Cell Death in Biology and Diseases

Wen-Xing Ding
Xiao-Ming Yin
Editors

Cellular Injury in Liver Diseases

Springer

Cell Death in Biology and Diseases

Series Editors

Xiao-Ming Yin
Zheng Dong

More information about this series at http://www.springer.com/series/8908

Wen-Xing Ding • Xiao-Ming Yin
Editors

Cellular Injury in Liver Diseases

Springer

Editors
Wen-Xing Ding
Department of Pharmacology, Toxicology and Therapeutics
The University of Kansas Medical Center
Kansas City, KS, USA

Xiao-Ming Yin
Department of Pathology and Laboratory Medicine
Indiana University School of Medicine
Indianapolis, IN, USA

Cell Death in Biology and Diseases
ISBN 978-3-319-53773-3 ISBN 978-3-319-53774-0 (eBook)
DOI 10.1007/978-3-319-53774-0

Library of Congress Control Number: 2017936480

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer International Publishing AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Series Preface

Cell death, or conversely cell survival, is a major biological phenomenon. Just as with cell proliferation and cell differentiation, cell death is a choice that a cell has to make, sometimes voluntarily, other times accidentally. As such, cell death serves a purpose in the biology of a multicellular organism. The machinery of cell death and that of cell protection are evolutionarily conserved and their elements can even be found in single-celled organism. The disruption of cell death mechanisms can often cause developmental abnormalities. Factors that can trigger cell death are diverse and the cell death process is intricately connected with other biological processes. Cell death directly contributes to the pathogenesis of many diseases, including cancer, neurodegenerative diseases, and tissue injury in organ failure.

The study of cell death and cell survival has become a multidisciplinary subject, which requires expertise from all fields of the modern biology. Exploring the role of cell death in disease development and the modulation of cell death for the prevention and treatment of devastating disease demands constant updating of our knowledge through the broadest interactions among all investigators, basic and clinical. The rapid expansion of our knowledge in this field has gone beyond what could be summarized in a single book. Thus, this timely series *Cell Death in Biology and Diseases* summarizes new developments in different areas of cell death research in an elaborate and systemic way. Each volume of this series addresses a particular topic of cell death that either has a broad impact on the field or has an in-depth development in a unique direction. As a whole, this series provides a current and encyclopedic view of cell death.

We would like to sincerely thank the editors of each volume in the series and the authors of each chapter in these volumes for their strong commitment and great effort towards making this mission possible. We are also grateful to our team of professional Springer editors. They have worked with us diligently and creatively from the initiation and are continuing this on the development and production of each volume of the series. Finally we hope the readers will enjoy the reading, find the content helpful to their work, and consider this series an invaluable resource.

Indianapolis, IN, USA — Xiao-Ming Yin
Augusta, GA, USA — Zheng Dong

Preface

Cell death is a fundamental biological phenomenon. It is evolutionarily conserved but can assume different forms under different conditions. While apoptosis, necrosis, and necroptosis are perhaps best studied, less known forms of cell death such as pyroptosis, ferroptosis, parthanatos, and entosis are increasingly found in various conditions or organisms. In multicellular organisms, cell death is important for development, shaping organ size, reforming tissue architecture, promoting functional differentiation, and determining mitochondrial inheritance. In the post-development stage, cell death determines the severity in tissue injury and the degree of subsequent response in inflammation, fibrosis, repair, regeneration, and tumorigenesis. The pathological changes in a complex organ, such as the liver, can be greatly affected by the death program in its cellular components.

As the major organ for metabolism and detoxification, the liver is constantly under challenges from both internal and external sources. Viral infection and xenobiotics are the two major environmental stimuli that can cause significant hepatocyte death. Metabolic disturbance (such as in autophagy function) and special food components (such as lipids, ethanol, cholesterol, sugars) are the major internal stress that can also take a significant token on the hepatocytes that leads to cell death. Furthermore, the liver may experience traumatic injury as in cholestasis or ischemia, which often leads to tissue injury to various degrees. The liver is composed of hepatocytes (the majority), cholangiocytes, stellate cells, fibroblasts, macrophages/Kupffer cells, sinusoidal cells, and many more. They respond to these insults with different sensitivities and contribute to the overall liver pathology in various ways. Thus different signaling pathways may be involved in a cell type-specific and/or in a stimulus-specific way, which triggers subcellular organelle (mitochondria and endoplasmic reticulum as well as lysosomes) stress to induce cell death. Significant progresses have been made in the past decades to characterize these cell death pathways and the cells that are involved in liver injury.

We are thus preparing two books devoted to the mechanisms of cell death in the liver and liver diseases. While the first volume mainly discusses liver injury and cell death caused by the various external and internal stimuli, the second volume discusses in great detail the type of cells and intracellular organelles involved in cell

death as well as several major signaling pathways involved. We have paid particular attention to the interaction of different cells and aimed to provide a global view of the liver pathology that connects injury/cell death to other pathological changes, such as inflammation, fibrosis, and tumor development. Notably we also discuss the interaction between the liver and the intestine where microbiota can both influence and be influenced by the liver pathology. Authors who are invited to contribute to the two volumes are respected experts in the subject, which should greatly enhance the authority of the chapter and the book. The volume editors are thus indebted to the authors for their outstanding contributions that make these two books so informative and thought-provoking.

Kansas City, KS, USA Wen-Xing Ding
Indianapolis, IN, USA Xiao-Ming Yin

Contents

Contributors

Xiaojuan Chao Department of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, Kansas City, KS, USA

John Y.L. Chiang Department of Integrative Medical Sciences, Northeast Ohio Medical University, Rootstown, OH, USA

Lily Dara Division of GI/Liver, Department of Medicine, Research Center for Liver Disease, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA

Wen-Xing Ding Department of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, Kansas City, KS, USA

Jia Fan Department of Liver Surgery, Liver Cancer Institute, Zhongshan Hospital, Fudan University, Shanghai, China

Key Laboratory of Carcinogenesis and Cancer Invasion of Ministry of Education, Shanghai, China

Gregory J. Gores Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN, USA

Julie Goswami Department of Surgery, University of Pittsburgh Medical Center, Pittsburgh, PA, USA

Michael Hinton Department of Microbiology and Immunology, Medical College of Virginia Campus, Virginia Commonwealth University, Richmond, VA, USA

McGuire VA Medical Center, Richmond, VA, USA

Petra Hirsova Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN, USA

I-Fang Hsin Division of Gastroenterology, Department of Medicine, Cedars-Sinai Medical Center, Los Angeles, CA, USA

Hai Huang Department of Surgery, University of Pittsburgh Medical Center, Pittsburgh, PA, USA

Nazmul Huda Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, IN, USA

Samar H. Ibrahim Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN, USA

Hartmut Jaeschke Department of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, Kansas City, Kansas, USA

Neil Kaplowitz Division of GI/Liver, Department of Medicine, Research Center for Liver Disease, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA

