), which contains probes for 710 human miRNAs. Each sample was labeled with Alexa Fluor® 3 and with Alexa Fluor® 5 to allow for dye swaps for each pair of UC and normal subjects. Data were processed and background-corrected, normalized using a Latin Squares algorithm by the NCode™ Profiler data analysis software (Invitrogen Japan K.K.), to identify genes from each pair showing differences with $P < 0.01$.

siRNA of FGF15. For silencing of FGF15 in myofibroblast cells, myofibroblast cells were treated with a commercially available silencing oligonucleotide (Santa Cruz Biotechnology, Inc. siRNA ID#sc-39473) or scrambled oligonucleotide (ID#sc-37007). In all cases, silencing oligonucleotides were complexed with siRNA transfection reagent (Santa Cruz Biotechnology, Inc. ID#sc-29528) in serum-free Optimem medium (Invitrogen). Myofibroblast cells were treated with the complexed oligonucleotides 1 day after plating (at 1×10^4 cells/cm^2) using a final concentration of 20 nM silencing oligonucleotides and 0.1 μl of Silentfect reagent per cm^2. The oligonucleotide and transfection reagent were individually diluted into Optimem medium (Invitrogen), and then mixed and allowed to complex for 20 min at room temperature. Media was removed, replaced with Optimem and complexed oligonucleotides added. After 60 min, complete medium was added and cells were returned to the incubator overnight. Silencing of FGF15 was monitored by examining FGF15 expression by real-time PCR.

The medium of myofibroblast cells treated with FGF15 siRNA was removed 48 h after siRNA treatment and new medium added to make the conditioned medium as described above except from FGF15-silenced myofibroblasts.

Statistical analysis. All results were expressed as means ± SE. The data were compared by two-way ANOVA, and difference were considered to be significant if the P value was <0.05 based on Scheff's multiple-comparison test. All analyses were performed using the StatView 5.0-J program (Abacus Concepts, Berkeley, CA).

Results

Wound healing assay by myofibroblast conditioned medium

To investigate the effect of CORM to myofibroblast related to YAMC restitution, CORM-treated-myofibroblast conditioned med-

ium was used to wound healing assay to YAMC monolayers. Compared to ruthenium-treated myofibroblast conditioned medium as a control, CORM-treated myofibroblast conditioned medium enhanced repair of wounded YAMC monolayer (Fig. 1).

micro RNA array analysis

All known miRNAs are registered in a public web-based registry, the "miRBase" database, which provides up-to-date information on all published miRNAs [22]. Among these candidates, miR-710 is the most down regulated by the treatment of CORM (Table 1).

FGF15 expression treated with CORM in myofibroblast

To determine whether CORM could affect the expression of mRNA and protein of FGF15, VUPF cells were stimulated with CORM. Incubation of VUPF cells with 10 mμM of CORM for 3 h induced FGF15 mRNA expression in VUPF cells (Fig. 2A). FGF15 protein expression was also induced by 10 mμM of CORM treatment for 6 h in VUPF cells (Fig. 2B).

Myofibroblast-derived FGF15 and wound healing assay

To determine whether FGF15 secreted from VUPF cells enhanced repair of wound YAMC monolayer, silencing RNA was used to knock down FGF15 expression in VUPF cells. The silencing oligonucleotide designed decreased FGF15 expression by almost 50% in all cases in VUPF cells treated with CORM (determined by real-time PCR in each experiment). VUPF cell conditioned medium treated with CORM enhanced YAMC wound repair compared with conditioned medium treated with ruthenium (Fig. 3B lanes 2 versus 3). Conditioned medium from VUPF cells that FGF15 was silenced did not enhanced YAMC wound repair (Fig. 3B lanes 2 versus 4). FGF15-silenced conditioned medium treated with ruthenium did not affect YAMC wound repair (Fig. 3B lanes 2 versus 5).

