Cholic Acid

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Matrix metalloproteinase-9 (MMP-9) plays a crucial role in cell invasion and cancer metastasis. In this study, we showed that cholic acid (CA), a major primary bile acid, can induce MMP-9 expression in colon cancer HT29 and SW620 cells. CA increased reactive oxygen species (ROS) production and also activated phosphorylation of ERK1/2, JNK, and p38 MAPK. Specific inhibitors and mutagenesis studies showed that ERK1/2 and JNK functioned as upstream signals in the activation of AP-1, and p38 MAPK functioned as an upstream signal in the activation of NF-κB. N-acetyl-L-cysteine (NAC, an ROS scavenger) and diphenyleneiodonium chloride (DPI, an NADPH oxidase inhibitor) inhibited CA-induced activation of ERK1/2, JNK, and p38 MAPK, indicating that ROS production by NADPH oxidase could be the furthest upstream signal in MMP-9 expression. Colon cancer cells pretreated with CA showed remarkably enhanced invasiveness. Such enhancement was partially abrogated by MMP-9-neutralizing antibodies. These results demonstrate that CA could induce MMP-9 expression via ROS-dependent ERK1/2, JNK-activated AP-1, and p38-MAPK-activated NF-κB signaling pathways, which in turn stimulate cell invasion in human colon cancer cells.

Keywords: cholic acid ; matrix metalloproteinase-9 ; reactive oxygen species ; AP-1 ; NF-κB ; MAPK ; cell invasion ; colon cancer cells

1. Colon Cancer

Colon cancer is the third most common human disease worldwide. The rate of relative survival following diagnosis is 65% at 5 years and 58% at 10 years [1]. Bile acid has been reported to be strongly associated with colon cancer development [2]. However, the molecular mechanisms for the role of bile acid in the development of colon cancer have not been elucidated yet. Bile acid, as the end product of cholesterol catabolism, accounts for a major fraction of daily cholesterol turnover in humans. It plays an important role in the absorption, transport, and metabolism of dietary fats and lipid-soluble vitamins in the intestine [3]. In the duodenum, more than 90% of bile acids are reabsorbed and returned to the liver, which again secretes primary bile acids, cholic acid (CA), and chenodeoxycholic acid (CDCA) [4]. Secondary bile acids deoxycholic acid (DCA) and lithocholic acid (LCA) are formed through bacterial 7α-dehydroxylation of primary bile acids CA and CDCA, respectively [5].

2. CA

CA, a major primary bile acid, plays an important role not only in the digestion and absorption of dietary lipids but also in cell invasion, growth, and apoptosis through various signaling pathways [6][7][8][9]. NADPH oxidases activated by CA are the major intracellular sources of reactive oxygen species (ROS), which play important roles in modulating signaling pathways, thus changing the cellular phenotype [10][11][12]. Several studies have shown that bile acids can induce ROS production via NADPH oxidase involved in multiple signaling cascades, such as ERK1/2 [13], JNK [14], p38 MAPK [15], and Akt [16].

3. Cell Invasion

Cell invasion is a fundamental process for cancer metastasis. It requires increased expression of proteases such as uroplaminogen-type activator (uPA) and matrix metalloproteinases (MMPs) [17]. MMPs are a family of zinc-containing enzymes that are involved in the degradation of different components of the extracellular matrix. There is sufficient evidence indicating that individual MMPs have important roles in tumor cell invasion [18][19]. MMP-9 is involved in cancer metastasis and tumor-induced angiogenesis [20][21]. Furthermore, it has been reported that ROS can activate MAPK (ERK1/2, JNK, and p38 MAPK), which leads to the expression of MMP-9 [22][23]. Some MAPK-activated transcription factors such as NF-κB and AP-1 can regulate the expression of MMP-9 by interacting with the binding site of the promoter of MMP [24].

In colon cell carcinomas, MMP-9 not only serves as a potential prognostic marker of tumor but also an indicator for tumor metastasis [25]. In addition, in a study with T3-T4 node-negative patients, it was found that MMP-9 could be an independent marker of poor prognosis [26]. Therefore, the detailed regulatory relationship between bile acid and MMP-9 should be clarified.

4. Findings

We demonstrated that CA, a major primary bile acid, can induce cell invasion through MMP-9 expression in human colon cells. We also elucidated the underlying molecular mechanism involved in such induction.

