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Review

Roland Reinehr, Annika Sommerfeld and Dieter Häussinger*

The Src family kinases: distinct functions of c-Src, Yes, and Fyn in the liver

Abstract: The Src family kinases Yes, Fyn, and c-Src play a pivotal role in regulating diverse liver functions such as bile flow, proteolysis, apoptosis, and proliferation and are regulated by anisoosmotic cell volume changes, death receptor ligands, and bile acids. For example, cell swelling leads to an integrin-sensed and focal adhesion kinase-mediated activation of c-Src-triggering choleresis, proteolysis inhibition, regulatory volume decrease *via* $p38^{MAPK}$ and proliferation *via* the activation of the epidermal growth factor receptor and extracellular regulated kinases 1 and 2. In contrast, hepatocyte shrinkage generates an almost instantaneous oxidative stress response that triggers the activation of c-Jun N-terminal kinase and the Src family kinases Fyn and Yes. Whereas Fyn activation mediates cholestasis, Yes triggers CD95 activation and apoptosis. This review will discuss the role of Src family kinases in the regulation of liver function with emphasis on their role in osmo-signaling and bile acid signaling.

Keywords: apoptosis; bile acids; osmolarity; proliferation.

***Corresponding author: Dieter Häussinger**, Clinic for Gastroenterology, Hepatology and Infectious Diseases, University Hospital Düsseldorf, Moorenstrasse 5, D-40225 Düsseldorf, Germany, e-mail: haeussin@uni-duesseldorf.de
Roland Reinehr and Annika Sommerfeld: Clinic for Gastroenterology, Hepatology and Infectious Diseases, University Hospital Düsseldorf, Moorenstrasse 5, D-40225 Düsseldorf, Germany

Introduction

The Src family kinases represent nonreceptor cytoplasmic protein tyrosine kinases. Initially described in the context of cell growth and differentiation, their roles in cell adhesion and motility, carcinogenesis and immune cell function, learning and memory as well as in osmo-sensing and osmo-signaling have been recognized [for reviews, see (1–5)]. Apart from c-Src, the Src kinase family also comprises other protein kinase members, i.e., Yes, Fyn, Fgr, Blk, Lck, Hck, Yrk, and Lyn (Table 1) (3). Yes, Fyn, and Src are expressed ubiquitously, whereas the other Src family kinases are more or less tissue-specific (Table 1) [reviewed in (1)]. The Src family kinases are closely related to Abl kinase and COOH-terminal Src kinase (Csk) and to Eph receptor tyrosine kinases (4).

Src family kinases are characterized by their modular architecture including the so-called Src homology (SH) domains [reviewed in (4, 5)]. Figure 1 schematically depicts the molecular structure and activation of the Src proteins. SH1 represents the catalytic domain with its tyrosine kinase activity. The inactive kinase is defined by the key tyrosine (Y^{416}), which blocks substrate binding to the kinase. Upon (auto-)phosphorylation, this tyrosine residue is displaced and substrate binding is unhindered. SH2 and SH3 domains represent the binding or interaction sites for other signaling proteins. Furthermore, the SH2 domain also provides a binding site for intramolecular interaction, i.e., binding of the Src kinase COOH-terminal tyrosine residue (Y^{527}) to this SH2 domain. This binding results in an inactivation of the Src kinase catalytic SH1 domain. The Csk and the Csk homologous kinase (Chk) were reported to induce Src family kinase Y^{527} phosphorylation, thereby inactivating the SH1 tyrosine kinase domain. Meanwhile, several phosphatases such as protein tyrosine phosphatase (PTP) 1B, PTPα, *SH2*-containing phosphatases SHP1 and SHP2 can dephosphorylate the Src family kinase residue Y^{527}, thereby activating the respective Src kinase [reviewed in (5)]. Also, the focal adhesion kinase (FAK), a nonreceptor tyrosine kinase, was reported to activate Src family kinases. FAK directly binds to the Src family kinase SH2 domains, thereby disrupting their inhibitory intramolecular interactions (4).

It is unclear how individual Src family kinases can play distinct roles within cells where all three are present, but one may speculate that specificity resides in distinct serine/threonine phosphorylation patterns and the unique region of each Src kinase. Functional specificity may also depend on other factors, such as cell type, upstream receptors, protein phosphatases, the subcellular localization of

Table 1 Expression of Src family kinases.

Src family kinase (gene)	Abbreviation (protein)	Cell type specifity
v-Src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog (avian)	Src	Ubiquitous
FYN oncogene related to SRC, FGR, YES	Fyn	Ubiquitous
v-Yes-1 Yamaguchi sarcoma viral oncogene homolog 1	Yes	Ubiquitous
Gardner-Rasheed feline sarcoma viral (v-fgr) oncogene homolog	Fgr	Myeloid cells, B cells
B lymphoid tyrosine kinase	Blk	B cells
Lymphocyte-specific protein tyrosine kinase	Lck	T cells, NK cells, brain
Hemopoietic cell kinase	Hck	Myeloid cells
Proto-oncogene tyrosine-protein kinase Yrk	Yrk	Ubiquitous
v-Yes-1 Yamaguchi sarcoma viral-related oncogene homolog	Lyn	B cells, brain, myeloid cells

Adapted from Thomas and Brugge (1) and Cohen (4).

the Src kinase (1, 6–8) and may occur upstream or downstream of Src kinase activation (4).

Receptor tyrosine kinases such as the epidermal growth factor receptor (EGFR) also can induce Src family kinase phosphorylation (1) and *vice versa*, Src family kinases can (trans)activate the EGFR (9, 10). Recent data suggest that c-Src directly interacts with hepatitis C virus (HCV)-encoded proteins. Here, c-Src binds the viral RNA-dependent RNA polymerase (NS5B) *via* its SH3 domain and the nonstructural phosphoprotein NS5A *via* its SH2 domain, which is required for efficient replication of HCV (11).