Bilon Khambu Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, IN, USA

Eric Kwong Department of Microbiology and Immunology, Medical College of Virginia Campus, Virginia Commonwealth University, Richmond, VA, USA

McGuire VA Medical Center, Richmond, VA, USA

Jiyoung Lee Department of Molecular Microbiology and Immunology, University of Southern California, Keck School of Medicine, Los Angeles, CA, USA

Yunzhou Li Department of Microbiology and Immunology, Medical College of Virginia Campus, Virginia Commonwealth University, Richmond, VA, USA

McGuire VA Medical Center, Richmond, VA, USA

Tiangang Li Department of Pharmacology, Toxicology and Therapeutics, Kansas University Medical Center, Kansas City, KS, USA

Harmeet Malhi Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN, USA

Jing-hsiung James Ou Department of Molecular Microbiology and Immunology, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA

Yuan-Fei Peng Department of Liver Surgery, Liver Cancer Institute, Zhongshan Hospital, Fudan University, Shanghai, China

Key Laboratory of Carcinogenesis and Cancer Invasion of Ministry of Education, Shanghai, China

Ekihiro Seki Division of Gastroenterology, Department of Medicine, Cedars-Sinai Medical Center, Los Angeles, CA, USA

Ying-Hong Shi Department of Liver Surgery, Liver Cancer Institute, Zhongshan Hospital, Fudan University, Shanghai, China

Key Laboratory of Carcinogenesis and Cancer Invasion of Ministry of Education, Shanghai, China

Meng-Xin Tian Department of Liver Surgery, Liver Cancer Institute, Zhongshan Hospital, Fudan University, Shanghai, China

Key Laboratory of Carcinogenesis and Cancer Invasion of Ministry of Education, Shanghai, China

Allan Tsung Department of Surgery, University of Pittsburgh Medical Center, Pittsburgh, PA, USA

Division of Hepatobiliary and Pancreatic Surgery, University of Pittsburgh, Pittsburgh, PA, USA

Han Wang Department of Liver Surgery, Liver Cancer Institute, Zhongshan Hospital, Fudan University, Shanghai, China

Key Laboratory of Carcinogenesis and Cancer Invasion of Ministry of Education, Shanghai, China

Shaogui Wang Department of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, Kansas City, KS, USA

Heather Waring Department of Surgery, University of Pittsburgh Medical Center, Pittsburgh, PA, USA

Benjamin L. Woolbright Department of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical CenterKansas City, Kansas, USA

Shengmin Yan Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, IN, USA

Xiao-Ming Yin Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, IN, USA

Huiping Zhou Department of Microbiology and Immunology, Medical College of Virginia Campus, Virginia Commonwealth University, Richmond, VA, USA

McGuire VA Medical Center, Richmond, VA, USA

Jun Zhou Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, IN, USA

Center of Minimally Invasive Surgery, Xiangya Second Hospital, Central South University, Changsha, Hunan, China

Chapter 1
Cell Death in Drug-Induced Liver Injury

Lily Dara and Neil Kaplowitz

Abbreviations

ALF	Acute liver failure
APAP	Acetaminophen
APAF1	Apoptotic peptidase-activating factor-1
ASK1	Apoptosis signal-regulating kinase 1
ASMase	Acid sphingomyelinase
ATF6	Activating transcription factor 6
ATG	Autophagy-related genes
Cbl-b1	Casitas B lineage lymphoma-B1
CCL4	Carbon tetrachloride
CHOP	CCAAT-enhancer-binding protein homologous protein
cIAP	Cellular inhibitor of apoptosis 1 and 2
Cr	Chromium
CTLA4	Cytotoxic T-lymphocyte-associated protein 4
DILI	Drug-induced liver injury
DR	Death receptor
ER Stress	Endoplasmic reticulum stress
ETC	Electron transport chain
FADD	Fas-associated protein with death domain
GRP78	Glucose regulatory peptide 78
GSH	Glutathione
GSK3b	Glycogen synthase kinase 3 beta
GWAS	Genome-wide association study

L. Dara • N. Kaplowitz (✉)
Division of GI/Liver, Department of Medicine, Research Center for Liver Disease, Keck School of Medicine, University of Southern California, 2011 Zonal Ave, HMR 101, Los Angeles, CA 90033, USA
e-mail: kaplowit@usc.edu

W.-X. Ding, X.-M. Yin (eds.), *Cellular Injury in Liver Diseases*, Cell Death in Biology and Diseases, DOI 10.1007/978-3-319-53774-0_1

X.-M. Yin and Z. Dong (Series eds.), *Cell Death in Biology and Diseases*

INH	Isonizaide
JNK	c-Jun N terminal kinase
K18	Keratin 18
KCC	King's college criteria
KO	Knockout
HLA	Human leukocyte antigen
HMGB1	High-mobility group box 1
IRE1α	Inositol-requiring enzyme 1α
IDILI	Idiosyncratic drug-induced liver injury
LPS	Lipopolysaccharides
LSEC	Liver sinusoidal endothelial cells
MAPK	Mitogen-activated protein kinase
MDSC	Myeloid-derived suppressor cells
MEF	Mouse embryonic fibroblasts
MELD	Model for end-stage liver disease
MHC	Major histocompatibility complex
miR	Micro RNA
MDB	Mallory-Denk bodies
MKK4	Mitogen-activated protein kinase kinase 4
MLKL	Mixed lineage kinase domain-like
MOMP	Mitochondrial outer membrane permeabilization
MPT	Mitochondrial permeability transition pore
mTOR	Mammalian target of rapamycin
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
NFκB	Nuclear factor κB
NK	Natural killer
PD-1	Programmed cell death protein-1
PERK	Protein kinase RNA-like ER kinase
PKC	Protein kinase C
POLG	Polymerase γ
PTPN6	Phospho-tyrosine phosphatase, non-receptor type 6
RIPK1	Receptor interacting protein kinase 1
RIPK3	Receptor interacting protein kinase 3
ROS	Reactive oxygen species
Sab	SH3BP5, SH3 domain-binding protein that preferentially associates with Bruton's tyrosine kinase
SMAC	Second mitochondria-derived activator of caspases
TAB2/3	TAK1-binding protein 2/3
TAK1	Transforming growth factor-β-activated kinase 1
TRAF2	TNF receptor-associated factor 2
TRAIL	TNF-related apoptosis-inducing ligand
TNF	Tumor necrosis factor
TNFR	Tumor necrosis factor receptor
TRADD	TNFR1-associated via death domain

UPR	Unfolded protein response
VPA	Valproic acid
WT	Wild type
XIAP	X-chromosome-linked inhibitor of apoptosis protein