Discussion

Carbon monoxide (CO) is now recognized as a ubiquitous cellular mediator capable of controlling fundamental physiological and signaling processes in mammalian tissues [23]. CO modulates sGC activity and subsequent stimulation of cGMP production and vari-

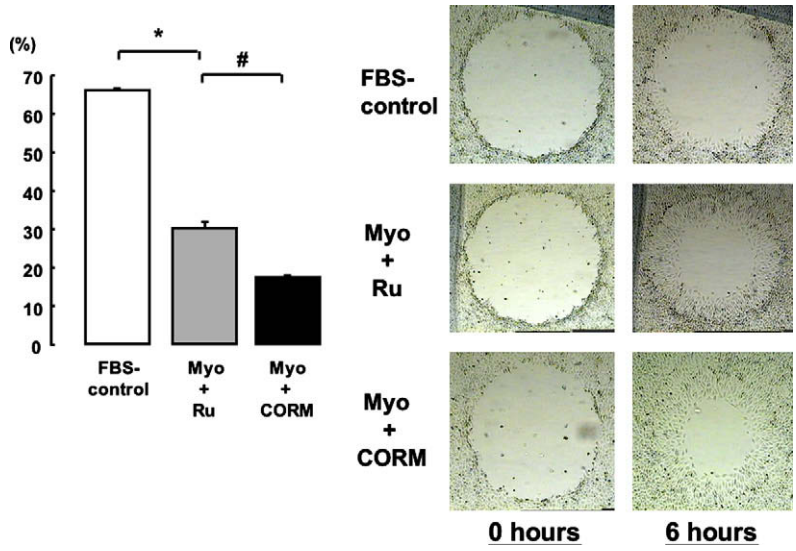

Fig. 1. Results of wound healing assay of YAMC cells treated with myofibroblast (VUPF) conditioned medium. Ru is ruthenium as a control of CORM. Wound remaining ratio is calculated based on the area of 0 h ($n = 3$) ($^{*}P < 0.01$, $^{\#}P < 0.05$).

Table 1
Result of micro RNA array of VUPF cells treated with CORM.

up regulation

	Fold Change	P-Value
mmu-miR-710	5.186217533	0.01000
cel-miR-53	4.513639048	0.01440
1569-shuf-mmu-mir-325	3.921727104	0.02200
dme-miR-275	3.907300506	0.02600
hsa-miR-208_mmu-miR-208_rno-miR-208	3.87601417	0.02540
1561-shuf-rno-mir-101b	3.813158794	0.02320
1562-mut1-mir-34c	3.642984362	0.03260
1546-mut1-cel-mir-244	3.628734869	0.03240
1577-shuf-dme-mir-317	3.583824723	0.04020
dre-miR-489	3.371908185	0.04240
cel-miR-43	3.320594228	0.04800

down regulation

	Fold Change	P-Value
mmu-let-7i_hsa-let-7i_rno-let-7i	-4.654859892	0.01200
hsa-miR-16_mmu-miR-16_rno-miR-16	-4.427750409	0.01400
hsa-miR-22_mmu-miR-22_rno-miR-22	-4.284328639	0.01740
hsa-let-7d_mmu-let-7d_rno-let-7d	-4.242179109	0.01840
hsa-miR-214_mmu-miR-214_rno-miR-214_dre-miR-214	-4.01321019	0.02540
cel-let-7_hsa-let-7a_rno-let-7a_dre-let-7a	-3.982872796	0.02520
hsa-let-7b_mmu-let-7b_rno-let-7b_dre-let-7b	-3.953024344	0.02500
dre-miR-26a	-3.925331731	0.02720
mmu-miR-199a_hsa-miR-199a_rno-miR-199a_dre-miR-199	-3.826702142	0.02800
dre-miR-143	-3.670565649	0.02980
hsa-let-7c_mmu-let-7c_rno-let-7c_dre-let-7c	-3.611259584	0.03180
hsa-miR-24_mmu-miR-24_rno-miR-24_dre-miR-24	-3.60152242	0.03120
dre-let-7i	-3.591251251	0.03760
mmu-miR-31_rno-miR-31	-3.585811577	0.03120
mmu-miR-99b_hsa-miR-99b_rno-miR-99b	-3.574999155	0.03860
hsa-miR-21_mmu-miR-21_rno-miR-21	-3.501085257	0.03760
hsa-miR-29a_mmu-miR-29a_rno-miR-29a	-3.471053961	0.03900
dme-let-7_mmu-let-7a	-3.464710257	0.03840
dre-miR-145	-3.43001563	0.04160
hsa-miR-103_mmu-miR-103_rno-miR-103_dre-miR-103	-3.380801951	0.04360
mmu-miR-709	-3.325653901	0.04220
mmu-miR-191_hsa-miR-191_rno-miR-191	-3.184527138	0.04860