The functional role of Src family kinases was studied in knockout animal models. Despite the fact that expression of the Src family kinases c-Src, Hck, and Fgr increases dramatically during myeloid cell development, c-Src-deficient mice exhibit functional abnormalities only in the osteoclasts, resulting in impaired bone remodeling and osteopetrosis (12), whereas Hck- or Fgr-deficient mice, respectively, exhibit only few and subtle myeloid cell deficiencies (13). However, double knockout mice for Hck and Fgr develop immunodeficiency (13). Fyn knockout was shown to affect the thymocytes and peripheral T cells (14) as well as hippocampal development, spatial learning, and long-term potentiation (15). Yes knockdown did not show discernible effects (16). However, Src/Yes and Src/Fyn double-mutant mice rarely survive past birth (16). Yes/Fyn double knockout shows a renal phenotype and develops glomerulosclerosis, with only about one-third of

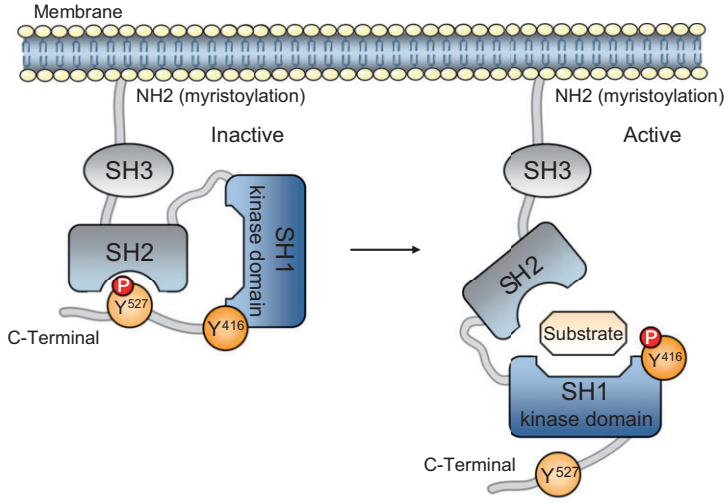


Figure 1 Molecular structure and activation of Src family kinases.
The Src protein is composed of three SH domains (SH1, SH2, and SH3), with SH1 representing the catalytic domain with its tyrosine kinase activity and SH2 and SH3 domains representing the binding or interaction sites for signaling proteins. The activity of Src kinases is regulated by conformational activation: The inactive kinase is defined by tyrosine Y^{416} (chicken), which blocks substrate binding to the kinase. Upon (auto-)phosphorylation, this tyrosine residue is displaced, and substrate binding is unhindered. The binding of the Src kinase COOH-terminal tyrosine residue Y^{527} (chicken) in its phosphorylated form to the SH2 domain results in an inactivation of the Src kinase catalytic SH1 domain. Adapted from Kim et al. (126).

the mice surviving until adulthood (16). From these observations, it was concluded that the Src family kinases have overlapping functions in many cell types. Nevertheless, one has to keep in mind that double-mutant phenotypes, e.g., Src/Yes, Src/Fyn, or Yes/Fyn, respectively, may also exhibit additive effects of the single mutations (17).

This short review focuses on the Src family kinases Yes, Fyn, and c-Src in the liver, with emphasis on their role in the regulation of cell volume, bile formation, apoptosis, and proliferation.

Src family kinases and cell volume regulation

Recent data suggest that Src family kinases are regulated by anisoosmotic cell volume changes. For example, hypoosmolarity can activate Src (18), Lyn (19), and Lck (20), whereas hyperosmolarity activates Yes (10), Fyn (21), Hck, and Fgr (22). The underlying mechanisms are cell type-specific and can involve integrin, EGFR, and ion channel activation (2, 4, 18, 23).

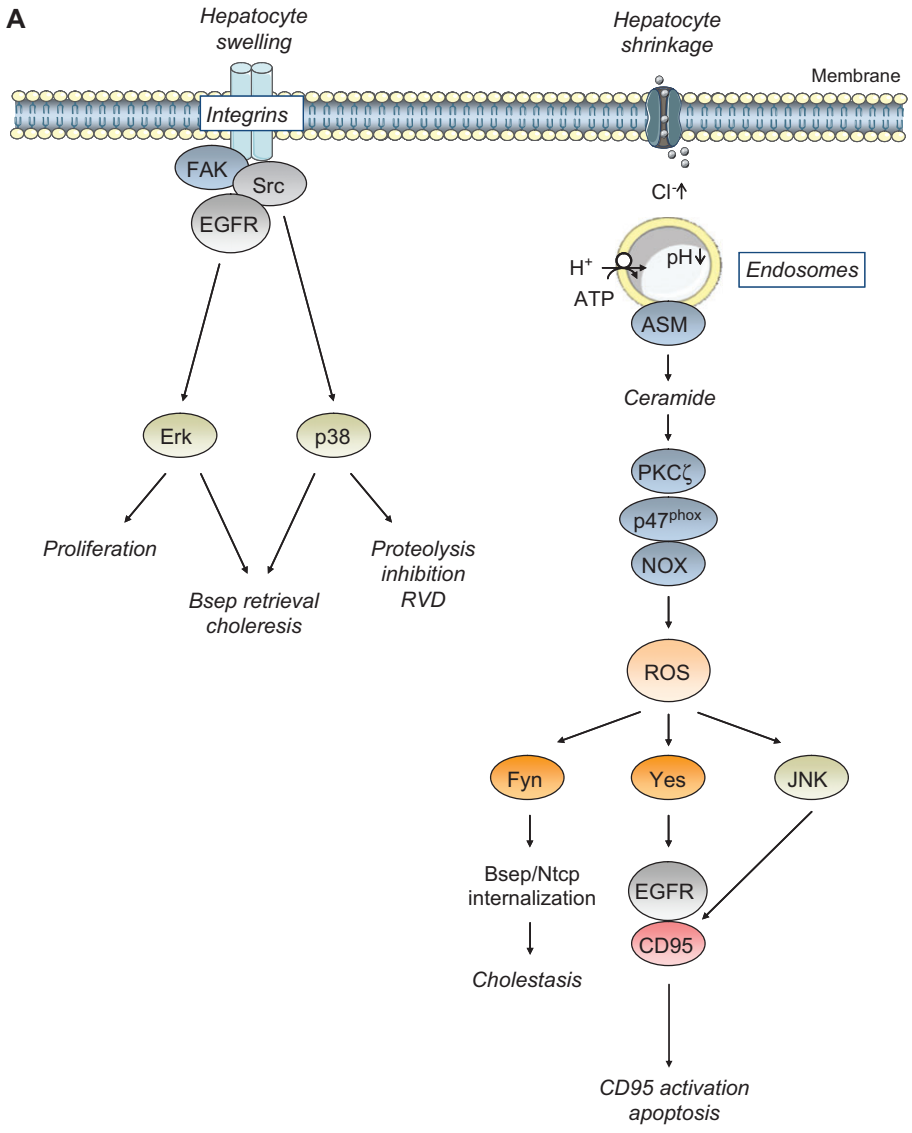
The Src family kinases are not only activated by changes in the ambient osmolarity but are also involved in cell volume regulatory responses such as regulatory volume decrease (RVD) or increase (RVI) [reviewed in (4, 24)]. In RVD, Src family kinases were reported to regulate large conductance channels [activation of BK_{Ca}; (25)], voltage-dependent potassium channels [both activation and inhibition were described; (26, 27)], and swelling-activated anion channels [(20), reviewed in (4)]. In addition, the Src family kinase-dependent activation of the electroneutral K^+-Cl^- symport in hippocampal neurons (28) and the rapid efflux of organic solutes, such as amino acids, sugars, methylamines, and polyols, *via* the volume-sensitive organic osmolyte-anion channel (29) were discussed to contribute to RVD. In the perfused rat, liver swelling-induced activation of $p38^{MAPK}$ is involved in RVD, whereas $p38^{MAPK}$ is apparently not involved in the net K^+ release induced by oxidative stress (30). The Src family kinase inhibitor PP-2 and the integrin-blocking RGD peptide inhibited hypoosmotic $p38^{MAPK}$ activation and blunted RVD, indicating that integrins and Src are upstream events in the osmo-signaling toward RVD (31).

