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Review Article

Mitochondria at the Crossroads of Cholestatic Liver Injury: Targeting Novel Therapeutic Avenues

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Abstract

Bile acids are byproducts of cholesterol metabolism in the liver and constitute the primary components of bile. Disruption of bile flow leads to cholestasis, characterized by the accumulation of hydrophobic bile acids in the liver and bloodstream. Such accumulation can exacerbate liver impairment. This review discussed recent developments in understanding how bile acids contribute to liver damage, including disturbances in mitochondrial function, endoplasmic reticulum stress, inflammation, and autophagy dysfunction. Mitochondria play a pivotal role in cholestatic liver injury by influencing hepatocyte apoptosis and inflammation. Recent findings linking bile acids to liver damage highlight new potential treatment targets for cholestatic liver injury.

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Introduction

Bile acids (BAs) are signaling molecules generated from cholesterol in the liver and subsequently modified by the gut microbiome in the intestines.[1,2] Synthesis of BAs in the human body involves both classical and nonclassical pathways.[3] In the classical pathway, primarily involving the liver, cholesterol 7-hydroxylase acts as the key rate-limiting enzyme, facilitating the production of two hydrophobic primary BAs: cholic acid and chenodeoxycholic acid (CDCA). The nonclassical pathway, which involves various tissues and macrophages, is initiated by sterol 27A-hydroxylase in mitochondria and steroid 7-hydroxylase in the endoplasmic reticulum (ER).[4,5] This pathway, as described by Axelson *et al*,[6] primarily becomes prominent in certain pathological conditions. When cholesterol 7-hydroxylase activity in the liver decreases, the nonclassical pathway plays a regulatory role *in vivo*, influencing metabolic equilibrium through the production of CDCA.

Cholestasis is a multifactorial condition characterized by stagnation of bile flow and accumulation of potentially harmful BAs in the liver and bloodstream.[7] The etiological factors of cholestasis include viral infections, inflammation, and autoimmune diseases.[8–10] Although researchers have extensively investigated the causes of cholestasis, the specific molecular pathways through which accumulated toxic BAs induce liver damage remain incompletely understood.[11,12] Earlier theories indicated that elevated hepatic levels of BAs led to hepatocyte death through direct cytolytic effects.[13,14] However, these studies exposed cultured human hepatocytes to submillimolar levels of toxic secondary BAs, concentrations rarely observed in patient serum.[12] The studies reported that BAs contribute to cholestatic liver injury through alternative mechanisms. Subsequent experiments implicated mitochondrial oxidative stress and ER stress as pivotal contributors to cholestatic liver injury,[15,16] although the specific molecular details remained elusive. As research advanced, novel mechanisms were identified, including the generation of inflammatory signals, infiltration of immune cells, and dysfunction of autophagy. Notably, mitochondria play a critical role in these processes, influencing the release of apoptotic signals and contributing to the production of inflammatory signals.

Mitochondria are complex organelles enclosed in a dual-layer membrane structure comprising the mitochondrial outer membrane (MOM) and mitochondrial inner membrane (MIM). The MOM is fairly permeable because it contains porins such as the voltage-dependent anion channel (VDAC) that enable the passage of small, uncharged molecules and ionic species up to approximately 5 kDa.[17] In contrast, the MIM acts as the primary barrier between the cytosol and the mitochondrial matrix, and it is predominantly impermeable. The MIM curves inward to shape the mitochondrial matrix, a highly intricate environment housing the electron transport chain (ETC), ATP synthase, and human mitochondrial DNA (mtDNA), which comprises 13 structural genes encoding partial proteins of respiratory chain complexes I, II, III, and IV.[18] Additionally, studies have highlighted the importance of the mitochondrial permeability transition pore (mPTP) located on the mitochondrial membrane, comprising adenine nucleotide translocase and F1F0 ATPase, and regulated by VDAC and

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cyclophilin D receptors.[19–21] The mPTP periodically opens under physiological conditions to maintain electrochemical balance within mitochondria. Mitochondria also play a central role in regulating cell death and inflammatory signaling processes.[22,23]

Pathways of hepatocyte apoptosis mediated by BAs

Death receptor-independent pathways

Mitochondria, the primary loci for oxidative phosphorylation within cells, play a pivotal role in the complex landscape of energy metabolism. In this process, NADH and $FADH_2$ act as electron carriers, facilitating the transfer of electrons obtained from the tricarboxylic acid cycle to the ETC. The majority of these electrons traverse the ETC, ultimately combining with oxygen molecules at cytochrome oxidase to form water. However, approximately 2% of electrons from complexes I and III deviate from the ETC.[24] These electrons directly interact with oxygen molecules, generating a superoxide anion, which is then converted to H_2O_2 by manganese MnSOD/SOD-2.[25] Hydrophobic BAs can directly impair ETC complex III, thereby reducing the respiratory control ratio of respiratory state 3. Within the iron-sulfur centers of proteins, superoxide anions can reduce Fe^{3+} to Fe^{2+}, leading to subsequent interaction of H_2O_2 with Fe^{2+}, which can yield ·OH.[26] Furthermore, the superoxide anion can react with N_2O, resulting in the formation of ONOO−. Both ·OH and ONOO− have high reactivity and can therefore exert detrimental effects on mitochondrial membranes, proteins, and mtDNA.