The human bile acid pool consists of four different bile acids: two primary bile acids (CA and CDCA) and two secondary bile acids (DCA and LCA) [27]. CA and CDCA are major bile acids in humans [28]. Biliary cholesterol secretion is increased by CA. The amount of cholesterol absorbed was found to be larger with CA (79%) than with CDCA (60%) [29]. Bile acid is involved in the progression of colon cancer. However, many authors are interested in the effect of the secondary bile acid DCA, a proinflammatory and procarcinogenic natural chemical, on bile-acid-sensing receptors such as farnesoid X receptor (FXR) and G-protein-coupled bile acid receptor (TGR5) or gut microbiota study of DCA-induced dysbiosis [30][31][32], while the relevant role of the major bile acid CA in colon cancer progression is ignored. CA, a naturally occurring bile acid, can stimulate cell invasion in human colon cancer cells through activation of multiple signaling pathways [8]. A previous study has shown that CDCA, the primary bile acid, can induce MMP-9 by FAK regulation at the AP-1 motif of the MMP-9 promoter via c-jun activation [33]. Previously, we also reported that bile acids can stimulate invasion of human colon cancer cells [34]. In the present study, we observed that CA treatment could increase colon cancer cell invasiveness and elucidated the molecular mechanisms of CA-induced MMP-9 expression.

ROS, such as superoxide and H_2O_2, can act as second messengers in intracellular signaling pathways. They are increasingly involved in cell invasion and migration [35][36]. Previous studies have reported that ROS can act as key regulators in mediating MMP gene expression [37]. AP-1 and NF-κB are involved in the regulation of MMP-9 expression [24]. Bile acids can promote tumor formation on the colon through the generation of ROS [38]. There are several ways that ROS can be produced by the action of bile acids: (i) bile acids can stimulate the release and oxygenation of arachidonate metabolism via cyclooxygenase and lipoxygenase pathways, thus leading to ROS production [39][40]; (ii) protein kinase C activation by bile acids is correlated with the stimulation of reactive oxygen production [41]; (iii) membrane perturbations caused by the hydrophobicity of bile acid can induce ROS production by activating the surface enzyme NADPH oxidase [42]. In our current study, NAC (an ROS scavenger) and DPI (an NADPH oxidase inhibitor) significantly inhibited H_2O_2 generation induced by CA, indicating a regulatory role of CA for ROS in MMP-9 expression and cell invasion through NADPH oxidation.

Invasion and metastases are properties of cancer cells and the final results of a sophisticated series of actions involving multiple signaling molecule interactions [20]. In this study, the blockage of CA-induced cell invasion was observed in SW620 cells with pretreatment of MMP-9 antibody, DPI, or NAC, indicating that ROS production by NADPH oxidase plays an important role in CA-induced MMP-9 expression as well as colon cancer cell invasion. Accumulated evidence shows that ROS production affects invasion and metastases through MAPK signaling pathways [43]. Consistent with our results (Figure 2), in hepatocytes, bile-acid-induced mitochondrial ROS can enhance the downsignaling of ERK1/2 through the ERBB 1-ERKl/2 signaling module [13]. In human breast cancer MCF-7 cells, JNK plays a crucial role in the ROS/MAPK molecular pathway, leading to synthetic lethality upon p53 activation and TrxR inhibition [14]; ROS/MAPK activation by TBBPA-induced NOX plays an important role in MMP-9 expression, and treatment with PD (ERK inhibitor), SP (JNK inhibitor), or SB (p38 MAPK inhibitor) blocked the ROS/MAPK molecular pathways [15]. Transcription factors AP-1 and NF-κB are known to be downstream signals for MAPK [20]. AP-1, a dimeric transcription factor, plays an important role in regulating cell invasion [44], and c-jun and c-fos are two main components of AP-1 [45]. As shown in Figure 5, CA induced both c-fos and c-jun phosphorylation. Consistent with our results, dimerumic acid can suppress H_2O_2-induced MMP-7 expression by inhibiting AP-1-mediated gene expression via the JNK/c-jun and ERK/c-fos signaling pathway in SW620 cells [46].

Cross talk and cooperativity between p38 MAPK and NF-κB have been reported [47]. However, the regulation of p38-dependent NF-κB has not been fully elucidated yet. In chondrocytes, COX-2 is expressed via p38 activation/NF-κB recruitment during both differentiation and inflammatory response [48]. Interestingly, it has been reported that mitogen- and stress-activated kinase 1 (MSK1), a potential p38 substrate, can upregulate p65-S276 phosphorylation [49][50]. CA induces phospho-p65 through the activation of p38 MAPK, revealing the regulation of p38 MAPK and NF-κB in human SW620 colon cancer cells.