Meanwhile, the role of Src family kinases in RVI is less clear. Although the hyperosmotic activation of the Na^+/H^+ antiporter Na^+/H^+ exchanger (NHE-1) is associated with Hck and Fgr activation in human polymorphonuclear leukocytes (22), inhibition of these kinases failed to impair NHE-1 stimulation in response to hyperosmolarity (32). In a model of apoptotic cell shrinkage induced by a CD95 (CD95 receptor, Fas, APO-1) agonistic antibody, the inhibition of NHE-3 required Lck activation in NIH fibroblasts (33). In addition, the inhibition of Src family kinases by PP-1, which is a rather unspecific Src family tyrosine kinase inhibitor also inhibiting c-Kit and Bcr-Abl (34), was reported to block the $Na^+/K^+/2Cl^-$ 1 cotransporter in calyculin A-stimulated erythrocytes (35). To further strengthen the role of Src family kinases in RVI, it should be mentioned that Swiss 3T3 cells genetically deficient for c-Src are more prone to hyperosmolarity-induced apoptotic cell death (36).

Src family kinases and regulation of liver cell function by ambient osmolarity

In rat liver, the role of different Src kinases, i.e., c-Src, Yes, and Fyn, in the cell volume-dependent regulation of liver function was studied extensively. Here, the regulation of liver cell function by changes in hepatocyte volume, which reflect on short-term time scale changes in hepatocyte hydration, requires structures, which pick up changes in liver cell volume (osmo-sensing) and which further signal this information toward effector sites (osmo-signaling). Our own studies identified the integrin system as a major osmosensor in hepatocytes (18, 31, 37). Integrins are a family of extracellular matrix (ECM) adhesion molecules involved in 'mechanotransduction' and growth factor signaling (38–40). In the liver, $\alpha_1\beta_1$, $\alpha_5\beta_1$, and $\alpha_9\beta_1$ integrins are of major importance (41–43). As shown recently, hypoosmotic swelling of hepatocytes induces a rapid activation of the β_1 integrin subunit in the plasma membrane (18). Also, hepatocyte cell swelling induced by insulin activates the β_1 integrins in the plasma membrane (37). Interestingly, the choleretic and anti-apoptotic bile acid tauroursodeoxycholate (TUDC) and urea can induce direct β_1 integrin activation in hepatocytes without triggering hepatocyte swelling (44, 45). The downstream consequence of swelling-, TUDC-, or urea-induced integrin activation is the activation of the FAK, c-Src, EGFR, and the mitogen-activated protein kinases (MAPKs) extracellular regulated kinase 1 and 2 (Erk-1/-2) and p38 mitogen-activated kinase ($p38^{MAPK}$) (18, 31, 44). Although hypoosmolarity activates c-Src in the liver, no activating phosphorylation of Yes or Fyn is observed (Reissmann and Häussinger, unpublished result).

Figure 2A summarizes the osmo-sensing and osmo-signaling pathways in response to hypoosmotic and hyperosmotic exposure of hepatocytes.