In mitochondria isolated from rat livers,[27] CDCA was found to directly trigger sudden opening of the mPTP. This opening allows unrestricted passage of molecules with a relative molecular mass exceeding 1.5×10^3, disrupting the mitochondrial membrane potential and hindering ATP production, ultimately causing mitochondrial swelling and rupture.[28,29] Due to the substantially larger surface area of the MIM compared to the MOM, swelling of the mitochondrial matrix induced by mPTP opening triggers rupture of the MOM, resulting in the release of cytochrome C (Cyto C) into the cytoplasm. Cyto C initiates the intrinsic pathway of apoptosis.[30] Activation of the cysteine aspartate protease family is a pivotal step in apoptosis induction. This family includes multiple members primarily categorized into two groups: executors, such as caspase-3, which directly degrade intracellular structural and functional proteins to induce apoptosis without undergoing autocatalysis or self-splicing, and promoters, such as caspase-9, which undergo self-splicing activation and subsequently initiate a cascade reaction of caspases without specific intracellular protein degradation.[31] Scientific investigations have confirmed that activation of caspase-9 depends on Cyto C and apoptosis-inducing factors within mitochondria, with the mPTP governing their release.[30] Cyclosporine A (CsA) was identified as the most potent inhibitor regulating mPTP opening.[27] CsA directly binds to cyclophilin D, hindering mPTP opening; this prevents MIM depolarization and caspase-3 activation, exerting a strong inhibitory effect on programmed cell death.[32] Experimental evidence has indicated that pretreatment of mitochondria with CsA significantly inhibits mitochondrial permeability transition.[33] Additionally, BAs have been observed to reduce mitochondrial biogenesis, as evidenced by a notable reduction in the levels and activities of mitochondrial transcription factors in bile duct-ligated (BDL) mice.[34,35]

Death receptor-dependent pathways

Studies have indicated that BAs can trigger apoptosis through death receptors. This process is typically initiated by BAs interacting with death receptors such as tumor necrosis factor receptor superfamily, member 6 (FAS) and TNF ligand superfamily, member 10 (TRAIL). Research has demonstrated that in BDL mice, deficiency in FAS reduces liver fibrosis and apoptosis.[36,37] Hydrophobic BAs facilitate FAS receptor phosphorylation, leading to its translocation to the cell membrane and subsequent activation.[38] Increased density of FAS receptors on the cell membrane renders hepatocytes more susceptible to the FAS receptor-induced cell death pathway.

Additionally, exposure of hepatocytes to glycochenodeoxycholate can induce transcriptional upregulation of TRAILR2 and inactivation of FAS-associated via death domain-like apoptosis regulator through phosphorylation.[39–41] Death receptors oligomerize, leading to the formation of a death-initiating signaling complex comprising FAS-associated via death domain and pro-caspase 8/10.[42] Upon activation of caspase 8, downstream caspases can be directly activated, initiating apoptosis. Simultaneously, BH3 interacting domain death agonist is activated by caspase 8, which then activates B-cell lymphoma-2 (Bcl-2)-associated X protein (BAX) and Bcl-2-antagonist/killer 1 (BAK).[43,44]

The Bcl-2 family proteins BAX and BAK play pivotal roles in the formation of the apoptotic pore, which facilitates mitochondrial permeabilization during apoptosis.[45,46] BAX and BAK are typically inactive, but when activated, conformational rearrangements occur in the MOM, leading to the opening of mitochondrial membrane pores of varying sizes.[47] Evidence indicates that Cyto C can be released when the membrane pore radius is as small as 2 nm.[48] Following such a release, Cyto C triggers the binding of apoptotic protease activator to the precursor caspase-9, activating it. This cascade of events within the caspase system ultimately leads to irreversible death of liver cells (Fig. 1).[49]

ER stress-dependent pathways

The ER is present in all eukaryotic cells and plays various roles, including protein synthesis, secretion, and storage and release of calcium ions (Ca^{2+}). It is essential for the synthesis, processing, folding, assembly, and transportation of new synthetic peptide chains, as well as for the modification of existing proteins.[50] Proteins designated for modification are initially synthesized on the ribosome and then transported to the ER for further processing. When proteins undergo appropriate modification and folding, mature proteins are produced, whereas those unable to fold correctly are identified and degraded. Unfolded proteins accumulate in the ER due to imbalances in protein folding and degradation processes; this phenomenon is referred to as ER stress.[51]

Research has indicated that cholestasis can trigger ER stress.[52–54] In cases of mild to moderate ER stress, liver cells can eliminate unfolded and misfolded proteins and restore ER homeostasis through three pathways mediated by inositol-requiring enzyme 1a (IRE1a), PKR-like ER kinase, and activating transcription factor 6a. However, prolonged cholestasis can result in severe ER stress, overwhelming hyperactivation of the unfolded protein response, and subsequent activation of the intrinsic apoptotic machinery.[55] Although the precise mechanisms governing apoptosis induced by ER stress remain incompletely understood, they may involve sustained release of ER calcium, activation of mitogen-activated protein kinase c-jun N-terminal kinase (JNK), upregulation of proapoptotic Bcl-2 family members, generation of reactive oxygen species (ROS), and induction of the proapoptotic transcription factor C/EBP homologous protein (CHOP).