1.1 Introduction

Drug-induced liver injury (DILI) presents as a spectrum of clinical presentations such as acute hepatocellular liver injury resulting in acute hepatitis, cholestasis and jaundice, nodular regenerative hyperplasia, sinusoidal obstruction syndrome, or subclinical injury which is detected during routine serum chemistries (Yuan and Kaplowitz 2013). Acute hepatocellular DILI can lead to acute liver failure (ALF), the most feared complication that triggers regulatory responses ranging from warnings, withdrawal, disapproval to recommendation for close monitoring. DILI can present sub-acutely with elevated liver enzymes or gradual jaundice or even manifest as a chronic progressive disease, the latter being less defined and somewhat controversial (Stine and Chalasani 2015). In all these instances, the ongoing liver damage from the insulting drug often leads to inflammation and direct and indirect liver cell death. Although it is unusual for DILI to persist and become chronic, in certain contexts such as in diabetics on long-term methotrexate, low grade injury can become chronic and tissue repair responses can be activated resulting in fibrosis and ultimately cirrhosis. In addition, some drugs can increase weight gain and insulin resistance aggravating underlying NAFLD and thus indirectly promoting NASH. DILI can be further classified into dose-dependent predictable (e.g., acetaminophen) and idiosyncratic injury. In either case, the major concern is hepatocellular death with the fear of massive parenchymal extinction leading to ALF.

Cell death due to DILI and hepatotoxicity can be apoptotic or necrotic depending on the inciting drug and which signaling pathways are activated. Apoptosis, the predominant cell death routine in parenchymal liver cells, hepatocytes, and cholangiocytes, can be activated through intrinsic and extrinsic pathways. Intracellular stress responses, such as mitochondrial toxicity or ER stress from drugs or toxic metabolites, can activate the intrinsic pathway of apoptosis, whereas the extrinsic pathway of apoptosis is activated by the engagement of surface death receptors with their respective ligands, usually via the immune system. Necrosis of hepatocytes to a large part also involves activation of cellular signaling pathways. While this is the subject of much of the current research in the field, the exact mechanistic pathways are not as clearly elucidated as in the case of apoptosis. It is important to point out that hepatotoxicity can occur due to toxic effects of drugs on other cells in the liver such as the liver sinusoidal endothelial cells (LSECs) or changiocytes resulting in their death. In this chapter, we focus on cell death in hepatocytes, the main parenchymal liver cells.

A few drugs such as acetaminophen (APAP), aspirin, and chemotherapeutic agents can cause predictable, dose-dependent, and direct hepatocyte-intrinsic hepatotoxicity.

However, most instances of liver injury from drugs are idiosyncratic. Idiosyncratic Drug-Induced Liver Injury (IDILI) is by definition an unpredictable injury due to a drug, usually occurring after a relatively long latency and only in a small proportion of exposed individuals. The pathogenesis if IDILI is multifactorial and complex but in most instances involves the adaptive immune system as multiple genome-wide association studies (GWAS) have identified strong correlations between certain HLA polymorphisms and IDILI occurrence. A seminal example is the GWAS study by Daly et al. in which patients with flucloxacillin hepatotoxicity were more likely to harbor the HLA-B*57:01 allele compared to exposed individuals with no DILI (Hazard Ratio of 100) (Daly et al. 2009). Additionally, the peripheral blood mononuclear cells of patients with flucloxacillin DILI showed immune activation on re-exposure to flucloxacillin. Flucloxacillin was also able to activate CD8+ T cells in drug naive individuals with the HLA-B*57:01 allele indicating a strong association of the allele with the resultant injurious phenotype (Monshi et al. 2013; Urban et al. 2014; Wuillemin et al. 2013). Other examples of strong associations between HLA polymorphisms and IDILI are the increased risk of amoxicillin-clavulanate toxicity (the single most common form of IDILI) in patients with DRB1*15:01, DQB1*06:02, and HLA-A*02:01 haplotype and ximelagatran in patients with the HLA-DRB1*07:01 polymorphism, among others (Table 1.1) (Kindmark et al. 2008; Mallal et al. 2008; Hautekeete et al. 1999; Lucena et al. 2011; O'Donohue et al. 2000; Chen et al. 2015; Sharma et al. 2002; Daly et al. 2009; Hirata et al. 2008; Kurosaki et al. 2000; Nicoletti et al. 2016; Phillips et al. 2013; Singer et al. 2010; Spraggs et al. 2011; Xu et al. 2015; Monshi et al. 2013; Urban et al. 2014; Wuillemin et al. 2013). Although little mechanistic evidence exists, it is currently hypothesized that once a drug is converted into a "reactive metabolite" or "hapten" it can activate the adaptive immune system through neoantigen formation or molecular mimicry in those individuals with the susceptible HLA haplotype. In addition to the hapten hypothesis two other hypotheses exist for why HLA polymorphisms result in aberrant immune activation and IDILI. These include the pharmacological interaction hypothesis (p-i hypothesis) and the altered peptide repertoire hypothesis. Interactions between antigen-binding grooves and proteins (covalent and noncovalent) are considered to be important in the activation of the adaptive immune system. The "pharmacological interaction hypothesis" postulates that drugs can directly contact MHC molecules on T cells via noncovalent interactions resulting in T cell activation without the presence of an antigenic peptide (Pichler 2002). An example of the possible clinical relevance of the pi hypothesis is in ximelegatran DILI since the drug does not covalently bind proteins to form neoantigens. However, in vitro studies have shown a direct inhibition by ximelegatran of peptide binding to HLA DRB1*0701, supporting direct interaction of the drug to this HLA-binding site, thus pointing to the p-i hypothesis as a possible mechanism for immune activation and DILI (Grove and Aithal 2015). Another potential mechanism is drug-induced mistargeting of endogenous peptides to the wrong HLA leading to autoimmunity, as described for abacavir rash and Stevens Johnson syndrome from carbamazepine (Ostrov et al. 2012; Wei et al. 2012). In experimental IDILI models it has been suggested that subsequent to these immune activation pathways, either CD4+-dependent antibody-mediated cytotoxicity (Chakraborty et al. 2015), or CD8+ T-cells-mediated cytotoxicity (Mak and Uetrecht 2015) results in targeting of hepato-

