Cellular mechanism of bile acid-accelerated hepatocyte polarity

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We recently discovered that the major mammalian bile acid, taurocholate, accelerated polarity in primary rat hepatocytes. Taurocholate increased cellular cAMP and signals through an Epac-Rap1-MEK-LKB1-AMPK pathway for its polarity effect. This review discusses possible mechanisms for how taurocholate affects different cell polarity factors, particularly AMPK, and thereby regulates events that generate polarity. These include tight junction formation, apical trafficking, recycling endosome dynamics, and cytoskeleton rearrangement. We also discuss whether the effects of taurocholate are mediated by other LKB1 downstream kinases, such as Par1 and NUAK1.

Introduction
Hepatocytes are the major epithelial cells in the liver, comprise 70–80% of the liver's cytoplasmic mass, and are polarized. The polarized surfaces of hepatocytes consist of a basolateral domain facing the circulation and an apical domain that forms the bile canaliculus, the smallest branch of the biliary tree. Tight junction proteins, including occludin, claudin and ZO-1, seal the apical domains of two adjacent hepatocytes, helping to create the bile canaliculus.1,2 A major function of the liver is biliary secretion which requires hepatocyte polarization. Loss of polarity causes bile secretory failure (cholestasis) and subsequent liver damage due to bile acid retention.3 The mechanisms controlling hepatocyte polarization are partially understood. Structurally they include cytoskeletal, tight junctional and intracellular trafficking components.1,3,4 Taurocholate, the major bile acid, is synthesized from cholesterol in hepatocytes and secreted into the canaliculus by ABCB11 (BSEP), an ATP binding cassette (ABC) transporter which couples ATP hydrolysis to transport.5 Approximately 85% of bile acids are absorbed and transported back to the liver via the enterohepatic circulation.5,6 The traditional function of bile acids is to emulsify dietary fat;7 however, bile acids are also signaling molecules,8,9 which are involved in many signaling pathways including increasing cellular cAMP6,10 activating protein kinase C,14 nuclear farnesoid X receptor (FXR) and pregnane X receptors (PXR,10,12 PPRK/ AKTglyoxyn synthase kinase 3 (GSK3),15 and also enhance liver regeneration.16 These properties of bile acids and their polarized secretion suggest that hepatocytes respond to bile acid. Recent work has shown that bile acids, including taurocholate regulate hepatocyte polarization.17 Here, we discuss possible mechanisms.

Taurocholate Accelerates Hepatocyte Polarity in Primary Hepatocyte Sandwich Culture
Recent work has revealed a novel and important role for bile acids in hepatocyte polarity.17 Addition of taurocholate to rat hepatocytes in a collagen sandwich culture system accelerated polarization.17 Using pharmacological activators and inhibitors as well as dominant negative constructs, we discovered that the taurocholate effect...
on bile canicular formation requires activation of adenyl cyclase and cAMP downstream kinase, Epac (Exchange Protein Activated by cAMP), and signaling through the downstream kinases Rap1-MEK pathway resulting in activation of LKB1 (Liver Kinase B1) and AMPK (AMP-activated Protein Kinase) (Fig. 1). This study links taurocholate with Epac and LKB1-AMPK, the key cellular metabolic kinases. Mass spectroscopic study of lysates of cultured hepatocytes revealed detectable levels of endogenous bile acids that increased by day 5 in culture, which correlates with canicular development. These observations suggest that endogenous bile acids may participate in normal polarity development in hepatocytes. Interestingly, during liver development, the fetal hepatocytes are not fully polarized and only have very small canaliculi, and bile acid synthesis is sparse. However, hepatocytes rapidly polarize shortly after birth in parallel with increased bile acid synthesis in the liver.

Possible Effect on Tight Junction Assembly

How LKB1-AMPK regulates polarization in primary hepatocyte culture is not known. Several studies suggest that AMPK regulates the tight junction assembly required for polarity. Inhibition of AMPK by overexpression of dominant negative-AMPK resulted in loss of tight junction structure and polarity. AMPK regulates myosin light chain (MLC) which may affect the actin cytoskeleton that is involved in tight junction formation. In addition, AMPK activation occurs in parallel with mitochondrial fusion, increased mitochondrial potential and ATP levels. These observations suggest that mitochondria are important in the effect of AMPK on metabolism and polarization. Thus, AMPK can regulate tight junction assembly through small GTPases directly or indirectly by altering the cellular energy status. The small GTPase Rab5a promotes junction formation and apical constriction, and involves myosin II signaling. Another small GTPase, Rab13, is involved in tight junction assembly. Epac downstream Rap1 also regulates junction formation.