In conclusion, our results demonstrate that CA can induce MMP-9 expression through ROS-dependent ERK1/2, JNK-activated AP-1, and p38-MAPK-activated NF-κB, thus promoting the invasion of human colon cancer cells.

References

1. America Cancer Society. Colorectal Cancer Facts & Figures 2017–2019; America Cancer Society: Atlanta, GA, USA, 2019.
2. Centuori, S.M.; Martinez, J.D. Differential regulation of EGFR-MAPK signaling by deoxycholic acid (DCA) and ursodeoxycholic acid (UDCA) in colon cancer. Dig. Dis. Sci. 2014, 59, 2367–2380.
3. Chiang, J.Y. Bile acid metabolism and signaling. Compr. Physiol. 2013, 3, 1191–1212.
4. Nakahara, M.; Fujii, H.; Maloney, P.R.; Shimizu, M.; Sato, R. Bile acids enhance low density lipoprotein receptor gene expression via a MAPK cascade-mediated stabilization of mRNA. J. Biol. Chem. 2002, 277, 37229–37234.
5. Engelking, L.R. Textbook of Veterinary Physiological Chemistry, 3th ed.; Elsevier Inc.: Amsterdam, The Netherlands, 2015.
6. Russell, D.W. The enzymes, regulation, and genetics of bile acid synthesis. Annu. Rev. Biochem. 2003, 72, 137–174.
7. Chiang, J.Y. Bile acids: Regulation of synthesis. J. Lipid Res. 2009, 50, 1955–1966.
8. Debruyne, P.R.; Bruyneel, E.A.; Karaguni, I.M.; Li, X.; Flatau, G.; Müller, O.; Zimber, A.; Gespach, C.; Mareel, M.M. Bile acids stimulate invasion and haptotaxis in human colorectal cancer cells through activation of multiple oncogenic signaling pathways. Oncogene 2002, 21, 6740–6750.
9. Deuschle, U.; Schuler, J.; Schulz, A.; Schlüter, T.; Kinzel, O.; Abel, U.; Kremoser, C. FXR controls the tumor suppressor NDRG2 and FXR agonists reduce liver tumor growth and metastasis in an orthotopic mouse xenograft model. PLoS ONE 2012, 7, e43044.
10. Ushio-Fukai, M.; Nakamura, Y. Reactive oxygen species and angiogenesis: NADPH oxidase as target for cancer therapy. Cancer Lett. 2008, 266, 37–52.
11. Hong, J.; Behar, J.; Wands, J.; Resnick, M.; Wang, L.; Delellis, R.A.; Lambeth, D.; Cao, W. Bile acid reflux contributes to development of esophageal adenocarcinoma via activation of phosphatidylinositol-specific phospholipase Cgamma2 and NADPH oxidase NOX5-S. Cancer Res. 2010, 70, 1247–1255.
12. Booth, D.M.; Murphy, J.A.; Mukherjee, R.; Awais, M.; Neoptolemos, J.P.; Gerasimenko, O.V.; Tepikin, A.V.; Petersen, O.H.; Sutton, R.; Criddle, D.N. Reactive oxygen species induced by bile acid induce apoptosis and protect against necrosis in pancreatic acinar cells. Gastroenterology 2011, 140, 2116–2125.
13. Fang, Y.; Han, S.I.; Mitchell, C.; Gupta, S.; Studer, E.; Grant, S.; Hylemon, P.B.; Dent, P. Bile acids induce mitochondrial ROS, which promote activation of receptor tyrosine kinases and signaling pathways in rat hepatocytes. Hepatology 2004, 40, 961–971.
14. Shi, Y.; Nikulenkov, F.; Zawacka-Pankau, J.; Li, H.; Gabdoulline, R.; Xu, J.; Eriksson, S.; Hedström, E.; Issaeva, N.; Kel, A.; et al. ROS-dependent activation of JNK converts p53 into an efficient inhibitor of oncogenes leading to robust apoptosis. Cell Death Differ. 2014, 21, 612–623.
15. Lee, G.H.; Jin, S.W.; Kim, S.J.; Pham, T.H.; Choi, J.H.; Jeong, H.G. Tetrabromobisphenol a induces MMP-9 expression via NADPH oxidase and the activation of ROS, MAPK, and Akt pathways in human breast cancer MCF-7 cells. Toxicol. Res. 2019, 35, 93–101.
16. Li, Q.; Fu, G.B.; Zheng, J.T.; He, J.; Niu, X.B.; Chen, Q.D.; Yin, Y.; Qian, X.; Xu, Q.; Wang, M.; et al. NADPH oxidase subunit p22(phox)-mediated reactive oxygen species contribute to angiogenesis and tumor growth through AKT and ERK1/2 signaling pathways in prostate cancer. Biochim. Biophys. Acta 2013, 1833, 3375–3385.
17. Stamenkovic, I. Matrix metalloproteinases in tumor invasion and metastasis. Semin. Cancer Biol. 2000, 10, 415–433.
18. Damodharan, U.; Ganesan, R.; Radhakrishnan, U.C. Expression of MMP2 and MMP9 (gelatinases A and B) in human colon cancer cells. Appl. Biochem. Biotechnol. 2011, 165, 1245–1252.
19. Wu, Y.C.; Chiu, C.F.; Hsueh, C.T.; Hsueh, C.T. The role of bile acids in cellular invasiveness of gastric cancer. Cancer Cell Int. 2018, 18, 75.
20. Khoi, P.N.; Park, J.S.; Kim, J.H.; Xia, Y.; Kim, N.H.; Kim, K.K.; Jung, Y.D. (-)-Epigallocatechin-3-gallate blocks nicotine-induced matrix metalloproteinase-9 expression and invasiveness via suppression of NF-kappaB and AP-1 in endothelial cells. Int. J. Oncol. 2013, 43, 868–876.