Table 1.1 DILI drugs with known HLA associations

Drug	Associated HLA haplotypes	Phenotype	Reference
Abacavir	B* 57:01	Hepatocellular and skin rash	Mallal et al. 2008
Amoxicillin-clavulanate	A*02:01, B*18:01, DRB1*1501, DGB1 *0602, DRB1*07	Spectrum	Kindmark et al. 2008, Hautekeete et al. 1999, Lucena et al. 2011
Anti-tuberculous drugs	DQB1*02:01, DQB1 *05:02, DQA1*01:02‡	Hepatocellular and mixed	Chen et al. 2015, Sharma et al. 2002
Fenofibrate	A*33:01	Cholestatic	(Aithal, Liver meeting 2015 Abstract 225)
Flucloxacillin	B* 57:01, DRB1*07:01, DQB1*03:03, DRB1*15‡	Cholestatic	Daly et al. 2009
Flupirtine	DRB1*16:01-DQB1*05:02	Hepatocellular	Nicoletti et al. 2016
Lapatinib	DQA1*02:01, DQB1*02:02, DRB1*07:01	Hepatocellular and mixed (delayed)	Spraggs et al. 2011
Lumiracoxib	DRB1*15:01, DQB1*06:02, DRB5*01:01, DQA1*01:02	Hepatocellular (delayed)	Singer et al. 2010
Minocycline	B*35:02	Hepatocellular	(Urban, Liver meeting 2015 Abstract 1930)
Nevirapine	DRB*01:02	Hepatocellular	Phillips et al. 2013
Pazopanib	B*57:01	Hepatocellular	Xu et al. 2015
Ticlopidine	A*33:03, A*33:01	Cholestatic	Hirata et al. 2008
Tiopronin	A*33	Cholestatic	Kurosaki et al. 2000
Terbinafine	A*33:01	Mixed	(Aithal, Liver meeting 2015 Abstract 225)
Ximelagatran	DRB1*0701, DQA1*0201	Hepatocellular	Kindmark et al. 2008

‡Haplotype decreases risk of toxicity

cytes and death via DR-initiated apoptosis (the de facto cell death pathway in hepatocytes), which will be discussed in detail below. However, the susceptible HLA polymorphism resulting in immune activation does not fully explain hepatotoxicity on its own as most identified HLA haplotypes associated with toxicity are quite common in the general population and IDILI is a rare event. Thus, not all individuals with the susceptible HLA exhibit clinical hepatotoxicity when exposed to the drug. This is likely due to the liver's extraordinary capacity for immune tolerance, a physiologic constant dampening of immune response in the liver which leads to "adaptation." When immune tolerance is inhibited in the liver, for example, by genetic knockout of immune check point inhibitors (such as Cbl-b1−/− mice or PD1−/− mice) or when CTLA4 inhibitors are used, drugs that normally would not result in liver injury can cause T cell activation and DILI (Chakraborty et al. 2015; Metushi et al. 2015; Mak and Uetrecht 2015). Two groups have recently explored this phenomenon using mice

deficient in one or more check point inhibitor components. Chakaborty et al. injected two doses of halothane (a drug known to cause allergic DILI), 14 days apart, to mice depleted of myeloid-derived suppressor cells (MDSC) using anti-Gr1 antibody, and reported more severe injury in the MDSC-deficient mice that lack the ability for adaptation (Chakraborty et al. 2015). The anti-Gr-1-treated mice displayed severe inflammation, necrosis, and eosinophilia (Chakraborty et al. 2015). Treatment with anti-CD4 antibody was able to abrogate this allergic response (Chakraborty et al. 2015).

Metushi et al. examined the toxicity of amodiaquine in Cbl-b1 and PD-1-deficient mice (both molecules are immune check point inhibitors). Mice knockout for either gene exhibited more severe DILI than wild-type (WT) mice; however, this resolved due to adaptation and the mice recovered (Metushi et al. 2015). Blocking an additional immune check point pathway using CTLA4 antibody in PD1−/− mice resulted in more severe and sustained injury from amodiaquine (Metushi et al. 2015). In contrast to the halothane immunoallergic model of IDILI, in this model, the resultant liver injury was dependent on the action of cytotoxic CD8+ T lymphocytes (Mak and Uetrecht 2015). These studies suggest that under normal conditions check point immune modulators have a pro-adaptation dampening effect on the activation on T cells in the liver, preventing constant inflammatory response and IDILI (Dara et al. 2015b). Thus, a failure of adaptation is likely an additional or simultaneous requirement for the development of IDILI but the details of this interesting phenomena are beyond the scope of this chapter and is discussed elsewhere (Dara et al. 2015b) (Fig. 1.1).

Morphologically and mechanistically cell death is broadly classified into apoptosis, necrosis, and autophagy (which has close ties to apoptosis). Necroptosis, a form of regulated necrosis, is also an important cell death subroutine that has been studied in the context of DILI. Below we will examine the current evidence and the role of each of these cell death modes in drug hepatotoxicity. It should be pointed out that when extensive injury occurs in humans, it may be very difficult to distinguish which form of cell death predominates. This is less of a problem in animal models where mechanistic biochemical studies can be deployed. Nevertheless, animal models of IDILI are only beginning to be identified (by modulating immune tolerance) but are thus far not very robust, probably because of redundancies in the mechanisms of immune tolerance and the restricted HLA associations specific to humans.

1.2 Apoptosis in DILI

Apoptosis is a programmed mode of cell death that occurs both physiologically and, in many forms of liver disease, as a pathologic process. Apoptosis occurs in two steps, an activation phase where a stimulus or death signal activates a series of biochemical events that lead to the activation of specific proteases termed initiator caspases. Subsequently, these initiator caspases lead to the execution phase (second step), which is carried out by the effector caspases. Apoptosis can be activated via two major and convergent routes: the extrinsic (death receptor) and the intrinsic

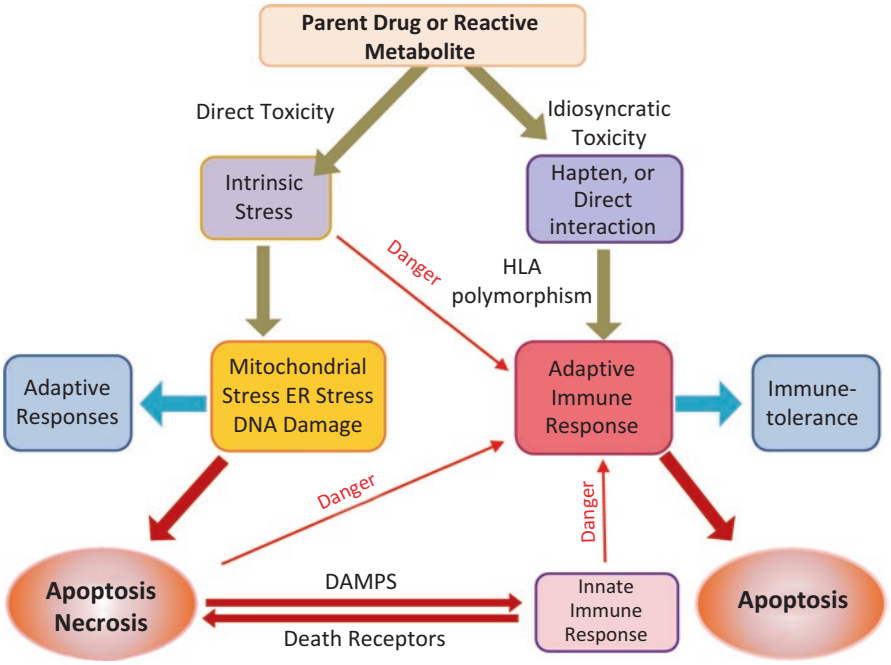

Fig. 1.1 Cell death in DILI. Drugs can be directly toxic to hepatocytes causing "intrinsic stress" or they can activate the adaptive immune system in certain individuals with susceptible HLA polymorphisms causing idiosyncratic, dose-independent, and unpredictable toxicity. Direct toxicity results in intrinsic stress (mitochondrial, ER stress, or DNA damage) that can lead to apoptosis and necrosis when a certain threshold is passed and adaptive responses cannot compensate. Apoptosis and necrosis subsequently lead to the release of DAMPs and a secondary innate immune response in the injured liver. Idiosyncratic DILI occurs via the adaptive immune system but is usually dampened by the process of immune tolerance leading to adaptation. However, if these adaptive responses are overcome then DILI ensues, resulting in cell death (apoptosis). (*ROS* reactive oxygen species, *ER* endoplasmic reticulum, *HLA* human leukocyte antigen, *DAMP* danger-associated molecular patterns)