Studies have suggested that IRE1a plays a role in modulating ER stress-induced apoptosis. Upon activation of IRE1a,

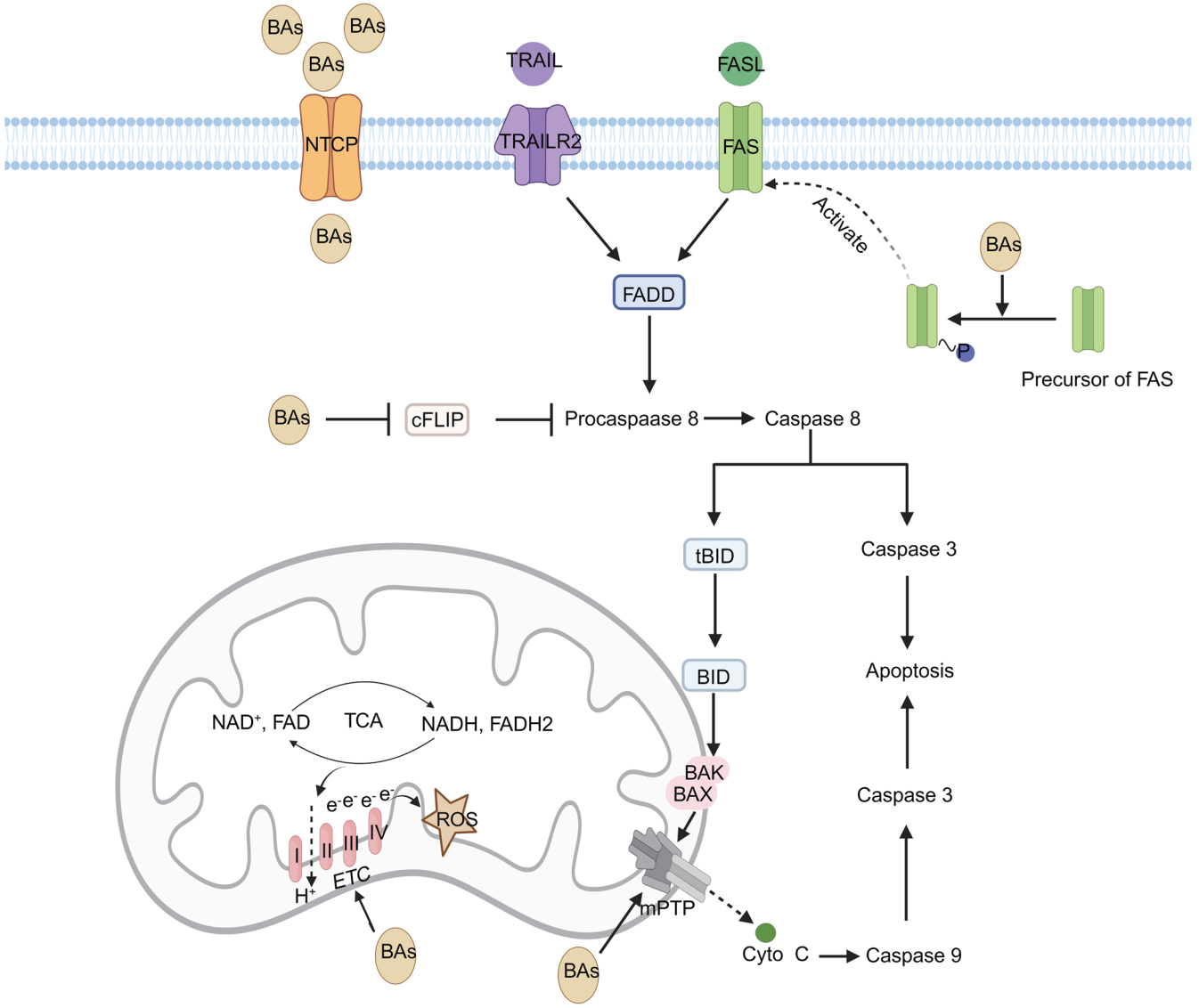

Fig. 1. BAs induce hepatocyte apoptosis by mediating mitochondrial dysfunction through death receptors via independent & dependent pathways. BAs, Bile acid; NTCP, sodium-taurocholate co-transporting polypeptide; FADD, FAS-associated via death domain; BID, BH3 interacting domain death agonist; BAX, Bcl-2-associated X protein; BAK, Bcl-2-antagonist/killer 1; ETC, electron transport chain; mtDNA, mitochondrial DNA; TCA, tricarboxylic acid; mPTP, mitochondrial permeability transition pore; ROS, reactive oxygen species; Cyto C, cytochrome C.