Effect on Apical Trafficking/Recycling

Knockdown of Rab11a or overexpression of myosin Vb motorless tail domain prevented canicular formation, suggesting that Rab11a is required for canicular formation. This study revealed that polarization of hepatocytes requires recruitment of Rab11a and myosin Vb for targeting ABC transporters to the apical plasma membrane. The recycling pathway for ABCB11 was initially characterized in WIFB cells, a hybrid of rat hepatoma and human fibroblast that retain a spherical canicular structure, and confirmed in hepatocytes in collagen sandwich cultures. Overexpression of dominant negative Rab11A or motorless tail domain of myosin Vb inhibited trafficking of ABCB11 to the canicular membrane; ABCB11 accumulated intracellularly and this was associated with loss of polarization. These observations confirm that Rab11a and myosin Vb dependent apical traffic is essential for hepatic polarity. Using FRAP (Fluorescent Recovery After Photobleaching) analysis, our study revealed that taurocholate enhances delivery of ABCB11 to the canicular membrane in primary hepatocytes. Moreover, our recent FRAP studies of hepatocytes from LKB1 knockout mice showed loss of taurocholate-enhanced ABCB11 trafficking to the apical membrane, suggesting that taurocholate enhancement of ABCB11 trafficking is LKB1 dependent. How taurocholate facilitates apical protein trafficking is under investigation. After activation by taurocholate, LKB1-AMPK may interact with Rab11a either directly or indirectly via Rab11a-Fip1 proteins (Rab11 family-interacting protein 1). Rab11a-Fip1 has constitutive AMPK phosphorylation sites. LKB1-AMPK may interact with Rab11a-Fip1 to regulate Rab11a activity and modulate apical protein trafficking. We are currently studying possible interactions of AMPK and Rab11a-Fip1 proteins.
Effect on Cytoskeleton

AMPK phosphorylates microtubule plus end protein CLIP-170, which is essential for polymerization and depolymerization of microtubules. AMPK-induced phosphorylation of CLIP-170 is important for lamellipodium formation and adhesion maturation, which is important for cell polarity.27 Taurocholate may activate AMPK-CLIP170 thereby inducing cytoskeleton arrangement for polarization. In addition, proteomic studies suggest that AMPK can interact with β2 tubulin and gamma actin, indicating a role for AMPK in cytoskeleton arrangement.28 Moreover, microtubule plus-ends can interact with the actin cytoskeleton which is required for apical protein trafficking/recycling. Recycling endosomes containing ABCB1, move along microtubules and are eventually transferred to an actin-based system. Therefore, taurocholate may accelerate apical protein traffic through activation of AMPK-CLIP170. Whether taurocholate increases phosphorylation of CLIP-170 is under examination in our lab.