(mitochondrial) pathways. Both lead to the eventual activation of executioner caspases which results in proteolysis, pyknosis (chromatin condensation), and karyorrhexis (nuclear fragmentation), which are the morphologic features of apoptosis, a.k.a., type I cell death (Galluzzi et al. 2012).

The extrinsic pathway of apoptosis is activated when death receptors (DR) such as TNF, FAS, Trail, etc. are engaged by their ligands resulting in caspase 8 activation and ultimately apoptosis and in some cases necroptosis depending on cell type. Death receptors are type 1 transmembrane proteins with intracellular C-terminal portions that contains a span of amino acids termed the death domain (Guicciardi and Gores 2009). All the members of the death receptor family including TNFR, Fas (CD95), TRAIL-R1 (DR4), and TRAIL-R2 (DR5) are highly expressed in the liver. Immune-mediated killing of hepatocytes occurs through T cells and cytokines (free and membrane bound) and involves these death receptors. Immune-mediated

destruction of hepatocytes is largely mediated by major histocompatibility complex (MHC) class I-restricted CD8+ cytotoxic T lymphocytes (CTLs). However, other immune cells such as NK cells and NKT cells may have important roles in the liver and Kupffer cells (KC) also contribute (Tian et al. 2013; Protzer et al. 2012). Death receptor engagement and activation by their ligands activates the extrinsic pathway of apoptosis. These ligands include FasL for FAS, TNF for TNFR, and TRAIL (TNF-related apoptosis inducing ligand) for TRAILR. Immune-mediated hepatocyte killing in IDILI likely occurs through one or more of the receptor-mediated pathways discussed here.

1.2.1 FAS

FAS receptor is ubiquitously expressed and is abundant in the liver among many other organs (pancreas, kidney, heart, brain, etc.). It is also highly expressed by activated mature T cells. FAS Ligand (FASL) is mainly expressed on the surface of activated T cells and also by KC to a lesser extent; in addition, a soluble form exists (Strasser et al. 2009; Malhi et al. 2010). FASL binding to its receptor leads to recruitment of FADD to activate caspase 8 and internalization of the receptor (Guicciardi and Gores 2009; Lee et al. 2006). The receptor oligomerizes and forms large lipid rafts and clusters, is internalized, and localizes to the endosomal compartment for death inducing signaling complex (DISC) assembly that includes FADD, caspase-8, and cFLIP (FLICE (i.e., caspase-8) inhibitory protein). It is important to point out in humans in addition to caspase 8, FAS activates another initiator enzyme, caspase-10, which is not present in mice (Strasser et al. 2009). Caspase 8 activation then leads to activation of down-stream effector caspases and apoptosis in most cells (type I cells) such as immune cells. In hepatocytes (type II cells) FAS-mediated apoptosis depends on caspase-8-mediated cleavage of Bid and subsequent mitochondrial apoptosis pathway involvement. FAS stimulation results in massive hepatocyte apoptosis in WT animals. Bid knockout hepatocytes are resistant to Fas-mediated apoptosis whereas thymocytes are not (Yin et al. 1999). Additionally, FAS-mediated apoptosis can be blocked by overexpression of Bcl-2 or Bcl-XL in type II cells (Scaffidi et al. 1999). In type II cells, X-chromosome-linked inhibitor of apoptosis protein (XIAP) associates with caspase 3, thereby preventing propagation of the caspase 8 signal (Jost et al. 2009). This association can be unhinged by second mitochondria-derived activator of caspases (SMAC/Diablo) which is released from the inter membrane space of mitochondria during MOMP (Jost et al. 2009). Therefore, the extrinsic pathway of apoptosis is insufficient to induce cell death and the intrinsic/mitochondrial pathway needs to be engaged for apoptosis to ensue via MOMP (discussed further in the TNF section). Theoretically, FAS stimulation can result in a mode of hepatocyte killing in IDILI. However, another possible mechanism of FAS contribution to hepatotoxicity is through low level (1 μg) FASL depletion of cellular GSH levels which exacerbates toxicity from

drugs such as acetaminophen. This could contribute to patients experiencing toxicity at lower levels when suffering from viral infections (Tinel et al. 2004). While FAS itself does not participate in acetaminophen-induced signaling, which is necrotic, FAS-deficient LPR mice have been shown to have a faster GSH recovery and rebound after APAP resulting in attenuated susceptibility to APAP injury (Williams et al. 2013).

1.2.2 TRAIL

TRAIL-R (a.k.a Apo 2) is a member of the TNF receptor super family that is expressed on hepatocytes. Indeed, TRAIL can induce proapoptotic caspase activity in isolated human hepatocytes (Volkmann et al. 2007). The ligand, TRAIL, is expressed by cells of the immune system but in particular, KC, macrophages, and NK and NKT cells (Malhi et al. 2010). In healthy liver explants TRAIL only induces apoptosis when combined with histone deacetylase inhibitors augmenting hepatotoxic effects of these chemotherapeutic agents, while in diseased liver (livers with HCV, steatosis) TRAIL stimulation resulted in massive apoptosis (Volkmann et al. 2007; Gores and Kaufmann 2001). TRAIL was first described in 1995 and thought to exclusively induce apoptosis in cancer lines, sparing healthy tissues and cells (Walczak et al. 1999; French and Tschopp 1999). Unlike the massive cell death and liver apoptosis seen with FASL, TRAIL administration appeared relatively safe in primates studies (Ashkenazi et al. 1999). Of the five known TRAIL death receptors only two transmit the apoptotic signal (DR4, DR5) through activation of caspases. Binding of TRAIL to DR4 or DR5 similar to FAS results in recruitment of proteins to form a signaling complex and assembly of the DISC. FADD and caspase 8 (and in humans, caspase 10) also play an important role in this death receptor pathway that also needs mitochondrial participation in hepatocytes.