apoptosis signal-regulating kinase 1 and JNK are activated, which recruits adaptor protein tumor necrosis factor receptor-associated factor 2. This activation triggers proapoptotic effects from JNK, including inactivation of antiapoptotic Bcl-2 proteins and phosphorylation-induced activation of BAX and BAK.[56,57] Activation of BAK and BAX leads to mitochondrial permeabilization, resulting in the opening of the mitochondrial membrane and the release of Cyto C and other small apoptotic precursors. The transcription factor CHOP, a well-studied proapoptotic pathway arising from ER stress, is primarily activated by recombinant activating transcription factor 4 and activating transcription factor 6a.[58,59] A study discovered reduced apoptosis in CHOP knockout mouse hepatocytes treated with toxic BAs, indicating that CHOP plays a pivotal role in apoptosis.[60] CHOP is implicated in apoptosis through various mechanisms, including controlling growth arrest and DNA damage-inducible 34 (hereinafter referred to as GADD34) expression. GADD34, upon binding to protein phosphatase 1, induces dephosphorylation of eukaryotic initiation factor 2A, thereby restoring protein translation when ER stress persists and consequently exacerbating ER stress.[61] Another study indicated that CHOP is essential for the apoptotic response to ER stress, as cells isolated from mice deficient in CHOP were found to be resistant to ER stress-induced cell death.[62] CHOP may transcriptionally upregulate expression of the death receptor TRAIL receptor 2,[63] a key receptor for death receptor-mediated mitochondrial dysfunction. Moreover, CHOP could upregulate proapoptotic Bcl-2-interacting mediator of cell death and BID proteins while suppressing the expression of antiapoptotic Bcl-2.[64]

Disturbances in ER calcium levels have been reported to be linked to apoptosis mechanisms. Hydrophobic BAs can trigger sustained release of Ca^{2+} from the ER. This process involves a protein complex consisting of VDAC, 75 kDa glu-