21. Bergers, G.; Brekken, R.; McMahon, G.; Vu, T.H.; Itoh, T.; Tamaki, K.; Tanzawa, K.; Thorpe, P.; Itohara, S.; Werb, Z.; et al. Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. Nat. Cell Biol. 2000, 2, 737–744.

22. Shin, I.; Kim, S.; Song, H.; Kim, H.R.; Moon, A. H-Ras-specific activation of Rac-MKK3/6-p38 pathway: Its critical role in invasion and migration of breast epithelial cells. J. Biol. Chem. 2005, 280, 14675–14683.

23. Huang, Q.; Shen, H.M.; Ong, C.N. Inhibitory effect of emodin on tumor invasion through suppression of activator protein-1 and nuclear factor-kappaB. Biochem. Pharmacol. 2004, 68, 361–371.

24. Mook, O.R.; Frederiks, W.M.; Van Noorden, C.J. The role of gelatinases in colorectal cancer progression and metastasis. Biochim. Biophys. Acta 2004, 1705, 69–89.

25. Roy, R.; Yang, J.; Moses, M.A. Matrix metalloproteinases as novel biomarkers and potential therapeutic targets in human cancer. J. Clin. Oncol. 2009, 27, 5287–5297.

26. Cho, Y.B.; Lee, W.Y.; Song, S.Y.; Shin, H.J.; Yun, S.H.; Chun, H.K. Matrix metalloproteinase-9 activity is associated with poor prognosis in T3-T4 node-negative colorectal cancer. Hum. Pathol. 2007, 38, 1603–1610.

27. Li, T.; Chiang, J.Y. Bile acid signaling in metabolic disease and drug therapy. Pharmacol. Rev. 2014, 66, 948–983.

28. Hofmann, A.F.; Hagey, L.R.; Krasowski, M.D. Bile salts of vertebrates: Structural variation and possible evolutionary significance. J. Lipid Res. 2010, 51, 226–246.

29. Reynier, M.O.; Montet, J.C.; Gerolami, A.; Marteau, C.; Crotte, C.; Montet, A.M.; Mathieu, S. Comparative effects of cholic, chenodeoxycholic, and ursodeoxycholic acids on micellar solubilization and intestinal absorption of cholesterol. J. Lipid Res. 1981, 22, 467–473.

30. Jia, W.; Xie, G.; Jia, W. Bile acid-microbiota crosstalk in gastrointestinal inflammation and carcinogenesis. Nat. Rev. Gastroenterol. Hepatol. 2018, 15, 111–128.

31. Farhana, L.; Nangia-Makker, P.; Arbit, E.; Shango, K.; Sarkar, S.; Mahmud, H.; Hadden, T.; Yu, Y.; Majumdar, A.P. Bile acid: A potential inducer of colon cancer stem cells. Stem Cell Res. Ther. 2016, 7, 181.

32. Cao, H.; Xu, M.; Dong, W.; Deng, B.; Wang, S.; Zhang, Y.; Wang, S.; Luo, S.; Wang, W.; Qi, Y.; et al. Secondary bile acid-induced dysbiosis promotes intestinal carcinogenesis. Int. J. Cancer 2017, 140, 2545–2556.