References
1. Vrisko M, Papile P, Strecker S, De Rey P, Hendriksen T, Chhoncharuk J, et al. Involvement of cell junctions in hepatocyte polarity. Gastroenterology. 2004; 126:999-1011. PMID:15080618; http://dx.doi.org/10.1053/j.gastro.2004.05.045
2. Kreut J, Yamamoto T, Murata M, Ohi H, Kobayashi N, Sakakura N. Regulation of the Microtubule-Actin Interactions between α-tubulin and γ-tubulin in Mammalian Cells. Biochem Biophys Res Commun. 1999; 263:420-425. PMID:10161869; http://dx.doi.org/10.1006/bbrc.1999.1723
3. Rodrigues-Boues E, Kreutz G, Musch A. Organization of vesicular trafficking in epithelia. Nat Rev Mol Cell Biol 2005; 6:233-47; PMID:15738988; http://dx.doi.org/10.1038/nrm1593
4. Mostov K, Su T, ter Beest M. Polarized epithelial cell function in the liver. Physiol Rev 2003; 83:287-93; PMID:12669082; http://dx.doi.org/10.1152/physrev.00030.2002
5. Bryant DM, Mostov KE. From cells to organs: building the mammalian liver. J Biol Chem 1998; 273:10046-50; PMID:9757103; http://dx.doi.org/10.1073/pnas.0503702102
6. Huang W, Ma K, Zhang J, Qatanani M, Cuvillier J, et al. Nuclear receptor-dependent bile acid signaling is required for normal liver regeneration. Science 2006; 312:235-6; PMID:16421413; http://dx.doi.org/10.1126/science.1124546
7. Babu DN, Wuthier RE. Bile acids inhibit ileal transepithelial electrolyte transport in vivo. J Clin Invest 1980; 66:760-8; PMID:7403970; http://dx.doi.org/10.1172/JCI110692
8. Babu DN, Wuthier RE. Bile acids induce intestinal electrolyte transport in vitro. J Clin Invest 1980; 66:760-8; PMID:7403970; http://dx.doi.org/10.1172/JCI110692
9. Wakabayashi Y, Dutt P, Lippincott-Schwartz J, Arumugam M, Ransohoff RM. The sister of P-glycoprotein (ABCB11) and the nucleoporin NUP60 are required for bile canalicular formation in WIF-B9 cells. Proc Natl Acad Sci USA. 2005; 102:15877-82. PMID:16321690; http://dx.doi.org/10.1073/pnas.0507822102
10. Grezegel V, Stumpner R, Badoual J, Maldonado I, Roled J, et al. The stress of Pglycoprotein represents the canalicule bile salt excretory pump of murine liver. J Cell Biol 1998; 140:1049-56. PMID:9495391; http://dx.doi.org/10.1083/jcb.140.6.1049
11. Therian C, Pelliccioni R, Pennazza M, Aruza J, Schiovone K. Targeting TGR-5 signaling for metabolic diseases. Nat Rev Drug Discov. 2008; 7:476-91. PMID:18678491; http://dx.doi.org/10.1038/nrd2523
12. Zhang J, Xiao Y, Xu T, Bauer M. Polarity of epithelial monolayer cell assembly and adhesion. J Cell Sci 2008; 121:2361-71. PMID:18544682; http://dx.doi.org/10.1242/jcs.018403-287
13. Hendershot D, Tijmink D, van Sic. The tubulopetal component: a traffic stream in mucin polarity development. J Cell Sci 2006; 119:2163-72. PMID:16574826; http://dx.doi.org/10.1242/jcs.011227
14. Wakahashi Y, Dutt P, Lippincott-Schwartz J, Arumugam M, Ransohoff RM. The sister of P-glycoprotein (ABCB11) and the nucleoporin NUP60 are required for bile canalicular formation in WIF-B9 cells. Proc Natl Acad Sci USA. 2005; 102:15877-82. PMID:16321690; http://dx.doi.org/10.1073/pnas.0507822102
15. Fang Y, Steiner E, Mitchell C, Granz S, Pandak WM, Hileman PB, et al. Constitutive bile acids regulate hepatocyte glycogen synthase activity in vitro and in vivo via Ga(4) signaling. Mol Pharmacol 2007; 71:1122-8. PMID:17288418; http://dx.doi.org/10.1124/mol.106.032060
16. Huang W, Ma K, Zhang J, Qatani M, Cuvillier J, et al. Nuclear receptor-dependent bile acid signaling is required for normal liver regeneration. Science 2006; 312:235-6; PMID:16421413; http://dx.doi.org/10.1126/science.1124546
17. Fu D, Wakahashi Y, Lippincott-Schwartz J, Arumugam M. Bile acid stimulates hepatocyte polarization through a G-protein coupled receptor (TGR-5). Proc Natl Acad Sci USA. 2011; 108:1640-9; PMID:21216520; http://dx.doi.org/10.1073/pnas.1008774108
18. Zheng B, Cantley LC. Regulation of epithelial tight junction assembly and disassembly by AMP-activated protein kinase. J Biol Chem 2004; 279:10414-9; PMID:14720693; http://dx.doi.org/10.1073/pnas.0307530100

LKB1 Downstream Kinases Part 1 and NUAK1 do not Mediate the Taurocholate Effect

In addition to AMPK, LKB1 also activates Par1, a downstream kinase involved in polarization. Par1, a downstream kinase involved in increased phosphorylation of MLC2 (myosin light chain 2) and activation of myosin II. Since myosin II is required for actin cytoskeleton arrangements,29 LKB1-induced phosphorylation of MLC2 and activation of myosin II may mediate the taurocholate effect on hepatocyte polarity. Blocking MLC2 (5 to 100 nM, IC50 = 5 nM), did not affect taurocholate-induced canalicular formation in hepatocytes, suggesting that taurocholate may not signal through LKB1 activation of MLC2 for its effect on canalicular formation. However, whether MLC2 activation of AMPK-related kinase NUAK1 resulting in increased phosphorylation of MLC2 (myosin light chain 2) and activation of myosin II. Since myosin II is required for actin cytoskeleton arrangements as cells polarize,3,7 we also tested whether NUAK1 mediates the taurocholate effect on hepatocyte polarity. Blocking NUAK1 with an inhibitor of NUAK1 (10 μM), did not affect taurocholate-induced canalicular formation in hepatocytes, suggesting that taurocholate may not signal through inhibition of NUAK1 after activation of LKB1 for its effect on canalicular formation.38

The mechanism whereby taurocholate activates adenyl cyclase in hepatocytes is uncertain. In other cell lines bile acids activate adenyl cyclase by interacting with the G-protein coupled receptor, TGR-5; however, in contrast to other cells, hepatocytes express TGR-5. We are investigating TGR5 function in hepatocytes using TGR5 knockout mouse liver and specific TGR-5 activator, FFN-777.