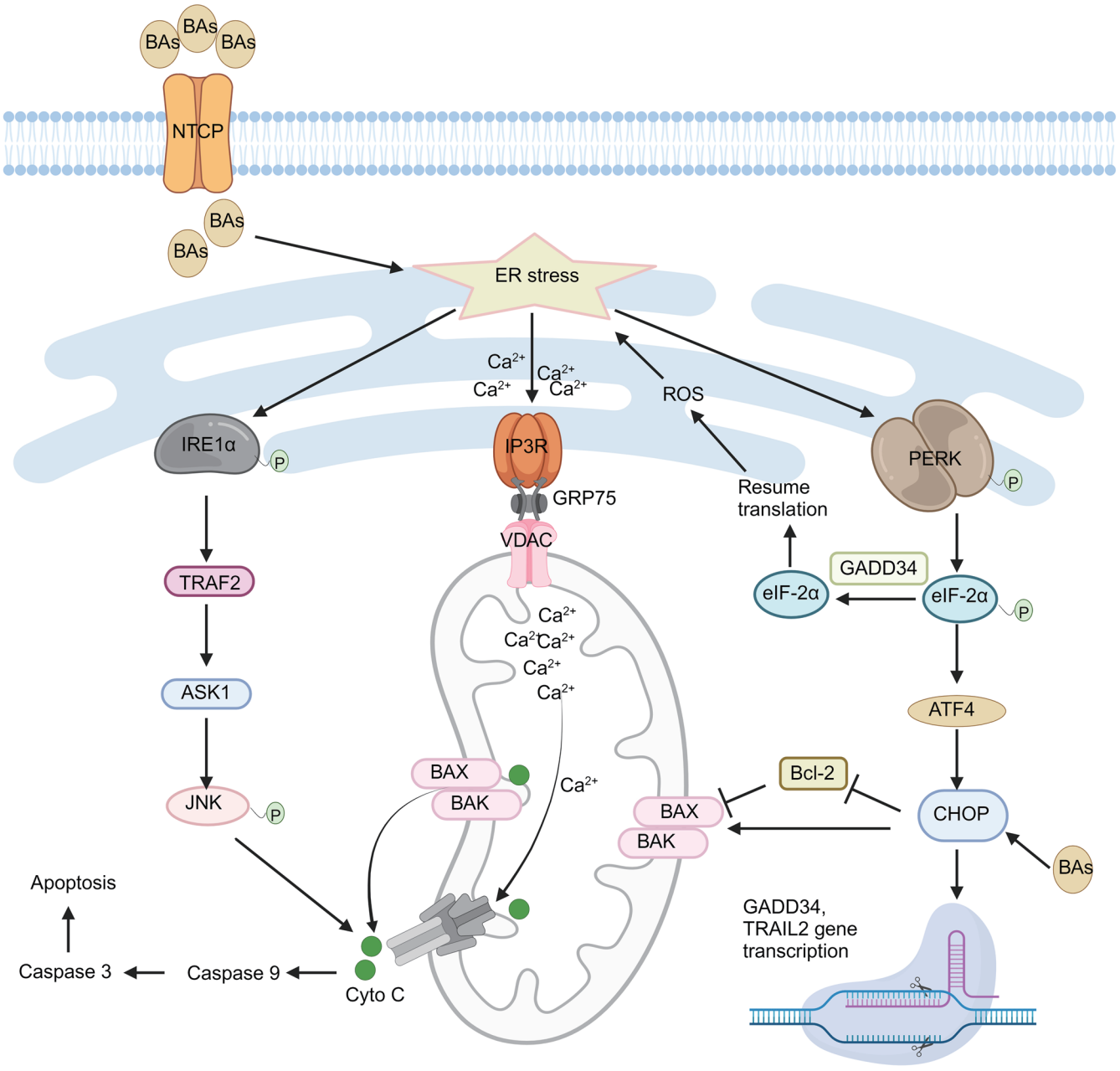

Fig. 2. Pathways leading to apoptosis induced by ER stress are depicted, including ER calcium release, activation of JNK, upregulation of pro-apoptotic BCL-2 family members, ROS production, and the proapoptotic transcription factor CHOP. BAs, Bile acid; ER, endoplasmic reticulum; NTCP, sodium-taurocholate co-transporting polypeptide; ROS, reactive oxygen species; IRE1a, inositol-requiring enzyme 1a; PERK, PKR-like ER kinase; eIF-2a, eukaryotic initiation factor 2A; BAX, Bcl-2-associated X protein; BAK, Bcl-2-antagonist/killer 1; Bcl-2, B-cell lymphoma-2; Cyto C, cytochrome C; CHOP, C/EBP homologous protein; ASK1, apoptosis signal-regulating kinase 1; TRAF2, tumor necrosis factor receptor-associated factor 2; ATF4, activating transcription factor 4; GADD34, growth arrest and DNA damage-inducible 34; GRP75, 75 kDa glucose-regulated protein; IP3R, inositol 1,4,5-trisphosphate receptor; VDAC, voltage-dependent anion channel; JNK, c-Jun N-terminal kinase; Ca^{2+}, calcium ions.

cose regulatory protein, and inositol 1,4,5-trisphosphate receptor, which facilitates the transfer of Ca^{2+} between the ER and mitochondria at the mitochondria-associated ER membrane.[65–67] Increased uptake of Ca^{2+} by mitochondria reduces the activities of MnSOD, catalase, and peroxidase in the mitochondria, leading to accumulation of oxygen radicals. Excessive oxygen radicals can damage the mitochondrial membrane, denature membrane proteins, increase membrane permeability, and disrupt mitochondrial structure. Moreover, research has demonstrated that mitochondrial Ca^{2+} overload is a crucial factor in the pathological opening of the mPTP (Fig. 2).[68]

Inflammatory response mediated by BAs

Apoptosis is an immune-silent form of cell death that typi-

cally does not elicit an inflammatory response. However, investigations observing neutrophil infiltration in cholestatic rodent models have suggested that BAs might induce liver injury through inflammation.[69,70] Additionally, elevated levels of cytokines have been detected in the liver and serum of patients with cholestasis as well as in BDL mice.[71–73] Research has also demonstrated that reducing inflammatory cytokines can alleviate liver damage in BDL mice,[74–76] further indicating the involvement of the inflammatory response in cholestatic liver injury. These findings imply that BAs can harm hepatocytes through nonapoptotic means, specifically by triggering inflammatory responses.

Research indicates that caspase-independent cell death, induced by BAX and BAK even in the absence of caspase activity, is strongly associated with changes in mitochondrial membrane permeability.[77] Typically, mtDNA is confined to the mitochondrial matrix and isolated from cytoplasmic components. However, when BAs activate BAX and BAK, these proteins gradually form larger supramolecular structures, facilitating the release of mtDNA. Simultaneously, mitochondrial calcium overload induced by BAs alters the permeability of the MIM, leading to the release of mtDNA from the mPTP. According to the endosymbiosis theory, mitochondria are considered to be damage-associated molecular patterns because they originated from aerobic bacteria phagocytosed by primordial eukaryotic cells, and mtDNA retains features of their bacterial ancestry. Toll-like receptors (TLRs) play a crucial role in initiating inflammatory responses to tissue damage caused by both endogenous sterile insults and pathogen recognition. BAs induce mitochondrial damage through various mechanisms, thereby exposing mtDNA to TLR9, an intracellular DNA receptor.[78] TLR9, located on the endosomal reticulum, has a high affinity for unmethylated CpG dinucleotides.[79] Since mitochondrial DNA contains unmethylated CpG DNA repeats, it serves as a ligand for TLR9.[80,81] Upon activation, TLR9 stimulates the production of inflammatory cytokines, which in turn promote chemotaxis of neutrophils as part of the innate immune response to damage-associated molecular patterns.[82] Stimulation by TLR9 and inflammatory cytokines activates the transcription factor nuclear factor kappa-B (NF-κB), triggering a cascade of intracellular signaling molecules that ultimately activate the NF-κB pathway, a key regulator of TLR-induced signaling pathways.[81] TLR9 binding to CpG DNA repeats activates the NF-κB signaling pathway, leading to transcription of a range of inflammatory factors.[80–84]

Neutrophils play a critical role in sterile inflammation. Under normal conditions, circulating neutrophils act as first responders to inflammatory signals, migrating to the site of inflammation due to their abundance, mobility, and potent cytotoxicity. Elevated levels of neutrophils have been observed around the hepatic sinusoids of BDL mice, detectable as early as 8 h after biliary ligation.[70,84,85] However, excessive tissue damage caused by neutrophil infiltration results in persistent inflammation, hindering the progression of injury to healing. Additionally, studies have revealed that ROS activated the NOD-like receptor thermal protein domain associated protein 3 (hereinafter referred to as NLRP3) inflammasome, composed of caspase-1 and NLRP3, thereby enhancing production of proinflammatory cytokines (Fig. 3).[86–88]

Mediation of autophagy dysfunction by BAs

Mitochondrial autophagy, as proposed by Lemasters *et al*,[89] becomes relevant following severe mitochondrial injury or fusion defects because it maintains intracellular stability. Studies indicate that autophagy serves as a survival mechanism in the liver, protecting against cholestasis-induced hepatocyte injury. Inhibiting autophagy with chloroquine exacerbates cholestasis-induced liver damage and hepatocyte death, while promoting autophagy with rapamycin partially mitigates cholestasis-related hepatotoxicity. Transcription factor EB (TFEB) regulates genes essential for lysosomal biogenesis and function.[90] Proteomic analysis has shown that glycochenodeoxycholic acid impairs the expression of transcription factor E3, a member of the TFEB family, synergistically influencing TFEB signaling and decreasing lysosomal production.[91] Research indicates compromised autophagy in human cholestatic conditions. *In vitro* experiments demonstrate that BAs accumulated during cholestasis induce the expression of the RUN domain and Beclin1-interacting protein in a farnesoid X receptor (FXR)-dependent manner. Furthermore, rubicon significantly impedes the fusion of autophagosomes and lysosomes, impairing the normal degradation process of autophagosomes.