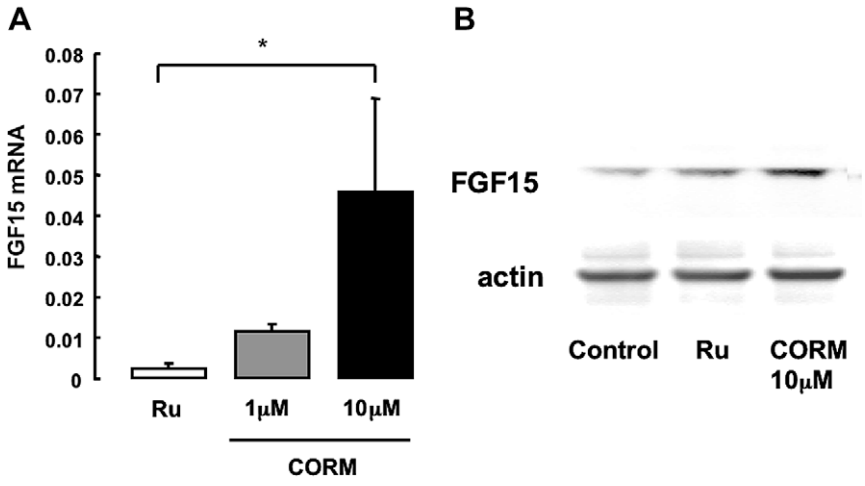

Fig. 2. Real time PCR and Western blotting against FGF15 treated with CORM ($n = 3$) ($^{*}P < 0.01$).

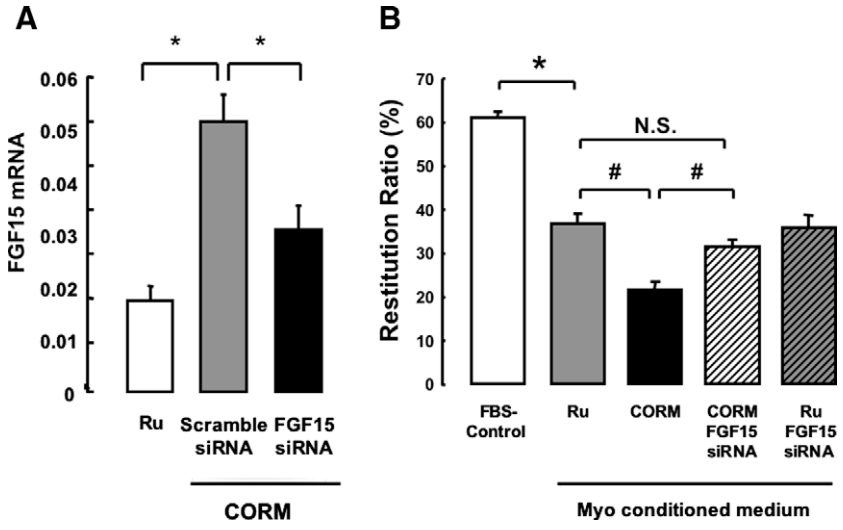

Fig. 3. (A) Real-time PCR of FGF15 transfected by FGF15 siRNA ($n = 3$) ($^{*}P < 0.01$). (B) Wound healing assay of YAMC cells with VUPF cells conditioned medium. VUPF cells were transfected by FGF15 siRNA ($n = 3$) ($^{*}P < 0.01$, $^{\#}P < 0.05$).