A
Hepatocyte swelling
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Integrins
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Src
EGFR
Cl-↑
H+
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Endosomes
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PKCζ
p47phox
NOX
ROS
Fyn
Yes
JNK
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Cholestasis
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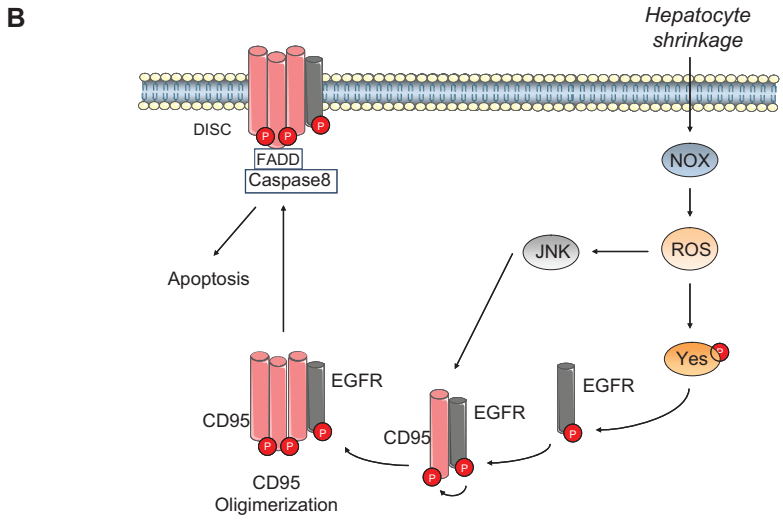
B
Hepatocyte shrinkage
DISC
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JNK
Apoptosis
Yes
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Figure 2 Hypoosmotic or hyperosmotic Src family kinase activation.
(A) The Src family kinases are differentially activated by anisoosmolarity in liver parenchymal cells and have different functions in osmo-signaling. Hepatocyte swelling triggers the activation of integrins, FAK, c-Src, and EGFR. Downstream consequences are the inhibition of autophagic proteolysis, RVD *via* $p38^{MAPK}$, proliferation *via* activation of Erk-1/-2, and stimulation of biliary excretion *via* dual MAPK activation. A similar pathway is activated by insulin and tauroursodesoxycholate (TUDC). Hyperosmotic hepatocyte shrinkage produces an almost instantaneous oxidative stress response that triggers the activation of JNK and the Src family kinases Fyn and Yes. Fyn activation leads to cholestasis and activation of Yes triggers CD95 activation. (B) EGFR becomes activated by a Yes-catalyzed tyrosine phosphorylation at Y^{845}, followed by an activating autophosphorylation of Y^{1173}. The activated EGFR thereafter associates with the CD95, which is strongly dependent on the JNK signal, and catalyzes tyrosine phosphorylation of CD95. CD95-tyrosine phosphorylation is required for CD95 oligomerization and the EGFR/CD95 complex translocates to the plasma membrane, where formation of the DISC, i.e., recruitment of Fas-associated death domain protein and caspase 8 occurs.

Src family kinases and bile formation

Bile formation is strongly regulated by changes in cell hydration in a way that hepatocyte swelling increases the canalicular secretion of bile acids, whereas hepatocyte shrinkage decreases it [reviewed in (2, 46, 47)]. A 10% increase in the hepatocyte's water content doubles the capacity of bile acid excretion into bile (48, 49). This is due to a rapid insertion of canalicular transporter molecules, such as the bile salt export pump (Bsep) (50) and the multidrug resistance-related protein (Mrp) 2 (51) into the canalicular membrane. These transporters are stored in subapical vesicles, which can be recruited to the canalicular membrane in response to hepatocyte swelling, whereas hepatocyte shrinkage is cholestatic due to transporter retrieval from the canalicular membrane (21, 50, 51). The Src family kinases play an important role in these processes: cell swelling-dependent c-Src activation is involved in osmo-signaling toward transporter insertion into the canalicular membrane and choleresis (18), whereas hyperosmotic Fyn activation contributes to transporter retrieval and cholestasis (21).

Osmo-dependent regulation of bile formation requires mechanisms of osmo-sensing and osmo-signaling. First, $\alpha_5\beta_1$integrins have been identified as a hepatocyte swelling-activated osmo-sensor that triggers downstream activation of osmo-signaling elements, such as FAK, c-Src, EGFR, Erk-1/-2, and $p38^{MAPK}$ [reviewed in (2, 47)]. Upon hepatocyte swelling, an RGD peptide-sensitive activation of the β_1-integrin subunit in the plasma membrane takes place, suggestive of an $\alpha_5\beta_1$ integrin/ECM interaction (18) corresponding to mechanotransduction (39). Here, the inhibitory RGD peptides prevent integrin binding to the RGD attachment sites of ECM proteins, such as fibronectin, and thereby impair the dynamics of integrin/matrix interactions, which are critical for an effective mechanotransduction (52). Recent data revealed an increase in the number of focal contacts upon hypoosmotic swelling of hepatocytes (53), suggestive of a swelling-induced increase in $\alpha_5\beta_1$ integrin/ECM protein interactions and subsequent β_1 integrin subunit activation. The role of $\alpha_5\beta_1$ integrins as osmo-sensors is underlined by the fact that integrin-inhibitory peptides containing an RGD motif abolish osmo-signaling toward c-Src and MAPKs as well as the stimulation of bile formation (18). Swelling-induced and $\alpha_5\beta_1$ integrin-dependent c-Src activation is mediated by FAK, which triggers c-Src-Y^{416} phosphorylation (18), thereby activating its kinase domain [reviewed in (5)]. Downstream dual activation of both Erks and $p38^{MAPK}$ is required for the swelling-induced choleretic effect, which is abolished in presence of pharmacological inhibitors of the integrin system (RGD peptide), c-Src (PP-2), or one of the two MAPKs (PD 98059 or SB203580, respectively) (18, 49, 54).

It is interesting to note that the bile acid TUDC also activates integrin-dependent osmo-sensing and osmo-signaling pathways in a cell volume-independent way (18) by a direct interaction of the bile acid with the binding pocket of the $\alpha_5\beta_1$ integrin (45). This may explain the integrin- and c-Src-dependent choleretic action of this bile acid (18). Likewise, urea and some of its derivatives can activate the β_1 integrin subunit in a volume-independent way and thereby partially mimic a 'swollen hepatocyte response' (55), which also involves c-Src activation (21).