Several studies have reported reduced expression of the Rab7 protein in a murine model of cholestasis.[92,93] Rab7 is involved in the fusion of autophagosomes with lysosomes through interactions with a new FYVE and coiled-coil domain-containing protein and is crucial for microtubule plus-end-directed transport.[94] Decreased Rab7 expression leads to impaired fusion between autophagosomes and lysosomes, resulting in p62 accumulation. This accumulation promotes the formation of intrahyaline bodies, contributing to cholestatic liver damage. Moreover, the accumulation of p62-containing intrahyaline bodies inhibits the FXR nuclear receptor and activates the NF-E2-related factor 2 (hereinafter referred to as NRF2) pathway.[95] Despite ongoing investigations, the precise mechanism by which impaired autophagy flux contributes to cholestatic liver injury remains unclear. Nonetheless, these findings underscore the pathological significance of the autophagy-p62-NRF2-FXR pathway (Fig. 4).[96]

Advances in clinical research of cholestatic diseases

Primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC) are the two most common chronic cholestatic liver diseases. Ursodeoxycholic acid (UDCA) has long been considered the first-line drug for treating PBC, as it can accelerate BAs excretion and alter BAs composition. However, approximately 40% of patients do not respond to UDCA and are therefore at risk of disease progression.[42,97] Furthermore, there is currently no available medical therapy for PSC. Recent advances in molecular biochemistry, specifically in BAs regulation and understanding of immunologic pathways, have led to the emergence of novel pharmacologic treatments.

Advances in clinical research of drugs targeting BAs metabolism

Most clinical trials focus on therapeutic interventions targeting the effector phase of BAs synthesis, metabolism, and inflammation. Obeticholic acid (OCA) exhibits a strong affinity for the farnesoid X receptor. In a clinical study, approximately 50% of patients who did not adequately respond to UDCA showed improvement with OCA, expediting its approval process by the Food and Drug Administration.[98] However, findings of a significantly increased incidence of itching in the POISE treatment group may pose a notable barrier to its clinical application.[98] Moreover, treatment with OCA led to decreased high-density lipoprotein cholesterol and triglyceride levels, along with an initial mild increase in low-density lipoprotein cholesterol levels; the long-term consequences of these changes in the lipid profile induced by OCA are yet to