ous MAPK activation, few are known about intracellular signaling. In the present study, we demonstrated up-regulation of FGF15 by CO via down-regulation of miR-710 in colonic myofibroblast. Though further study of detail mechanism is needed, it is the first time to show that CO modulate the expression of microRNA. Furthermore, it is also the first time to show the modulation of colonic epithelial restitution by FGF15.

It has been reported that colonic epithelial cell restitution involves complex interactions between epithelial cells, cells within the lamina propria, and components of the extracellular matrix. Previous studies have shown that TGF-β derived from subepithelial colonic myofibroblasts enhance the restitution of IEC-6 and T84 cell lines [24]. In this study, TGF-β mRNA expression did not increased by CORM treatment in myofibroblasts (data not shown). Though CORM did not increase TGF-β expression in myofibroblasts, CORM-treated myofibroblasts conditioned medium enhanced epithelial cell restitution. To determine the factor that enhanced epithelial cell restitution in CORM-treated myofibroblasts conditioned medium, we performed microRNA array. The microRNA array revealed that CORM significantly down regulated miR-710 expression in myofibroblasts and one of the target gene of miR-710 is FGF15 [22]. Real-time PCR and Western blotting of FGF15 treated with CORM.

In order to determine if myofibroblasts-derived FGF15 is involved in epithelial cell restitution, we silenced FGF15 mRNA in myofibroblasts by siRNA. The conditioned medium from FGF15 silenced myofibroblasts treated with CORM canceled the enhancement of epithelial cell restitution by CORM, suggesting that this effect was mediated by FGF15. Fibroblast growth factors (FGF) family of signaling molecules plays important roles in development, angiogenesis, and cancer [25]. It has been reported that several FGFs such as FGF-1, FGF-2 [19], FGF-4 [26], FGF-10 [27] and FGF-20 [28] enhance intestinal epithelial restitution. It has been reported that FGF15, the human ortholog of FGF19, is expressed in small intestine and control bile acid secretion [29]. However, little is known about which cells secrete FGF15 and the roles of FGF15 to colonic epithelial cells. In the present study, it is the first time to report that colonic myofibroblasts-derived FGF15 enhanced colonic epithelial cell restitution.

The present study demonstrates that FGF15 derived from murine colonic myofibroblasts induced by endogenous CO is one of the important molecules to control epithelial cell restitution.

References

[1] L.E. Otterbein, F.H. Bach, J. Alam, M. Soares, H. Tao Lu, M. Wysk, R.J. Davis, R.A. Flavell, A.M. Choi, Carbon monoxide has anti-inflammatory effects involving the mitogen-activated protein kinase pathway, Nat. Med. 6 (2000) 422–428.

[2] J.T. Chapman, L.E. Otterbein, J.A. Elias, A.M. Choi, Carbon monoxide attenuates aeroallergen-induced inflammation in mice, Am. J. Physiol. Lung Cell. Mol. Physiol. 281 (2001) L209–L216.

[3] S. Brouard, L.E. Otterbein, J. Anrather, E. Tobiasch, F.H. Bach, A.M. Choi, M.P. Soares, Carbon monoxide generated by heme oxygenase 1 suppresses endothelial cell apoptosis, J. Exp. Med. 192 (2000) 1015–1026.