Early endosomes were identified as chloride-governed osmo-sensors that are activated in response to hyperosmotic hepatocyte shrinkage (56). Here, the acidification of early endosomes is brought about by vacuolar-type H^+-ATPase, which is directly activated by chloride (57, 58) and requires an anion conductance to maintain electroneutrality during electrogenic proton pumping into the vesicular interior [for a review, see (59)]. Studies with endocytosed FITC-dextran showed an osmo-sensitivity of acidification in the endosomal (pH around 6), but not in the lysosomal (pH~5) compartment (60). Only in these presumably endosomal vesicles do hypoosmotic hepatocyte swelling increased, whereas hyperosmotic hepatocyte shrinkage lowered the apparent pH_{ves} (60, 61). DIDS and bafilomycin largely abolished the hyperosmotic vesicular acidification, suggesting that hyperosmolarity

interferes with the activity of H^+-ATPase in a chloride-dependent way (56). Hyperosmotic hepatocyte shrinkage increases the cytosolic Cl^- concentration (62) due to the osmotic water shift out of the hepatocyte and due to the ionic mechanisms of RVI. The latter involve a hyperosmotic activation of Na^+/H^+ exchange and Na^+/K^+-ATPase together with HCO_3^-/Cl^--exchange, resulting in net accumulation of Na^+, K^+, and Cl^- in rat hepatocytes (63). Evidence has been presented for a direct activation of the vesicular proton pump by Cl^- (58), independent of the role of chloride channels in shunting the endosomal membrane potential, which is generated by the proton pump. A hyperosmolarity-induced increase of the cytosolic chloride concentration may thus indirectly augment vesicular acidification, whereas a decrease in cytosolic chloride could explain the vesicular alkalinization, which is observed in response to cell swelling (64). Regardless, the mechanisms underlying the osmo-sensitivity of endosomal pH, modulation of endosomal pH must be seen as one site of hepatocellular osmo-sensing through the modulation of acidic sphingomyelinase (ASM) activity and subsequent ceramide formation, which have been described as a downstream consequence of endosomal acidification in this experimental setting (56).

The role of ASM for the hyperosmolarity-induced ceramide formation in hepatocytes has been studied in detail. Cell fractionation studies on hepatocytes revealed the presence of ASM not only in the plasma membrane and the cytosolic fraction but also in an endosomal compartment, which sediments at 100,000 g, and pharmacological inhibition of ASM as well as ASM protein knockdown using an siRNA approach largely abolished the otherwise observed hyperosmolarity-induced ceramide formation, suggestive of an ASM-driven ceramide formation in an early endosomal compartment (56). Ceramide can directly activate protein kinase Cζ (PKCζ) (65–67), which is also observed in response to hyperosmotic stimulation of hepatocytes (56). PKCζ then phosphorylates the regulatory NADPH oxidase (NOX) (68) subunit $p47^{phox}$ on serine residues resulting in NOX activation (69, 70). As a consequence, hyperosmotic hepatocyte shrinkage produces oxidative stress (71–73), which triggers the hyperosmotic retrieval of Mrp2 and Bsep from the canalicular membrane (50, 51, 74). In line with the findings that oxidative stress contributes to the cholestatic state after hyperosmotic hepatocyte shrinkage is the observation that hydrophobic bile acids not only induce cholestasis but also oxidative stress *via* NOX activation (75, 76). This hyperosmolarity-induced and NOX-mediated reactive oxygen species (ROS) formation leads to an activation of the Src family kinases Yes and Fyn (10) and of c-Jun N-terminal kinase (JNK) (73), whereas c-Src is not activated by hyperosmolarity (10). It was shown in the perfused rat liver that the hyperosmotic retrieval of Bsep and Mrp2 from the canalicular membrane is sensitive to the inhibition of Fyn, whereas Yes and JNK are not involved in hyperosmolarity-induced changes in bile acid transporter localization (21). In line with this, Fyn knockdown using an siRNA approach in rat hepatocyte couplets as well as pharmacological inhibition of Fyn largely abolished the hyperosmolarity-induced cholestasis (21). It was suggested that cortactin may act as a target of hyperosmolarity-induced Fyn phosphorylation in rat hepatocytes (21), as also found in fibroblasts (32). The phosphorylation of cortactin favors its dissociation from the actin cytoskeleton (21, 77). The phosphorylated cortactin exhibits a diminished F-actin cross-linking activity (77) and a cytoskeletal F-actin disarrangement, which accompanied the oxidative stress-mediated Bsep internalization in rat hepatocyte couplets (78). Therefore, one might speculate that hyperosmotic Fyn activation interferes with the actin cytoskeleton in a manner that favors transporter retrieval from the canalicular membrane (21, 79).

Src family kinases and apoptosis

Src family kinases also play a pivotal role in the regulation of apoptotic cell death [for a review, see (80)] and distinct roles for the Src family kinase Yes in hepatocyte and c-Src in hepatic stellate cell (HSC) apoptosis have been identified within the last years [for a review, see (81)].

In general, apoptosis is characterized by cell shrinkage, nuclear condensation, DNA fragmentation, and apoptotic body formation. These features distinguish apoptosis from other types of cell death, such as necrosis (82). Although some signs of apoptosis, such as externalization of phosphatidylserine, altered mitochondrial function, or activation of caspases, are cell type- and death signal-dependent, apoptotic cell volume decrease (AVD) is an early and ubiquitous event (33, 83, 84), and therefore, cell volume-mediated effects on Src family kinase activation may come into play. The contribution of AVD to apoptotic signal transduction is not yet clear, but hyperosmolarity can mimic AVD and thereby stimulate apoptosis (see below). This, however, is cell type-specific and depends on the efficacy of RVI mechanisms (73, 83, 85). In rat hepatocytes, hyperosmotic cell shrinkage triggers ligand-independent and Src family kinase Yes-dependent CD95 activation and subsequent CD95 trafficking to the plasma membrane, thereby sensitizing hepatocytes toward CD95 ligand (CD95L)-induced apoptosis or even

executing apoptosis when the hyperosmotic challenge is strong enough (10, 73, 86). In contrast, in HSCs, proapoptotic stimuli such as CD95L lead to a c-Src-mediated EGFR activation and subsequent cell proliferation (94).