Additionally, TRAIL binding can result in c Jun N-terminal Kinase (JNK) activation, through RIPK1-dependent (early phase) and independent (late phase) pathways (Zhang et al. 2015). Sustained and prolonged JNK activation can also contribute to apoptosis and cell death (see more details in the JNK/MAPK section). Furthermore, JNK itself can upregulate DR4 and DR5, increasing sensitivity to TRAIL in certain contexts (Fu et al. 2010; Malhi 2007; Zou et al. 2004, 2008). Bile acids can also induce DR5 expression (while inhibiting cFLIP function), thereby sensitizing hepatocytes to TRAIL-mediated cell death (Higuchi et al. 2004). This also occurs through a JNK-mediated pathway (Higuchi et al. 2004). The livers of cholestatic mice (bile duct ligation model) also upregulate TRAIL expression, and TRAIL deficiency significantly rescues the liver injury from the cholestasis resulting from bile duct ligation (Takeda et al. 2008; Kahraman et al. 2008). In addition, TRAIL has been shown to play a role in FASL-induced apoptosis in liver through a JNK-dependent mechanism involving activation of proapoptotic BIM (Corazza et al. 2006).

1.2.3 Perforin/Granzyme

Perforin or granzyme-induced apoptosis is the main pathway used by CD8+ cytotoxic lymphocytes and NK cells to eliminate virus-infected cells. While no direct evidence for this exists in DILI models, this is a well-described rout of eliminating virus-infected hepatocytes (Knolle and Thimme 2014). CD8 T cells and NK cells target infected cells, bind to them (conjugate), and release cytotoxic granules containing perforin, granzymes, and granulysin (Voskoboinik et al. 2015). Defects in this pathway result in many human diseases such as familial hemophagocytic lymphohistiocytosis and increased susceptibilities to viral infections and hematological malignancies (Voskoboinik et al. 2015). Although no data exists regarding the role of this pathway in IDILI, possible antigen mimicry can trigger cytotoxic T cells to kill hepatocytes expressing drug antigens thereby killing them and causing DILI.

1.2.4 TNF

The cell death pathways following TNFR stimulation by its ligands are among the most extensively studied signaling cascades in molecular biology (Fig. 1.2). When TNF binds its receptor, multiple proteins are recruited to a membrane-bound supramolecular structure termed complex 1. These include TNFR-associated death domain (TRADD), RIPK1, cellular inhibitor of apoptosis 1 and 2 (cIAP1 and 2), TNFR-associated factor 2 (TRAF2), or TRAF5. cIAPs, which are E3 ubiquitin ligases, are recruited to complex 1 via TRAF2 that prevents their polyubiquitylation. cIAPs catalyze the Lys63-linked polyubiquitylation of RIPK1 which serves as a scaffold for NFκB activation through transforming growth factor-β-activated kinase 1 (TAK1) and TAK1-binding proteins 2 and 3 (TAB2 and TAB3). In order for TNF to result in cell death, a second complex, receptor-free complex II, has to assemble in the cytoplasm. This consists of FADD, RIPK1, or TRADD, and caspase-8 to activate caspase-3 and caspase-7. In many cells such as hepatocytes, formation of complex II is blocked by Fas-associating protein with death domain-like interleukin-1 beta-converting enzyme (FLICE) inhibitory protein (c-FLIP) and NFκB target genes such as A20 (Karin and Lin 2002; Micheau and Tschopp 2003; Irmler et al. 1997).

Initiator and executioner caspases are present in a latent or catytically inactive zymogen form in the cytosol. Once the canonical pathway is engaged, the executioner caspases are activated by cleavage into 10 and 20 kD fragments by the initiator caspase. Activation of the executioner caspases, i.e., caspase −3, −6, and −7, which are effectors of both the extrinsic and intrinsic pathways, results in proteolysis and activation of nucleases leading to DNA fragmentation. In type I cells such as lymphocytes, activation of caspase −8 is sufficient to activate caspase-3 and other executioner caspases resulting in apoptosis. However, in type 2 cells such as hepatocytes, extrinsic pathway apoptosis induction first requires caspase −8-mediated

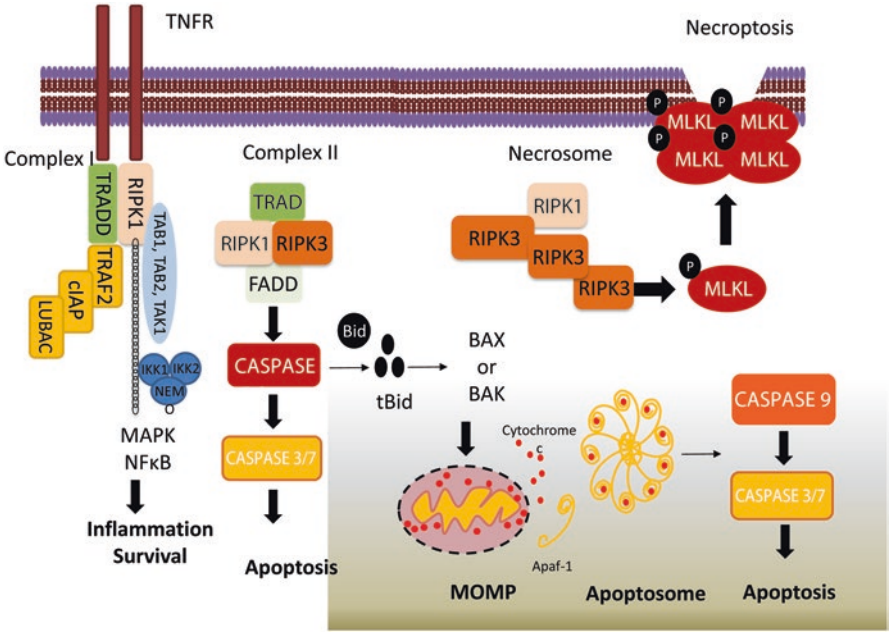

Fig. 1.2 TNF receptor-mediated cell death. TNF binding to TNF receptor (TNFR) results in the formation of complex I that includes TRADD, RIPK1, TRAF2, IAP1, IAP2, and LUBAC. Ubiquitination of RIPK1 results in a platform formation and recruitment of the IKK complex (made up of NEMO, IKK1, and IKK2) and TAB/TAK-1 complexes. This results in the activation of NF-κB and mitogen-activated protein kinase (MAPK). Complex II is made up of TRADD, FADD, and caspase-8 (IIa) or RIPK1/RIPK3, FADD, Caspase 8, and FLIPL (not shown) (IIb) which leads to caspase-8-mediated activation of caspase 3/7 and apoptosis in type I cells. In type II cells caspase 8 mediates cleavage of Bid to tBid resulting in BAX and BAK-mediated MOMP and Cytochrome C release. Cytochrome C binds to APAF-1 to form a wheel-like structure called the apoptosome that activates caspase 9, which in turn activates the effector caspases (caspase 3/7) to induce apoptosis. In certain cells when caspase-8 is inhibited the necrosome is formed, made up of RIPK1, RIPK3, and MLKL which leads to phospho-activation of MLKL, leading to its oligomerization and translocation to cell membrane to induce pore opening and necroptosis