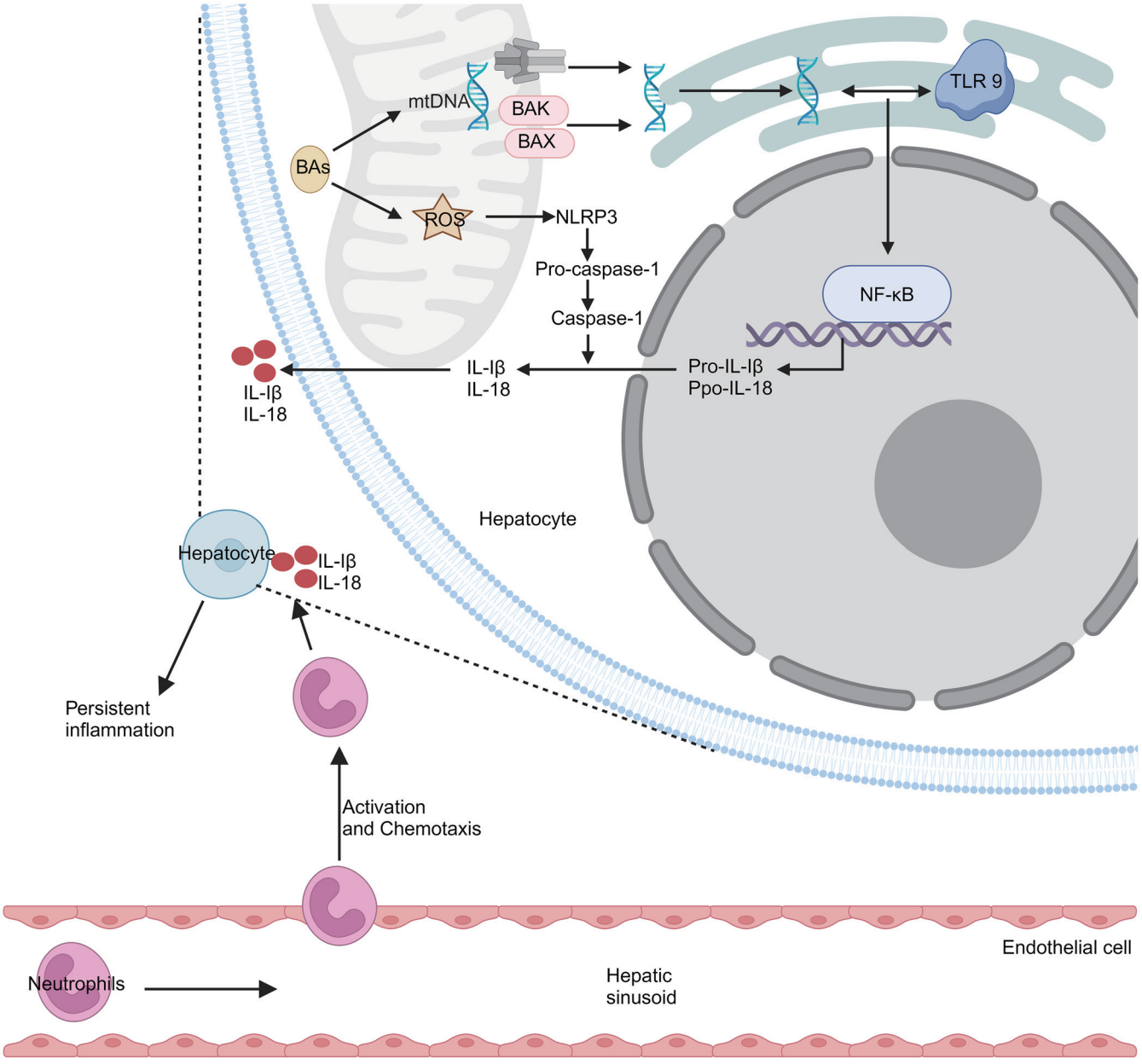

Fig. 3. Continuous accumulation of BAs in liver cells leads to an inflammatory response. BAs, Bile acid; NF-κB, nuclear factor kappa-B; ROS, reactive oxygen species; NLRP3, NOD-like receptor thermal protein domain associated protein 3; BAX, Bcl-2-associated X protein; BAK, Bcl-2-antagonist/killer 1; mtDNA, mitochondrial DNA.

be determined. New molecules with alterations to fibroblast growth factor 19's amino acid sequence have been developed to limit its procarcinogenic properties and enhance its anticholestatic effect.[99] NGM282 is one such nontumorigenic derivative of fibroblast growth factor 19. In a phase II multicenter, randomized, double-blinded, placebo-controlled trial involving 45 PBC patients who were inadequate responders to UDCA, NGM282 was administered as a daily subcutaneous injection of 0.3 mg or 3 mg versus placebo for 28 days.[100] Treated patients showed statistically significant reductions in ALP levels and other liver biochemistries, but some adverse events were reported, including diarrhea, nausea, and headache. It remains unclear whether NGM282 will undergo further examination in PSC.

In recent years, researchers have shifted their focus toward interventions targeting inflammatory effects, such as cytokine neutralizers and their signal transducers.[101,102] Rituximab, a monoclonal antibody targeting the CD20 antigen on B cells, has been investigated for potential benefits in PBC. In one study, six PBC patients with inadequate response to UDCA showed improvement in ALP levels following rituximab infusions.[100] However, another study involving 14 PBC patients reported less pronounced reductions in liver biochemistries despite improvements in pruritus.[103,104] Abatacept, a monoclonal antibody targeting CD80 and CD86 on antigen-presenting cells and interfering with T-cell activa-