[4] L.E. Otterbein, B.S. Zuckerbraun, M. Haga, F. Liu, R. Song, A. Usheva, C. Stachulak, N. Bodyak, R.N. Smith, E. Csizmadia, S. Tyagi, Y. Akamatsu, R.J. Flavell, T.R. Billiar, E. Tzeng, F.H. Bach, A.M. Choi, M.P. Soares, Carbon monoxide suppresses arteriosclerotic lesions associated with chronic graft rejection and with balloon injury, Nat. Med. 9 (2003) 183–190.

[5] I. Bak, L. Szendrei, T. Turoczi, G. Papp, F. Joo, D.K. Das, J. de Leiris, P. Der, B. Juhasz, E. Varga, I. Bacskay, J. Balla, P. Kovacs, A. Tosaki, Heme oxygenase-1-related carbon monoxide production and ventricular fibrillation in isolated ischemic/reperfused mouse myocardium, FASEB J. 17 (2003) 2133–2135.

[6] T. Fujita, K. Toda, A. Karimova, S.F. Yan, Y. Naka, S.F. Yet, D.J. Pinsky, Paradoxical rescue from ischemic lung injury by inhaled carbon monoxide driven by derepression of fibrinolysis, Nat. Med. 7 (2001) 598–604.

[7] G. Paul, F. Bataille, F. Obermeier, J. Bock, F. Klebl, U. Strauch, D. Lochbaum, P. Rummele, S. Farkas, J. Scholmerich, M. Fleck, G. Rogler, H. Herfarth, Analysis of intestinal haem-oxygenase-1 (HO-1) in clinical and experimental colitis, Clin. Exp. Immunol. 140 (2005) 547–555.

[8] Y. Erbil, M. Giris, S.D. Abbasoglu, U. Barbaros, B.T. Yanik, A. Necefli, V. Olgac, G.A. Toker, Effect of heme oxygenase-1 induction by octreotide on TNBS-induced colitis, J. Gastroenterol. Hepatol. 22 (2007) 1852–1858.

[9] C. Varga, F. Laszlo, P. Fritz, M. Cavicchi, D. Lamarque, K. Horvath, A. Posa, A. Berko, B.J. Whittle, Modulation by heme and zinc protoporphyrin of colonic heme oxygenase-1 and experimental inflammatory bowel disease in the rat, Eur. J. Pharmacol. 561 (2007) 164–171.

[10] M. Giris, Y. Erbil, S. Dogru-Abbasoglu, B.T. Yanik, H. Alis, V. Olgac, G.A. Toker, The effect of heme oxygenase-1 induction by glutamine on TNBS-induced colitis. The effect of glutamine on TNBS colitis, Int. J. Colorectal Dis. 22 (2007) 591–599.

[11] R.A. Hegazi, K.N. Rao, A. Mayle, A.R. Sepulveda, L.E. Otterbein, S.E. Plevy, Carbon monoxide ameliorates chronic murine colitis through a heme oxygenase 1-dependent pathway, J. Exp. Med. 202 (2005) 1703–1713.

[12] R. Motterlini, A. Gonzales, R. Foresti, J.E. Clark, C.J. Green, R.M. Winslow, Heme oxygenase-1-derived carbon monoxide contributes to the suppression of acute hypertensive responses in vivo, Circ. Res. 83 (1998) 568–577.

[13] I.A. Sammut, R. Foresti, J.E. Clark, D.J. Exon, M.J. Vesely, P. Sarathchandra, C.J. Green, R. Motterlini, Carbon monoxide is a major contributor to the regulation of vascular tone in aortas expressing high levels of haeme oxygenase-1, Br. J. Pharmacol. 125 (1998) 1437–1444.

[14] G.C. Actis, R. Pellicano, E. David, A. Sapino, Azathioprine, mucosal healing in ulcerative colitis, and the chemoprevention of colitic cancer: a clinical-practice-based forecast, Inflamm. Allergy Drug Targets (2009). September 1. [Epub ahead of print].