cleavage of Bid, a Bcl-2 family protein, and subsequent mitochondrial participation and mitochondrial outer membrane permeabilization (MOMP) for executioner caspase activation. Proapoptotic Bcl-2 family members that include Bax, which translocates to Bak, which resides in mitochondrial outer membrane are activated by cleaved Bid (tBid) or Bim which leads to MOMP, releasing cytochrome *c* and other intermembrane proteins (Kaufmann et al. 2009; Yin et al. 1999). This Bcl-2 family participation and mitochondrial amplification via activation of the apoptosome is necessary for apoptosis to ensue in liver cells as hepatocytes are extremely resistant to the lethal actions of TNF because formation of the DISC not only activates the apoptosis machinery (and in some cases necroptosis) but also serves to activate NFκB. The resistance to TNF in hepatocytes can be overcome by using translation or transcription inhibitors (such as actinomycin-D or galactosamine), or depletion

of glutathione (GSH) (Feng and Kaplowitz 2000; Nagai et al. 2002; Pierce et al. 2000; Lou and Kaplowitz 2007). In addition, generation of free radicals and ROS has also been implicated in hepatocytes to interfere with NFκB signaling and sensitize to TNF in hepatocytes (Han et al. 2006). This could be occurring through inhibition of IKK or the indirect effect of reactive oxygen species mediated by the alteration of cellular redox status (GSH/GSSG). Regardless, interference with NFκB is necessary for cell death via TNF in hepatocytes (Han et al. 2006; Lou and Kaplowitz 2007).

As noted above, apoptosis in hepatocytes and other type II cells occurs through the activation of a complex termed the "apoptosome" that forms as a result of MOMP and cytochrome *c* release. The primary component of the apoptosome is apoptotic peptidase-activating factor-1 (Apaf-1). Under normal conditions, Apaf-1 exists in a monomeric form. A series of WD-40 repeats at the C termus keep Apaf-1 autoinhibited (these bind cytochrome *c*), while a caspase-binding domain at the N terminal recruits caspase −9, and a nucleotide binding and oligomerization domain in the middle of the protein exhibits ATP-ase activity. When apoptosis signals are sensed, activated Bcl-2 family members translocate to the mitochondria, causing permeabilization of the outer mitochondrial membrane resulting in cytochrome *c* release that then binds to the WD-40 region of Apaf-1 molecule releasing its autoinhibitory effect (Kim et al. 2005; Liu et al. 1996; Zou et al. 1997). Apaf-1 then forms a wheel-like structure called the apoptosome that promotes self-activation of caspase 9 which then cleaves executioner caspases (Acehan et al. 2002).

1.3 JNK in DILI

In addition to the Bcl2 family, the mitochondria, and caspases, the mitogen-activated protein kinase (MAPK) family are critical regulators of hepatocyte death. MAPKs are a tiered cascade of kinases and the principal regulators of stress-responsive pathways in hepatocytes (Chang and Karin 2001). MAPKs are governed by their phosphorylation status. C Jun-N-terminal kinase (JNK), an extensively studied member of this family in the context of DILI, plays a critical role in liver homeostasis and disease (Chang and Karin 2001; Sabapathy et al. 2004). Once JNK is activated, the most critical factors dictating cell fate in hepatocytes are the duration of sustained JNK activation and the protein's subcellular localization. Prolonged JNK activation, which likely corresponds to the magnitude and duration of stress, promotes cell demise, while transient JNK activation does not (Bradham et al. 1997; Gunawan et al. 2006; Hanawa et al. 2008; Win et al. 2011). JNK activates the intrinsic mitochondrial pathway of apoptosis by phosphorylation of Bcl2 proteins, Bim and Bax, and mediates phosphorylation and inactivation of antiapoptotic Bcl2 proteins, Bcl-xl and mcl-1 (Lei and Davis 2003; Lei et al. 2002; Tournier et al. 2000). Interestingly, JNK activation and translocation to mitochondria is not only important for apoptosis in hepatocytes but is a critical step in APAP-induced hepatocyte necrosis as well (Win et al. 2011). This delicate balance between JNK activation, mitochondrial ROS generation, and NFκB transcriptional regulation determines

hepatocyte death or survival. TNF treatment in hepatocytes causes ROS formation leading to sustained MAP kinase (JNK) activation (Win et al. 2011). ROS formation from mitochondria is required for prolonged JNK activation. JNK can lead to degradation of cFLIP, a pro-survival protein induced by NFκB, and NFκB-responsive genes, such as XIAP, have been implicated in inhibition of JNK (Chang et al. 2006; Liu et al. 2002). Inhibition of NFκB in the presence of TNFR engagement results in sustained JNK activation and promotes TNF-induced hepatocyte death (Chang et al. 2006; Liu et al. 2002). When JNK signals to mitochondria, it binds to SH3BP5 or Sab, which spans the outer mitochondrial membrane (Wiltshire et al. 2002, 2004). Preventing JNK binding to Sab abrogates liver cell death both in apoptotic and in necrotic models of liver injury including DILI from acetaminophen (necrotic model), ER stress apoptosis models, TNF (plus D-galactosamine), and palmitic acid-induced liver cell apoptosis, further supporting the notion that JNK translocation and mitochondrial participation are critical for hepatocyte death in these models (Win et al. 2011, 2014, 2015). The intra-mitochondrial downstream pathways are discussed further in the programmed necrosis section (see below). It should be mentioned that JNK participation in hepatocellular death is not universal; thus in models of direct toxicity of CCL4 and furosemide, JNK is dispensable (Gunawan et al. 2006; McGill et al. 2015).

Some hepatotoxic agents cause injury by activating cellular pathways leading to apoptosis. Actinomycin D and the mushroom toxin α-amanitin induce a transcriptional arrest, thereby inhibiting NFκB and sensitizing to liver cell apoptosis via TNF stimulation and apoptosis (Leist et al. 1994, 1997). Drugs that cause idiosyncratic DILI (IDILI) do so in large part due to aberrant adaptive immunity in susceptible individuals with HLA polymorphisms. This cell death mode in immune-mediated liver injury is death receptor mediated and therefore primarily apoptotic. As an example, diclofenac, a known idiosyncratic hepatotoxin, has been suggested to cause toxicity in large part by inducing MOMP and apoptosis (Siu et al. 2008). In HepG2 cells, diclofenac induced apoptosis through the caspase8/Bid/Apaf1 pathway leading to caspase 3 cleavage. Furthermore, diclofenac induced sustained JNK activation and decreased NFκB transcriptional activity, thereby sensitizing cells to apoptosis through multiple mechanisms (Fredriksson et al. 2011). Interestingly while IDILI toxicity is presumed to be carried out by the adaptive immune system, in vitro drug panels exploring cytotoxicity of IDILI drugs in cultured primary human hepatocytes have demonstrated a direct induction of cytotoxicity by IDILI drugs as measured by decreases in GSH and ATP, increased ROS/ATP ratio, and activation of caspase 3 (Zhang et al. 2016).