Although under normo-osmotic control conditions, CD95 is localized inside the hepatocyte and exhibits almost no detectable membrane localization, this death receptor is targeted within 1–2 h to the plasma membrane in response to proapoptotic stimuli, such as CD95L, hyperosmolarity, or hydrophobic bile acids (54, 73, 86–88). All these different challenges trigger the activation of the Src family kinase Yes in liver parenchymal cells (10, 54, 89). The detailed mechanisms of hyperosmolarity-induced ROS formation have been discussed in detail above (see also Figure 2A) and resemble those triggered by CD95L (89) or hydrophobic bile acids (75, 76). Although mitochondria or the endoplasmic reticulum were discussed as potential sources of oxygen radical formation as a consequence of hepatocyte shrinkage (90), it is now clear that the rapid ROS generation in response to hyperosmotic hepatocyte shrinkage is largely mediated by NOX isoforms (86). Hyperosmolarity-induced ROS generation was identified as an important upstream event for activation of both the Src family kinase Yes and of JNK (56, 73). Here, the ROS-induced Yes activation was suggested to be mediated by an inhibition of a phosphatase (54). The activated Yes associates with the EGFR, and the latter becomes tyrosine-phosphorylated on position Y^{845} within the tyrosine kinase domain, followed by autophosphorylation at Y^{1173} and EGFR activation. Although Yes activates the EGFR, the JNK signal mediates the association of EGFR with CD95, as shown by co-immunoprecipitation and fluorescence resonance energy transfer studies (54, 73, 86–88). CD95 then becomes tyrosine-phosphorylated at tyrosine residues Y^{232} and Y^{291} by the EGFR tyrosine kinase activity (88), which is a signal for a microtubule-dependent targeting of the EGFR/CD95 complex to the plasma membrane (73, 88), where the formation of the death-inducing signaling complex (DISC) occurs including subsequent activation of caspase 8 (73, 91) (see also Figure 2B).

Quiescent HSCs, i.e., cells that have been in culture for 24–48 h only and do not express detectable amounts of α-smooth muscle actin, represent a hepatic stem/progenitor cell compartment in their niche (92, 93) and are fairly resistant toward apoptotic cell death (94, 95). In quiescent HSC, CD95L also induces EGFR tyrosine phosphorylation and the activation of the receptor tyrosine kinase activity, but in contrast to hepatocytes, CD95L induces an EGF-dependent EGFR activation (94). This is due to a c-Src-dependent activation of EGF shedding by matrix metalloproteinase 9 (MMP9), leading to ligand-dependent EGFR activation (94). CD95L-induced EGFR activation then couples to an Erk-mediated HSC proliferation, as indicated by an increase in bromodeoxyuridine (BrdU) incorporation and in cell number. In addition to CD95L-induced EGFR activation and stimulation of cell proliferation, CD95L also triggers apoptosis resistance through CD95 tyrosine nitration in quiescent HSC (94). This may be important in a hostile cytokine milieu during liver injury, which allows quiescent HSC to fulfill their role as resident liver stem cells (92, 93). In contrast to hepatocytes, CD95L failed to induce a sustained JNK activation in quiescent HSC (94, 95), which is a prerequisite for CD95/EGFR association and apoptosis induction (73). In line with the proliferative and anti-apoptotic action of CD95 ligand in HSC, which reflect stem cells, CD95 engagement was shown to accelerate liver regeneration after partial hepatectomy (96). Apart from HSC, hepatocytes may also contribute to liver regeneration in a CD95-dependent fashion because liver regeneration after partial hepatectomy is impaired after hepatocyte-specific CD95 knockout (97). In activated HSC, i.e., cells cultured for 7–14 days with transformation into a myofibroblast phenotype, CD95L-induced CD95 tyrosine nitration no longer occurs, whereas CD95L still triggers EGFR activation *via* the c-Src/MMP9/EGF/EGFR pathways, similar to the situation found in quiescent HSC (94). This results in cell proliferation, which, however, can be switched to apoptosis upon co-administration of cycloheximide (CHX). Here, CHX triggers sustained JNK activation, which is required for CD95/EGFR association, subsequent EGFR-dependent CD95 tyrosine phosphorylation, membrane translocation and DISC formation, and finally CD95-mediated HSC apoptosis (94). Thus, depending on the HSC activation state (quiescent vs. activated) and the underlying signaling context (absence vs. presence of a sustained JNK signal), CD95L-mediated EGFR activation can couple to both HSC proliferation and apoptotic cell death (Figure 3).

Similar to findings in liver parenchymal cells (54), hydrophobic bile acids were shown to induce an antioxidant-sensitive EGFR tyrosine phosphorylation in multiple-passaged and thereby culture-activated HSC (98). In contrast to hepatocytes, in quiescent HSC, bile acid-induced EGFR activation is coupled to cell proliferation (104) (see also Figure 3). The underlying mechanisms were recently studied in primary quiescent HSC cultures and involve an ASM-dependent and NOX-driven ROS formation, which finally leads to a Yes-mediated EGFR transactivation (99). However, as reported for CD95L (94), hydrophobic bile acids also fail to induce a sustained JNK activation in quiescent HSC, and therefore, CD95/EGFR association does not occur upon bile acid administration in those cells. However, when CHX or hydrogen peroxide (H_2O_2), i.e., well-known inducers of