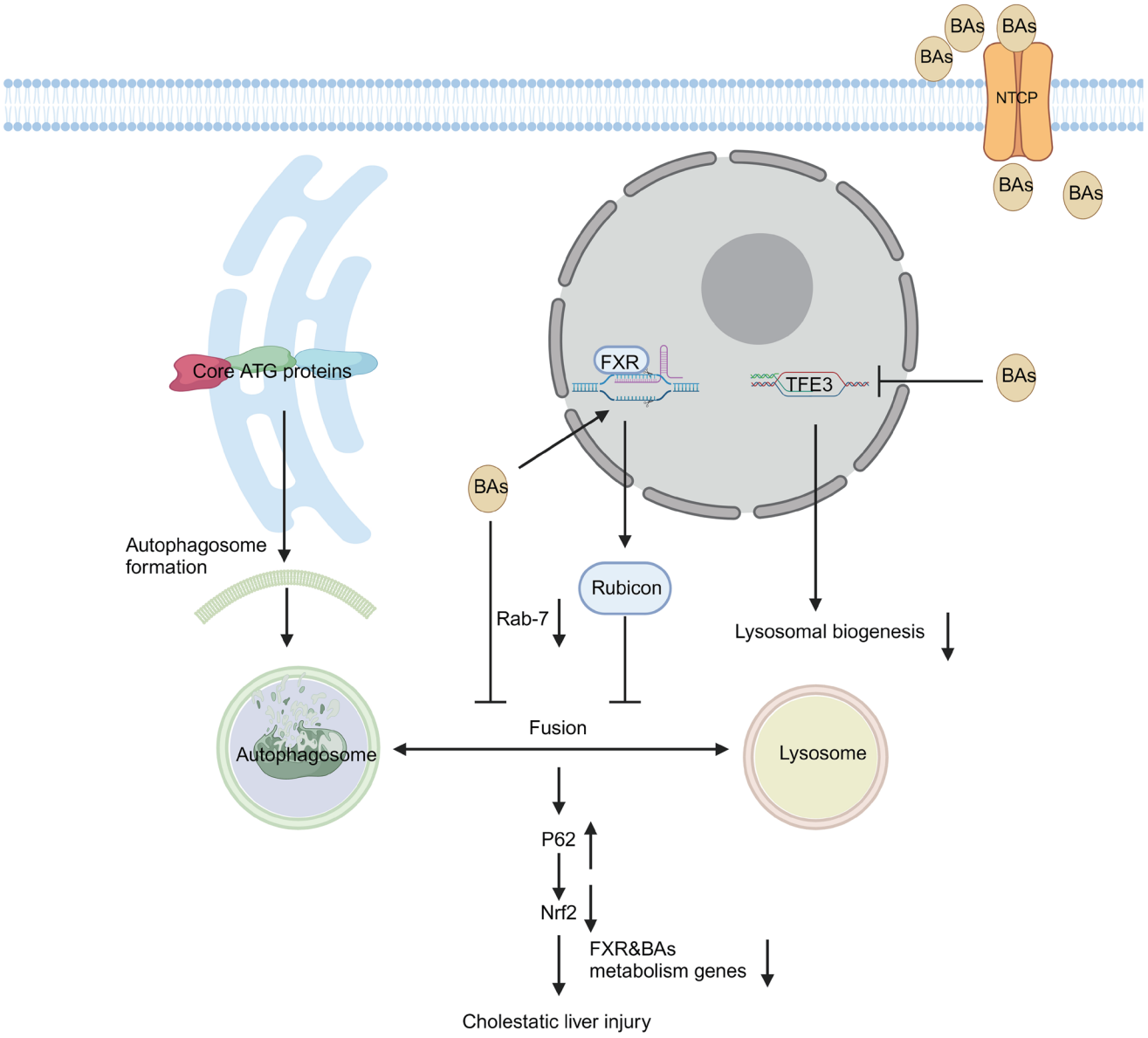

Fig. 4. Accumulation of BAs in cholestatic conditions impairs the fusion of autophagosomes and lysosomes. ↑, expression increased; ↓, expression decreased; BAs, Bile acid; FXR, farnesoid X receptor; NRF2, NF-E2-related factor 2; NTCP, sodium-taurocholate co-transporting polypeptide; Rubicon, RUN domain and Beclin1-interacting protein; TFE3, transcription factor E3.

tion, failed to meet the primary endpoint in a small study for PBC.[105] New therapeutic targets are urgently needed.

Advances in clinical research of mitochondria-targeted drugs

The regulation of mitochondrial membrane barrier function is of medical interest, as it can modulate access to mitochondrial contents, including DAMPs, into the cytosol, thereby influencing downstream cell death and inflammatory signaling pathways. Drugs that effectively modulate mitochondrial pore formation and DAMPs release could potentially be used in therapeutics. Research on potential treatments for cholestasis targeting mitochondria is rare, reflecting the novelty of this research area and the scarcity of pharmacological interventions specifically targeting mitochondrial function, especially mitochondrial outer membrane permeabilization (MOMP) and mPTP. CsA, a commonly used inhibitor of MOMP and mPTP, has been approved for clinical trials due to its inhibitory effect on mPTP when used in conjunction with mitochondrial recombinant cyclophilin D. CsA has demonstrated protective effects against ischemia/reperfusion injury in the heart. Although the therapeutic efficacy of CsA in cholestasis remains unclear and requires further investigation, findings regarding this drug provide valuable insights into the study of drugs targeting MOMP and mPTP.[106]

Discussion and future prospects

The etiology of cholestatic liver injury is multifaceted, and its pathogenesis is complex. A key pathological feature of chole-

static liver injury is elevated levels of BAs in the blood and liver. BAs, especially hydrophobic ones, act as pathological factors that can exacerbate liver cell damage. Mitochondria are key regulatory targets in apoptosis and inflammation induced by cholestasis. A growing body of evidence from various experimental environments suggests a close relationship between the structural and functional integrity of mitochondria and cholestatic liver injury.

However, several questions and obstacles remain to be addressed for the development of clinically viable mitochondria-targeting agents for the treatment of cholestatic liver injury. First, although abundant correlational evidence linking mitochondrial dysfunction to the pathogenesis of cholestatic liver injury exists, well-established mechanistic links are often missing and urgently need to be established. Second, additional work is needed to understand the crosstalk between autophagy and apoptotic caspases in regulating mitochondrial DAMP-driven inflammation. Third, although recent discoveries have allowed important progress in our understanding of the mPTP, we still lack a comprehensive view of the pore architecture and the molecular connections between MOM and MIM, which calls for the development of more refined experimental tools. Finally, studies on potential treatments of cholestasis with drugs targeting mitochondria are rare, which not only reflects the novelty of research targeting mitochondrial function but also indicates that the clinical trial basis for targeted mitochondrial drugs is insufficient. This hypothesis has only been confirmed in the laboratory and lacks clinical evidence. Therefore, further elucidation of the molecular mechanisms underlying mitochondrial dysfunction and cholestatic liver injury is crucial, as it will pave the way for the development of mitochondria-targeting drugs aimed at effectively managing cholestatic liver injury.

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Manuscript writing and reference collection (XL, TR), manuscript review and editing (SW, XS), and final critical revision for the manuscript (CL, YP, YT). All authors have read and agreed to the published version of the manuscript.

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