MINI REVIEW

Roles of the scaffolding proteins NHERF in liver biology

Audrey Clapéron a, b, Martine Mergey a, b, Laura Fouassier a, *, b

a Inserm UMR 938, CdR Saint-Antoine, 75012 Paris, France
b UPMC université Paris-06, 75012 Paris, France

Available online 28 January 2011

Summary Scaffold proteins are defined by the presence of specific protein-binding domains (e.g., PDZ domains) that assemble several proteins into functional complexes. Thus, scaffolds are critical for spatio-temporal organization and for proper regulation of intracellular signalling upon specific stimulus. Identified 15 years ago, NHERF scaffold proteins contain several PDZ modules and were initially viewed as “passive linkers” between transmembrane proteins and the cortical cytoskeleton underlying the plasma membrane. New NHERF-binding molecules involved in cell signalling have been recently identified. Thus, NHERFs are now viewed as “active” key players in regulating cellular functions. EBP50 and PDZK1, two members of the NHERF family, are highly expressed in the liver where they link receptors, channels, transporters and cytosolic components. This review aims to give an overview of the emerging functions of NHERF proteins in liver physiology.

© 2011 Elsevier Masson SAS. All rights reserved.

Abbreviations: PDZ, Postsynaptic density protein (PSD95); Drosophila disc large tumor suppressor (DlgA), and zonula occludens-1 protein (zo-1); NHERF, Na+/H+ Exchanger Regulatory Factor; EBP50, Ezrin-radixin-moesin-Binding Phosphoprotein 50; E3KARP, NHE3, Kinase A Regulator Protein; PDZK1, PDZ Domain Protein Kidney 1; IKK, Intestinal and Kidney-Enriched PDZ Protein; ERM, Ezrin-radixin-moesin; EB, Ezrin-radixin-moesin Binding; SR-BI, scavenger receptor class B, type I; HCC, hepatocellular carcinoma.

* Corresponding author. Tel.: +33 1 40 01 13 53; fax: +33 1 40 01 13 52.
E-mail address: laura.fouassier@inserm.fr (L. Fouassier).

2210-7401/$ - see front matter © 2011 Elsevier Masson SAS. All rights reserved.
doi: 10.1016/j.clinre.2010.11.009
The NHERFs family: Scaffold proteins with PDZ domains

Scaffolds are defined by the presence of protein-protein interaction modules that play a role in protein complex assembly. Thus, they are involved in the orchestration of numerous events including regulation of transmembrane protein activity (e.g. transporters) and signal transduction pathways. By physically tethering proteins, scaffolds locally concentrate, compartmentalize and position transporters or enzymes in close proximity to their substrates or regulatory proteins, thereby avoiding background and nonspecific interactions. Furthermore, recent studies have highlighted a new function of scaffold proteins, which is to act on the conformation of their partners. Finally, scaffolds may not only promote but also limit cell signalling by recruiting negative regulatory components (e.g. phosphatases) modulating the overall signalling [1—4] (Fig. 1).

The NHERF proteins belong to the family of PDZ-scaffolding proteins and are characterized by at least two PDZ modules that bind the C-terminus of target proteins in a sequence-specific manner. PDZ domains, which comprise 70 to 90 amino acids, are among the commonest protein domains in the human proteome sequenced genome [5,6]. Through their PDZ domains, scaffolds also have the ability to dimerize and to interact with each other further extending the potential of assembling effectors [7—9].

The NHERF family comprises four members ranging in size between 337 and 519 amino acids. EBP50 (or NHERF1), E3KARP (or NHERF2), PDZK1 (or NHERF3) and IKEPP (or NHERF4) [10—18] (Fig. 2). EBP50 and E3KARP display two PDZ domains, while PDZK1 and IKEPP possess four. Sequence and structural analysis indicate that the four members of NHERF family are evolutionarily related [13,14]. Among NHERFs, EBP50 and E3KARP have an additional protein-interacting domain called EB that allows interaction with the actin cytoskeleton through the ERM proteins [11].

Hepatic expression of NHERFs

NHERF proteins are mainly expressed in polarized epithelial cells at the apical side. Together with kidney and small intestine, liver is the tissue that exhibits the highest expression levels of EBP50 mRNA and protein [11,13,19,20]. In liver, EBP50 is concentrated beneath the canalicular/apical membrane of hepatocytes and of cholangiocytes. In addition, extrahepatic biliary epithelia such as the gallbladder epithelium displays a high expression level of EBP50 mRNA [21]. Interestingly, in contrast to mature liver epithelial cells, fetal hepatocytes and biliary epithelial cells show a strong immunopositivity for EBP50 both in the cytoplasm and nuclear compartments (L. Fouassier, S. Colnot and C. Godard, unpublished results).