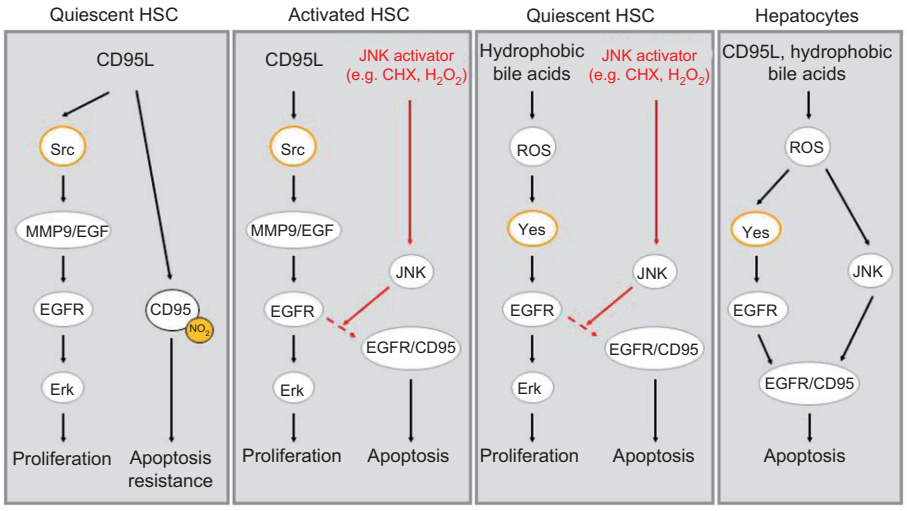

Figure 3 Signaling of Src family kinases toward proliferation and apoptosis in HSCs and hepatocytes.
Hydrophobic bile acids and CD95L are potent inducers of apoptosis by triggering a Yes-dependent activation of the EGFR and subsequent activation of CD95 in liver parenchymal cells (hepatocytes). In quiescent HSC, CD95L does not induce apoptotic cell death but stimulates HSC proliferation *via* a c-Src-dependent shedding of EGF and ligand-dependent activation of the EGFR. Simultaneously, CD95L triggers an inactivating CD95 tyrosine nitration. Like in quiescent HSC, CD95L induces a c-Src- and ligand-dependent EGFR activation, but no inactivating CD95-tyrosine nitration occurs in activated HSC. As a result, proliferation is stimulated unless a JNK signal comes into play, which triggers CD95/EGFR association and apoptosis induction. Hydrophobic bile acids trigger a rapid formation of ROS and Yes-dependent EGFR activation in quiescent HSC, which leads to proliferation. In contrast to hepatocytes, hydrophobic bile acids and CD95L do not induce a JNK signal in HSC. However, when JNK-activation is induced by the co-administration of either CHX or H_2O_2, the mitogenic signal is shifted to an apoptotic one.

a sustained JNK signal, were administered together with hydrophobic bile acids, CD95/EGFR association, EGFR-dependent CD95 tyrosine phosphorylation, membrane translocation, DISC formation, and apoptotic cell death occurred (99). In contrast to CD95L, no CD95 tyrosine nitration was induced by bile acids in quiescent HSC. Therefore, depending on the signaling context, i.e., presence of a transient or a sustained JNK signal, respectively, Yes-mediated EGFR activation by hydrophobic bile acids in HSC can couple to both cell proliferation and apoptosis. In activated HSC, bile acid-induced EGFR activation is also coupled to proliferation (98, 99) and follows the same activation mechanism as described in liver parenchymal cells (54) and quiescent HSC (99). These include an ASM-dependent and NOX-driven ROS formation, which finally leads to a Yes-mediated EGFR transactivation (99). However, hydrophobic bile acids fail to induce a sustained JNK activation in activated HSC, and therefore CD95/EGFR association does not occur upon bile acid administration in those cells. When, however, a sustained JNK signal comes into play, CD95/EGFR association, EGFR-dependent CD95 tyrosine phosphorylation, membrane translocation, DISC formation, and apoptotic cell death together induced by hydrophobic bile acids occurred (99).

Src family kinases and proliferation

In contrast to the hyperosmotic activation of apoptotic cell death pathways, cell swelling is a prerequisite for cell proliferation. Hypoosmolarity-induced hepatocyte swelling involves an $\alpha_5\beta_1$ integrin-mediated c-Src activation as discussed above, which triggers EGFR phosphorylation, subsequent Erk-1/-2 activation and proliferation (18, 100).

Swelling-induced activation of Erk-1/-2 is largely, but not completely inhibited by AG1478, an inhibitor of the EGFR tyrosine kinase activity (100). The remaining activation of Erk-1/-2 in the presence of this inhibitor may be due to a c-Src-mediated Y^{925} phosphorylation of FAK, which may trigger the Grb2/Ras/Raf/MEK/Erk pathway (101).

Also, cell swelling induced by insulin triggers an $\alpha_5\beta_1$ integrin-dependent activation of FAK, c-Src, Erks, and $p38^{MAPK}$, the latter of which mediates the anti-proteolytic effect of insulin (37). Apart from its metabolic effects, insulin stimulates proliferation in the liver and other organs (102–104). Much effort has been devoted to the understanding of insulin signaling and its complexity (105–107), which involves tyrosine phosphorylation of the insulin receptor substrate 1 and activation of a variety of protein kinases such as MAPKs Erk-1/-2 and $p38^{MAPK}$ (108,

109). In rat liver, insulin stimulates Na^+/H^+ antiport and $K^+/Na^+/2Cl^-$ cotransport, thereby inducing hepatocyte swelling (110). Recent data (100) point to an important contribution of insulin-induced cell swelling to the known proliferative effects exerted by the hormone (102–104). Both insulin and EGF increase DNA synthesis in hepatocytes kept under serum-free conditions (111, 112), but it remained unclear whether there is convergence of insulin- and EGF-derived signals. As shown in perfused rat liver and primary rat hepatocytes, insulin leads to an activation of the EGFR, which is triggered by insulin-induced hepatocyte swelling in a c-Src-dependent manner (100). EGFR activation by insulin was sensitive to bumetanide (which prevents insulin-induced hepatocyte swelling) and to inhibition by an integrin-antagonistic RGD peptide, an integrin β_1 subunit-blocking antibody, and the Src family kinase inhibitor PP-2. These findings point to an important role of the recently described swelling-induced and integrin-dependent osmo-sensing/osmo-signaling pathway in insulin signaling [detailed description see above (18, 37)]. Both insulin and hypoosmolarity induced a significant increase in BrdU uptake in primary rat hepatocytes suggestive of hepatocyte proliferation, which was again sensitive to inhibition by a RGD motif containing hexapeptide, an integrin β_1-blocking antibody, the Src kinase inhibitor PP-2, AG1478, and PD 98059, suggestive of an involvement of $\alpha_5\beta_1$-integrins, c-Src, EGFR, and Erk-1/-2 (18).