33. Das, A.; Yaqoob, U.; Mehta, D.; Shah, V.H. FXR promotes endothelial cell motility through coordinated regulation of FAK and MMP-9. Arterioscler. Thromb. Vasc. Biol. 2009, 29, 562–570.

34. Baek, M.K.; Park, J.S.; Park, J.H.; Kim, M.H.; Kim, H.D.; Bae, W.K.; Chung, I.J.; Shin, B.A.; Jung, Y.D. Lithocholic acid upregulates uPAR and cell invasiveness via MAPK and AP-1 signaling in colon cancer cells. Cancer Lett. 2010, 290, 123–128.

35. Nishikawa, M. Reactive oxygen species in tumor metastasis. Cancer Lett. 2008, 266, 53–59.

36. Storz, P. Reactive oxygen species in tumor progression. Front. Biosci. 2005, 10, 1881–1896.

37. Nelson, K.K.; Melendez, J.A. Mitochondrial redox control of matrix metalloproteinases. Free Radic. Biol. Med. 2004, 37, 768–784.

38. Bernstein, H.; Bernstein, C.; Payne, C.M.; Dvorakova, K.; Garewal, H. Bile acids as carcinogens in human gastrointestinal cancers. Mutat. Res. 2005, 589, 47–65.

39. Craven, P.A.; Pfanstiel, J.; DeRubertis, F.R. Role of reactive oxygen in bile salt stimulation of colonic epithelial proliferation. J. Clin. Investig. 1986, 77, 850–859.

40. DeRubertis, F.R.; Craven, P.A.; Saito, R. Bile salt stimulation of colonic epithelial proliferation. Evidence for involvement of lipoxygenase products. J. Clin. Investig. 1984, 74, 1614–1624.

41. Craven, P.A.; Pfanstiel, J.; DeRubertis, F.R. Role of activation of protein kinase C in the stimulation of colonic epithelial proliferation and reactive oxygen formation by bile acids. J. Clin. Investig. 1987, 79, 532–541.

42. Payne, C.M.; Bernstein, C.; Dvorak, K.; Bernstein, H. Hydrophobic bile acids, genomic instability, Darwinian selection, and colon carcinogenesis. Clin. Exp. Gastroenterol. 2008, 1, 19–47.

43. Jung, J.S.; Ahn, Y.H.; Moon, B.I.; Kim, H.S. Exogenous C2 ceramide suppresses matrix metalloproteinase gene expression by inhibiting ROS production and MAPK signaling pathways in PMA-stimulated human astroglioma cells. Int. J. Mol. Sci. 2016, 17, 477.

44. Ozanne, B.W.; Spence, H.J.; McGarry, L.C.; Hennigan, R.F. Transcription factors control invasion: AP-1 the first among equals. Oncogene 2007, 26, 1–10.

45. Angel, P.; Karin, M. The role of Jun, Fos and the AP-1 complex in cell-proliferation and transformation. Biochim. Biophys. Acta 1991, 1072, 129–157.

46. Ho, B.Y.; Wu, Y.M.; Chang, K.J.; Pan, T.M. Dimerumic acid inhibits SW620 cell invasion by attenuating H2O2-mediated MMP-7 expression via JNK/C-Jun and ERK/C-Fos activation in an AP-1-dependent manner. Int. J. Biol. Sci. 2011, 7, 869–880.
47. Hoesel, B.; Schmid, J.A. The complexity of NF-kappaB signaling in inflammation and cancer. Mol. Cancer 2013, 12, 86.
48. Ulivi, V.; Giannoni, P.; Gentili, C.; Cancedda, R.; Descalzi, F. p38/NF-kB-dependent expression of COX-2 during differentiation and inflammatory response of chondrocytes. J. Cell Biochem. 2008, 104, 1393–1406.
49. Deak, M.; Clifton, A.D.; Lucocq, L.M.; Alessi, D.R. Mitogen- and stress-activated protein kinase-1 (MSK1) is directly activated by MAPK and SAPK2/p38, and may mediate activation of CREB. EMBO J. 1998, 17, 4426–4441.
50. Vermeulen, L.; De Wilde, G.; Van Damme, P.; Vanden Berghe, W.; Haegeman, G. Transcriptional activation of the NF-kappaB p65 subunit by mitogen- and stress-activated protein kinase-1 (MSK1). EMBO J. 2003, 22, 1313–1324.