Conclusion

Bile acids play multiple roles in many cellular events. Taurocholate has a novel role in hepatocyte polarity linked to its effect on cellular energy metabolism, through AMPK. Given their diverse pharmacological effects, bile acids may accelerate hepatocyte polarity through multiple downstream effectors that regulate tight junction formation, apical trafficking and cytoskeleton rearrangements occurring during cell polarization. Given their novel role in hepatocyte polarity, bile acids may be potential therapeutics for polarity relevant disorders.
19. Zhang L, Li J, Young LH, Caplan MJ. AMP-activated protein kinase regulates the assembly of epithelial tight junctions. Proc Natl Acad Sci USA. 2006;103:17272-7. PMID:17088526; http://dx.doi.org/10.1073/pnas.0608531103

20. Fu D, Wakahashi Y, Ido Y, Lippincott-Schwartz J, Arain B. Regulation of bile canalicular network formations and maintenance by AMP-activated protein kinase and LRKK1. J Cell Sci. 2010;123:3296-329. PMID:20824848; http://dx.doi.org/10.1242/jcs.084998

21. Miyazaki T, Muramatsu Y, Sugimoto A, Mitani T, Nokida Y. Essential roles of myosin phosphatase in the maintenance of epithelial cell integrity of Drosophila imaginal disc cells. Dev Biol. 2007;308:79-86. PMID:17607293; http://dx.doi.org/10.1016/j.ydbio.2007.04.021

22. Terry SJ, Zikaki C, Ebbesen A, Veldhuis E, Leung Cheng Sun JY, Buda MS, et al. Spatially restricted activation of RhoA signaling at epithelial junctions by p114RhoGEF drives junction formation and morphogenesis. Nat Cell Biol. 2005;7:1944. PMID:21256939; http://dx.doi.org/10.1038/ncb1256

23. Wong K, Van Keymeulen A, Bourne HR. PDZRhoGEF and myosin II localize RhoA activity to the back of polarizing neutrophil-like cells. J Cell Biol. 2007;179:1141-8. PMID:17608513; http://dx.doi.org/10.1083/jcb.20070514

24. Marzesco AM, Donia E, Panditaan H, Racourmont M, Dinavene D, Benevetti RL, et al. The small GTPase Rab13 regulates assembly of functional tight junctions in epithelial cells. Mol Biol Cell. 2005;16:1819-31. PMID:15895815; http://dx.doi.org/10.1083/jcb.200506040

25. Bos JL, de Rooij J, Reedquist KA. Rap1 signaling: adhering to new models. Nat Rev Mol Cell Biol. 2001;2:369-77. PMID:11331911; http://dx.doi.org/10.1038/35073073

26. Wakabayashi Y, Fu D, Lippincott-Schwartz J, Arain B. Rab11A and myosin VB are required for canalicular network formation and ABC transporter cycling in primary hepatocytes. Hepatology. 2009;50:718A.

27. Yokota A, Kato H, Watanabe T, Miura K, Yamashita A, Amano Y, et al. AMPK controls the speed of microtubule polymerization and directional cell migration through CLIP-170 phosphorylation. Nat Cell Biol. 2010;12:583-90. PMID:20495555; http://dx.doi.org/10.1038/ncb2060

28. Tuerk RD, Zilch RF, Ande V, Rodenmier H, Brunsfeld LA, Schramm U, et al. New candidate target of AMP-activated protein kinase in mouse brain revealed by a novel multidimensional substrate-screen for protein kinases. J Proteome Res. 2007;6:344-7. PMID:17548852; http://dx.doi.org/10.1021/pr061045u

29. Cohen D, Fernandez D, Laroche-Dupuis F, Mendoza A. The serine/threonine kinase Par1 regulates epithelial lumen polarity via (hypothetical) cell-ECM signaling. J Cell Biol. 2011;200:535-44. PMID:21282462; http://dx.doi.org/10.1083/jcb.201101042

30. Zagórska A, Dukh M, Campbell DG, Hanus S, Hainov M, Aasen B, et al. New role for the LKB1-NUAK pathway in controlling myosin phosphatase complexes and cell adhesion. Nat Signal. 2010;5:1-5. PMID:20354225; http://dx.doi.org/10.1126/natsignal.2004416