1.4 Mitochondria in DILI

Mitochondria are crucial organelles in liver cell fitness and survival and they play an indispensable role in the execution of liver cell apoptosis. Therefore, it is not surprising that many hepatotoxins kill liver cells by targeting mitochondria. It is important to distinguish essential mitochondrial participation downstream of DR

signaling, DNA damage, and ER stress from DILI due to direct targeting of drugs to the mitochondria. For example, Reye and colleagues described in the 1960s that children with febrile illness given aspirin developed encephalopathy, profound hypoglycemia, lactic acidosis, and microvesicular steatosis. Mitochondria is a key target of APAP toxicity will be discussed in another chapter. Other drugs such as valproic acid, antiretrovirals, tetracycline, tamoxifen, and amiodarone can also target mitochondrial disrupting oxidative phosphorylation resulting in steatosis and liver injury and even acute liver failure (Lemasters et al. 1998; Silva et al. 1997; Fromenty and Pessayre 1995; Lewis et al. 1989). Drug-induced mitochondrial toxicity can result from interference with mitochondrial DNA synthesis. Fialuridine, an antiviral that was being developed for hepatitis B, exemplifies this type of toxicity. During the clinical trial, patients were noted to have elevated liver enzymes, pancreatitis, lactic acidosis, and even liver failure and death after a long latency. Disruption of mitochondrial function was determined to be the underlying mechanism as evidenced by depletion of mitochondrial DNA and classic clinical features of mitochondrial toxicity (McKenzie et al. 1995). A similar syndrome has been reported with the antiretrovirals zidovudine and didanosine (Bissuel et al. 1994). Due to the serious nature of these toxicities, all antiviral nucleoside and nucleotide analogue drugs have a black-box warning regarding potential mitochondrial toxicity (Dara et al. 2012). Valproic acid (VPA), an anticonvulsant and mood stabilizer, is another drug that kills liver cells by targeting mitochondria in multiple ways. VPA is metabolized by the cytochrome P-450 system to a reactive intermediary, 4-ene VPA, which is conjugated with coA to 4-ene-CoA, which enters mitochondria and then can be converted by β-oxidation to 2,4-diene VPA a highly electrophilic metabolite (Kassahun et al. 1991, 1994). Therefore, these reactive metabolites are generated in larger amounts when patients take other anticonvulsants such as carbamazepine or phenytoin concomitantly (Levy et al. 1990). Also, VPA itself is a fatty acid and can therefore easily cross the mitochondrial membrane to uncouple respiration by interfering with the proton gradient, resulting in microvesicular steatosis and Reye's syndrome (Levy et al. 1990). Valproate also sequesters CoA by forming valproyl-CoA moieties; thus, interfering with natural fatty acyl-CoA-oxidation, as well as pyruvate dehydrogenase, which requires CoA (Ponchaut et al. 1992; Silva et al. 1997). Another important determinant of valproate toxicity is amino acid substitutions that decrease the activity of mt-DNA polymerase γ (POLG) (Naviaux and Nguyen 2004). Alpers-Huttenlocher syndrome, a rare childhood disorder characterized by epilepsy, developmental delay, and liver disease, is associated with severe toxicity from VPA. This VPA toxicity occurs in up to a third of patients with this disorder and is due to the POLG mutation (Naviaux and Nguyen 2004). POLG mutations seem to correlate well with risk of VPA toxicity and interestingly even patients with *heterozygous* mutations in the POLG gene were associated with an increased risk of liver toxicity from valproate in the Drug-Induced Liver Injury Network (DILIN)

(Stewart et al. 2010). The presumption is that the mutation in POLG, by impairing mitochondrial DNA synthesis, sensitizes to the toxicity of VPA although this remains to be proven in model systems. In the general population, VPA-induced

DILI occurs in a small proportion of treated individuals which therefore can be considered IDILI. It is unclear if this represents direct toxicity to mitochondria alone or if an immune mechanism might contribute with sublethal mitochondrial impairment acting as a danger signal costimulating the adaptive immune system. Many IDILI drugs directly impair the function of isolated mitochondria (Porceddu et al. 2012). In addition, antibiotics are the major contributor drugs to IDILI and given mitochondria are descendants of bacteria it is intriguing to speculate that these drugs may cause liver toxicity in part by targeting liver mitochondria.

1.5 ER Stress in DILI

ER stress is another important intracellular stress pathway in hepatocytes that can result in cell death (Dara et al. 2011). ER stress occurs in specialized secretory cells when the amount of protein entering the ER lumen exceeds the organelle's capacity to fold them resulting in the activation of a stress response to cope with and compensate for the resulting gridlock (Hetz et al. 2013; Ron and Walter 2007). Thus, ER stress initiates a series of adaptive mechanisms termed the unfolded protein response or UPR to restore protein folding and homeostasis (by upregulating chaperones, selectively degrading mRNA of secretory proteins to decrease work load, or inhibiting protein synthesis) (Hetz et al. 2013; Ron and Walter 2007). Additionally, the UPR seems to be an essential signaling pathway in many physiologic processes such as lipid and cholesterol metabolism, oxidative stress, inflammatory response pathways, and cancer development (Wang and Kaufman 2012). The three major stress sensors that control the UPR are inositol-requiring enzyme 1α (IRE1α), protein kinase RNA-like ER kinase (PERK), and activating transcription factor 6 (ATF6). These three transmembrane proteins are anchored to the ER and activate downstream signals that travel to the nucleus to activate genes to restore protein folding. The IRE1α branch of the UPR activates X-box-binding protein 1 or XBP1s which is a transcription factor that activates ER-associated protein degradation in the cytosol and protein quality control. When the ER stress is too severe to overcome, IRE1α can activate the JNK-mediated apoptosis pathway (via ASK1 and TRAF2) to induce cell death (Urano et al. 2000). P-JNK then translocates to mitochondria where it induces ROS production; uptake of CA2+ released by the ER amplifies this effect of JNK on mitochondria. Sustained JNK then results in modulation of Bcl2 family leading to MOMP (Win et al. 2014). It has been shown that covalent binding of reactive chemicals activates ER stress, though the precise mechanism is unclear regarding whether covalent binding of client proteins leads to misfolding or direct impairment of UPR mediators (Dara et al. 2011). It is therefore intriguing to hypothesize that certain drugs cause DILI and hepatocyte death via the ER stress pathway. While many proteins are activated through the UPR, one of the main elements of ER stress-mediated apoptosis is CHOP, a transcription repressor that is activated downstream of the PERK and IRE1 pathways of the UPR. CHOP expression in the liver seems to be upregulated with various stressors such as