As EGFR activation participates in both cell swelling-/c-Src-dependent proliferation and cell shrinkage-/Yes-induced apoptosis, the question of which mechanisms provide the switch between EGFR-based proliferation and EGFR-dependent apoptosis arises. Apart from a differential activation of Src family kinases by either cell swelling or shrinkage, JNKs are a strong candidate because JNKs provide the signal for CD95/EGFR association and subsequent EGFR-mediated CD95 tyrosine phosphorylation, which activates the CD95 death machinery (73). Recent data suggest that insulin-induced swelling-/c-Src-dependent EGFR activation can be converted into a CD95-mediated death signal upon the addition of free fatty acids, which trigger a sustained JNK activation (Reinehr, Sommerfeld, and Häussinger, unpublished data).

c-Src activates mitogenic signaling in normal and malignant cells, thereby triggering cell proliferation, survival, metastasis, and angiogenesis (1, 113). c-Src can participate in cell cycle regulation by phosphorylating the cyclin-dependent kinase inhibitory protein p27 on tyrosines 74 and 88 in human breast cancer, thereby reducing the inhibitory action of p27 (114). In addition, the activation of the phosphatidylinositol 3-kinase (PI3 kinase)/phosphatase and tensin homolog (PTEN)/Akt pathway can play a role in the development of many cancers, including human hepatocellular carcinoma (115, 116). Src family kinases are involved in the regulation of this pathway through mechanisms, such as phosphorylation of PI3 kinase and tyrosine phosphorylation of PTEN that results in inhibition of PTEN (117–124). As PTEN-deficient mice exhibit liver tumors (125) and steatohepatitis (116), c-Src may also be involved in hepatocarcinogenesis.

Concluding remarks

The ubiquitously expressed Src family kinases c-Src, Yes, and Fyn play distinct roles in the regulation of various liver functions such as bile flow, proteolysis, apoptosis, and proliferation and are extensively regulated by hepatic cell volume and bile acids.

Cell swelling by either hypoosmolarity or insulin leads to an $\alpha_5\beta_1$ integrin-sensed and FAK-mediated activation of c-Src, which then leads to a dual MAPK activation of Erks and $p38^{MAPK}$. The functional consequence of this pattern of kinase activation is an increase in bile flow, inhibition of proteolysis, and induction of cell proliferation. It is interesting to note that swelling-independent $\alpha_5\beta_1$ integrin activation by either the anti-apoptotic bile acid TUDC or urea can mimic these processes at least in part.

Cell shrinkage by hyperosmolarity, pro-apoptotic bile acids, or CD95 ligand leads to an acidification of early endosomes, which in turn activates ASM-driven ceramide formation, subsequent PKCζ-mediated NOX activation, and ROS generation. This provides a signal for activation of JNK and the Src family kinases Yes and Fyn. Although Fyn mediates hyperosmotic cholestasis, Yes triggers EGFR-dependent CD95 activation and subsequent apoptosis. It is not yet clear to what extent the different Src family kinases can substitute each other and what mechanisms are governing the specific involvement of individual Src family members under different functional states of the cell. We hope that this review may stimulate further research in this interesting area.

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Prof. Dr. Roland Reinehr studied medicine at the Heinrich-Heine-University Düsseldorf (Germany), University Hospital Bern (Switzerland), and Mount Sinai School of Medicine, City University of New York (USA). He obtained his MD degree and finished his MD doctoral thesis in 2001. From 2001 to 2012, he underwent clinical and scientific training at the Clinic for Gastroenterology, Hepatology and Infectious Diseases at the University Hospital Düsseldorf under the supervision of Prof. Dr. D. Häussinger. He is a member of the Collaborative Research Center SFB 575 and 974. In 2006, he joined the habilitation at the Heinrich-Heine-University Düsseldorf. From 2009 to 2012, he joined the Junges Kolleg of the North Rhine-Westphalia Academy for Science and Arts. In 2011, he became an adjunct professor in internal medicine at the Heinrich-Heine-University Düsseldorf.

Dr. rer nat. Annika Sommerfeld received her degree in biology in 2004 at the Ruhr University Bochum. From 2005 to 2009, she studied for her PhD in the Integrated Research Training Group of the Collaborative Research Center SFB 575 (Experimental Hepatology) at the University of Düsseldorf, obtaining her PhD in 2009. Since 2011,

she is doing postdoctoral work at the Collaborative Research Center SFB 974 Communication and System Relevance in Liver Injury and Regeneration).

Prof. Dr. med. Dieter Häussinger was born on June 22, 1951, in Nördlingen/Bavaria. Since 1994, he is a full professor in internal medicine at the Heinrich-Heine-University Düsseldorf and the director of the Clinic for Gastroenterology, Hepatology and Infectious Diseases and the Center for Liver and Infectious Disease. He is the coordinator of the Colloborative Research Centers SFB 575 (Experimental Hepatology) and SFB 974 (Liver Injury and Regeneration) and the Clinical Research Group Klinische Forschergruppe 217 (Hepatobiliary Transport). He is also a member of the National Academy of Sciences Leopoldina and the North Rhine-Westphalia Academy for Science and Arts. In 2005, he became the founding president of the International Society of Hepatic Encephalopathy and Nitrogen Metabolism (ISHEN). He is also a senator of the Scientific Society Leibniz (WGL). He won the Thannhauser-Prize in 1989, the Gottfried-Wilhelm-Leibniz-Prize in 1991, the Dr. Robert Pfleger Prize in 2002, and the Order of Merit of the German Federal Republic in 2011.