E3KARP expression was first detected in human liver by Yun et al. [13]. Thereafter, rat E3KARP was cloned from liver and observed both in hepatocytes and cholangiocytes [22]. In contrast to EBP50, which is found at the apical membrane, staining of E3KARP is prominent in the perinuclear compartment of cholangiocytes [22]. PDZK1 is highly detected in liver with a subcellular distribution similar to that of EBP50 [15,16,23] (L. Fouassier and M. Mergey, unpublished results). The last member of the NHERF family, IKEPP, has not been detected in the liver [17,18].

NHERFs functions in the liver epithelia

NHERFs regulate cell surface expression and functional activity of transporters

Accumulating evidence suggests that EBP50 is involved in the retention, the localization and the regulation of transporters. Most transporters identified as binding partners of NHERFs in the liver belong to the super family of ABC transporters. An interaction between Cystic Fibrosis Transmembrane conductance Regulator (CFTR), a PKA-regulated chloride channel, and EBP50, and also with E3KARP and PDZK1, has been initially demonstrated in human airway epithelial cells [16,24—26]. In the liver, CFTR expression is restricted to biliary epithelial cells where EBP50 is highly expressed [21,27]. In these cells, we have shown that a dominant-negative form of EBP50 (i.e. PDZ1 domain of EBP50) down-regulates PKA-dependent Cl— channel activity [22]. As proposed for NHE3 [28], it is likely that EBP50 tethers within the same complex CFTR and ERM proteins which serve as docking platform for PKA. Therefore, at the plasma membrane of cholangiocytes, EBP50 may provide appropriate and specific regulation to the CFTR channel.

More recently, MRP2 (aka ABCC2 or cMOAT) and MRP4 (ABCC4) have been shown to interact with EBP50 [29—31]. MRP2 is involved in liver detoxification and formation of the bile by transporting a wide variety of organic anions. Mutations in the human MRP2 gene are associated with the Dubin-Johnson syndrome characterized by conjugated hyperbilirubinemia and defects in the secretion of organic

![Figure 1](image.png)

**Figure 1** Function of scaffold proteins. A. In absence of scaffold protein, membrane proteins (A) are free to interact with different effectors (B and C), resulting in a random and non-specific activation with low signalling efficiency. B. Scaffold protein (D) allows the close proximity and correct conformation of membrane protein (A’) and its associated effector (B’). Therefore, presence of scaffold proteins facilitates signalling efficiency.
Figure 2  Na+/H+ Exchanger Regulatory Factor family (NHERF) members and structures. Partners that link NHERF proteins in biliary epithelial cells (BEC) or hepatocytes (Hep) are indicated below their PDZ domain. Alternative names for NHERFs are mentioned.

Anions. In the liver, MRP2 and EBP50 have been shown to colocalize at the apical membrane of rat hepatocytes [30]. Interestingly, using EBP50 knockout mice [32,33], Boyer’s group first evidenced that EBP50 exerts in vivo crucial functions in the liver. Indeed, EBP50 by tethering MRP2 may contribute to the maintenance of canalicular expression of MRP2 and to its functions as a determinant of glutathione-dependent bile flow [30]. MRP2 has recently been shown to link PDZK1 in a hepatocyte cell line [34]. Even though, PDZK1 seems to play a role in apical localization of MRP2, the functionality of this interaction remains to be explored in native liver cells.

In nonliver cells, an association between EBP50 and MRP4 has been demonstrated. As a result, EBP50 modulates internalization and drug efflux of MRP4 [31,35]. However, since MRP4 is expressed at the basolateral membrane of hepatocytes [36] and EBP50 at the canalicular membrane, interaction between both proteins may not occur in the liver. Indeed, in contrast to MRP2, no modification of MRP4 expression was detected in the liver of EBP50−/− mice [30]. However, further investigations will be needed regarding functional interaction between EBP50 and MRP4 in cholestatic diseases, in which MRP4 is up regulated.

Recently, the organic anion transport protein, Oatp1a1, has been shown to interact with PDZK1 in rat liver membrane [37]. However, studies performed in PDZK1−/− mice demonstrated no modification of Oatp1a1 expression in the liver. Further studies will be needed to address the impact of PDZK1 on Oatp1a1-mediated transport functions.

PDZK1 controls the HDL receptor scavenger receptor class B, type I in the liver

Interaction between PDZK1 and the SR-BI has been identified in liver tissue. SR-BI is a high-density lipoprotein receptor predominantly expressed in the liver that contributes to HDL cholesterol uptake and cholesterol secretion into bile. In the liver, a growing number of studies supports a major contribution of PDZK1 in hepatic regulation of SR-BI. Indeed, targeted disruption of PDZK1 in mice results in an increase of plasma cholesterol attributed primarily to a decrease of hepatic content of SR-BI [38–41]. However, the precise mechanism by which PDZK1 mediates regulation of SR-BI expression remains to be explored.