[15] G.R. Lichtenstein, P. Rutgeerts, Importance of mucosal healing in ulcerative colitis, Inflamm. Bowel Dis. (2009). July 27. [Epub ahead of print].

[16] D.K. Podolsky, Healing the epithelium: solving the problem from two sides, J. Gastroenterol. 32 (1997) 122–126.

[17] W. Silen, S. Ito, Mechanisms for rapid re-epithelialization of the gastric mucosal surface, Annu. Rev. Physiol. 47 (1985) 217–229.

[18] A.U. Dignass, D.K. Podolsky, Cytokine modulation of intestinal epithelial cell restitution: central role of transforming growth factor beta, Gastroenterology 105 (1993) 1323–1332.

[19] A.U. Dignass, S. Tsunekawa, D.K. Podolsky, Fibroblast growth factors modulate intestinal epithelial cell growth and migration, Gastroenterology 106 (1994) 1254–1262.

[20] D.B. Polk, Epidermal growth factor receptor-stimulated intestinal epithelial cell migration requires phospholipase C activity, Gastroenterology 114 (1998) 493–502.

[21] M. Riegler, R. Sedivy, T. Sogukoglu, E. Cosentini, G. Bischof, B. Teleky, W. Feil, R. Schiessel, G. Hamilton, E. Wenzl, Epidermal growth factor promotes rapid response to epithelial injury in rabbit duodenum in vitro, Gastroenterology 111 (1996) 28–36.

[22] S. Griffiths-Jones, R.J. Grocock, S. van Dongen, A. Bateman, A.J. Enright, miRBase: microRNA sequences, targets and gene nomenclature, Nucleic Acids Res. 34 (2006) D140–D144.

[23] S.W. Ryter, D. Morse, A.M. Choi, Carbon monoxide: to boldly go where NO has gone before, Sci STKE 2004 (2004) 230. p. re6.

[24] B.C. McKaig, S.S. Makh, C.J. Hawkey, D.K. Podolsky, Y.R. Mahida, Normal human colonic subepithelial myofibroblasts enhance epithelial migration (restitution) via TGF-beta3, Am. J. Physiol. 276 (1999) G1087–G1093.

[25] D.M. Ornitz, N. Itoh, Fibroblast growth factors, Genome Biol 2 (2001). REVIEWS3005. 2001 March 9. [Epub ahead of print].

[26] H. Sasaki, K. Hirai, H. Yamamoto, H. Tanooka, H. Sakamoto, T. Iwamoto, T. Takahashi, M. Terada, T. Ochiya, HST-1/FGF-4 plays a critical role in crypt cell survival and facilitates epithelial cell restitution and proliferation, Oncogene 23 (2004) 3681–3688.

[27] D.S. Han, F. Li, L. Holt, K. Connolly, M. Hubert, R. Miceli, Z. Okoye, G. Santiago, K. Windle, E. Wong, R.B. Sartor, Keratinocyte growth factor-2 (FGF-10) promotes healing of experimental small intestinal ulceration in rats, Am. J. Physiol. Gastrointest. Liver Physiol. 279 (2000) G1011–G1022.

[28] M. Jeffers, W.F. McDonald, R.A. Chillakuru, M. Yang, H. Nakase, L.L. Deegler, E.D. Sylander, B. Rittman, A. Bendele, R.B. Sartor, H.S. Lichenstein, A novel human fibroblast growth factor treats experimental intestinal inflammation, Gastroenterology 123 (2002) 1151–1162.

[29] T. Inagaki, M. Choi, A. Moschetta, L. Peng, C.L. Cummins, J.G. McDonald, G. Luo, S.A. Jones, B. Goodwin, J.A. Richardson, R.D. Gerard, J.J. Repa, D.J. Mangelsdorf, S.A. Kliewer, Fibroblast growth factor 15 functions as an enterohepatic signal to regulate bile acid homeostasis, Cell Metab. 2 (2005) 217–225.