Estrogens control EBP50 and PDZK1 expression

EBP50 was shown to be under hormonal regulation by estrogens. Indeed, the promoter of EBP50 gene (SLC9A3R1) contains 13 half-estrogen responsive elements [19,42]. Our group has recently demonstrated that estrogens stimulate EBP50 expression in biliary epithelial cells [21]. Since estrogens regulate EBP50 and proliferation of the biliary epithelial cells [43], these data support a role of EBP50 in the estrogen-mediated proliferative response of biliary epithelium. As demonstrated for EBP50, steroid hormones also regulate PDZK1 expression, which is induced by estradiol and repressed by tamoxifen, an antagonist of the estrogen receptor, in breast and ovarian cancer cells [44,45].

EBP50 and cancer

A role for EBP50 in liver cancer has been documented but whether EBP50 acts as an oncoprotein or as a tumor suppressor remains unclear [46]. In HCC, high expression of EBP50 mRNA has been ascertained in 45% of the cases whereas mRNA level remains unchanged in 55%. A delocalization of EBP50 to the cytoplasm and nucleus of tumor cells has been also observed [47]. Global transcriptome analyses performed on human HCC specimens have identified EBP50 over expression in a specific HCC subgroup defined by differential upregulation of genes implicated in nucleus trafficking and in cell-cycle
check points [48] (J. Zucman-Rossi and L. Fouassier, unpublished results). In cholangiocarcinomas, analyses of a large series demonstrated a delocalization of EBP50 in the cytoplasm of tumor cells [49].

The activation of the WNT/β-catenin pathway is one of the major signalling pathways deregulated in liver carcinogenesis [50]. Shibata et al. was the first to show a direct interaction between EBP50 and β-catenin. This association promotes transcriptional activity of β-catenin in the hepatoma cells [47]. Therefore, it has been proposed that EBP50 may participate to the malignant phenotype of HCC by stimulating the WNT pathway. However, other studies argue for a role of EBP50 as a tumor suppressor. In embryonic hepatic cells, EBP50 assemblies EGFR, β-catenin and merlin/NF2 into a multiprotein complex at the plasma membrane, contributing to the integrity of epithelia [51]. In human biliary carcinoma cells, we have recently shown that loss of EBP50 causes upregulation of EGFR expression at the plasma membrane as well as sustained activation of the receptor. Subsequently, invalidation of EBP50 drives adherence junction disruption, cell dispersion and migration, suggesting that loss of EBP50 at the plasma membrane contributes to cholangiocarcinogenesis through EGFR activation [49].

Tyrosine kinase receptors and intracellular proteins (e.g. EGFR, β-catenin), related to cancer development and progression, represent a broad group of molecules known to interact with NHERFs, suggesting a critical role of these proteins in the coordination and regulation of cell signalling during carcinogenesis. However, the precise function of the only member of the NHERF family involved so far in carcinogenesis (e.g. EBP50) is still poorly understood. The ambiguous functions of EBP50 are likely related to the nature of its partners and to its subcellular localization as well as to the cellular context.

**Conclusion**

The liver epithelium expresses high levels of different NHERFs (e.g. EBP50 and PDZK1). Deregulation of their expression (e.g. EBP50) has been involved in various diseases, including immune disorders, nephrolithiasis and bone demineralization [52–54]. So far, the expression of NHERFs in liver pathology has been investigated mostly in cancer events but remains to be explored in nontumoral diseases. Beyond the assembly of transporters/channels into complex networks beneath the apical membranes, it is becoming apparent that NHERFs link intracellular proteins both in the cytoplasm and nuclear compartment of the liver cells. Considering the diversity of NHERF partners, it is not surprising that these scaffold proteins are integrated into several aspects and play ambiguous functions in liver cell biology. Future drug development, as already suggested, targeting NHERF-mediated protein-protein interaction requires to characterize binding partners and functions of NHERF [55,56]. Understanding the functions of two NHERF’s members (e.g. EBP50 and PDZK-1) in liver pathology, and more specifically in liver cancer, will be one of the major challenges in the future.

**Conflicts of interest statement**

No potential conflicts of interest are disclosed.

**Acknowledgment**

Authors are grateful to Dr. C. Desbois-Mouthon, Dr. N. Chignard and Dr D. Debray for helpful discussions and critical reading of the manuscript.

Grant support: this work was supported by Naturalia and Biologia (to A.C.).

**References**


**Grant support**

This work was supported by Naturalia and Biologia (to A.C.).
1q21, interacts with cMOAT (MRP2), the multidrug resistance-associated protein. Lab Invest 1999;79:1161–70.


